

Journal of

Zeitschrift für Neuroforschung

Biosciences

Indexed 1946 in the libraries
of Max-Planck-Gesellschaft

Volume 42 Number 1/2
January/February 1987



Journal of Zeitschrift für Neuroforschung

Tübingen

ZEITSCHRIFT FÜR NATURFORSCHUNG

Section C

A Journal of Biosciences

ISSN 0341-0382

Managing Editor:

H. Hausen, Tübingen

Council:

E. Bünning, Tübingen
A. Butenandt, München
M. Eigen, Göttingen

Editors:

A. Hager, Tübingen
K. Hahlbrock, Köln
W. Hasselbach, Heidelberg
P. Karlson, Marburg
F. Kaudewitz, München
J. Klein, Tübingen
J. St. Schell, Köln
E. Wecker, Würzburg

Associate Editors:

N. Amrhein, Bochum
B. A. Askonas, London
W. Barz, Münster
P. Böger, Konstanz
G. Bornkamm, Freiburg
D. Bückmann, Ulm
K. G. Götz, Tübingen
G. Gottschalk, Göttingen
P. Gruss, Heidelberg
G. Isenberg, Köln
R. Jaenicke, Regensburg
V. ter Meulen, Würzburg
G. F. Meyer, Tübingen
M. Rajewsky, Essen
H. Schimassek, Heidelberg
D. Schulte-Frohlinde,
Mühlheim/R.
G. Schulz, Freiburg
F. F. Seelig, Tübingen
J. Seelig, Basel
H. Simon, München
W. Steglich, Bonn
H. Stieve, Aachen
J. Suko, Wien
A. Trebst, Bochum
G. Weissenböck, Köln
G. Wick, Innsbruck
V. Zimmermann, Würzburg

Information for Contributors

Contributions to Z. Naturforsch., Section C (biological sciences), may be (i) original papers, (ii) research notes not exceeding two pages in print and (iii) reports on current research of special interest. Two copies of each contribution should be sent to Dr. Helga Hausen, P.O. Box 2645, D-7400 Tübingen. (Section B (chemical sciences): Dr. Heide Voelter, P.O. Box 2645, D-7400 Tübingen; Section A (physical sciences): Mrs. Tamara Littmann, Lenzhalde 21, D-7082 Oberkochen).

Contributors are encouraged to suggest possible reviewers of their papers. The manuscript should be type written using double spacing throughout.

The title should be concise but informative. The names of the authors, with first names and initials, and of the institution where the work was carried out follow the title. A running title with not more than 60 characters should be indicated if the title is longer than this.

In a footnote on the first page, please give an address for reprint requests.

The title, a selfconsistent abstract, suitable for direct use by the abstracting journals (no references to the main text), and five keywords, all in English, must precede the main text of each contribution.

The main text should meet the highest standards as to novelty of the material, organization and conciseness. A qualified colleague and, if the text is in a foreign language, a person who thoroughly knows it, should have been given the opportunity to check the paper before its submission for publication.

Papers, reporting mainly spectroscopic, X-ray or other data of no general relevancy should not be submitted.

References and footnotes should be numbered (e.g. [1]) and listed at the end of the paper (e.g. [1] A. Meyer, Z. Naturforsch. **30c**, 633 (1976)).

Tables with the appropriate captions and a list of the figure legends should follow at the end of the paper.

Allowance should be made for the reduction in printing of the drawings (line thickness, lettering!). Original drawings larger than 21 × 30 cm should be replaced by copies of reduced size, when the manuscript is submitted and only be sent in when the manuscript is accepted. On all illustrations, the figure number and the author's name must be written in pencil.

The authors will receive page proofs.

Changes in the text after acceptance of the paper and drawings not fit for direct reproduction cause delay and create extra costs which may be charged to the author. 50 reprints are free of charge.

Information for Subscribers

The subscription prices per year are for normal subscribers:

Section A	Section B	Section C
DM 510.—	DM 610.—	DM 425.—

for authors and their institutions if they order directly from the publishers:

Section A	Section B	Section C
DM 408.—	DM 488.—	DM 340.—

plus postage and handling

Section A	Section B	Section C
DM 35.—	DM 35.—	DM 25.—

Single copies and back-numbers are available.

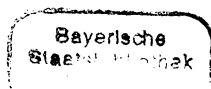
Subscriptions will remain standing for the following year unless cancellations are made by postage October 1st.

Informations for Advertisers

The price for a small advertisement (breadth 43 mm, height 57 mm) in all three sections of the journal is DM 60.—.

For larger advertisements please order the price list from the publishers.

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG, TÜBINGEN
P.O. Box 2645, D-7400 Tübingen (Postcheck-Konto Stuttgart 8039-700).



ZEITSCHRIFT FÜR NATURFORSCHUNG

SECTION C

A EUROPEAN JOURNAL OF
BIOSCIENCES

Council

E. BÜNNING, Tübingen
A. BUTENANDT, München
M. EIGEN, Göttingen

Editorial Board

A. HAGER, Tübingen
K. HAHNBROCK, Köln
W. HASSELBACH, Heidelberg
P. KARLSON, Marburg
F. KAUDEWITZ, München
J. KLEIN, Tübingen
J. ST. SCHELL, Köln
E. WECKER, Würzburg

Advisory Editorial Board

N. AMRHEIN, Bochum
B. A. ASKONAS, London
W. BARZ, Münster
P. BÖGER, Konstanz
G. BORNKAMM, Freiburg
D. BÜCKMANN, Ulm
K. G. GÖTZ, Tübingen
G. GOTTSCHALK, Göttingen
P. GRUSS, Heidelberg

G. ISENBERG, Köln
R. JAENICKE, Regensburg
V. TER MEULEN, Würzburg
G. F. MEYER, Tübingen
M. RAJEWSKY, Essen
H. SCHIMASSEK, Heidelberg
D. SCHULTE-FROHLINDE, Mülheim/R.
G. SCHULZ, Freiburg
F. F. SEELIG, Tübingen

J. SEELIG, Basel
H. SIMON, München
W. STEGLICH, Bonn
H. STIEVE, Aachen
J. SUKO, Wien
A. TREBST, Bochum
G. WEISSENBOCK, Köln
G. WICK, Innsbruck
V. ZIMMERMANN, Würzburg

EDITED IN COLLABORATION

WITH THE INSTITUTES OF THE MAX-PLANCK-GESELLSCHAFT

Volume 42c

1987

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG
TÜBINGEN

Anschrift des Verlages: Postfach 26 45, D-7400 Tübingen
Satz und Druck: Allgäuer Zeitungsverlag GmbH, Kempten

Nachdruck – auch auszugsweise – nur mit schriftlicher Genehmigung des Verlages

Section a
Physics, Physical Chemistry, Cosmic Physics

Section b
Inorganic and Organic Chemistry

Contents

Contents of Number 1/2

Original Communications

X-Ray Structure Analysis and Spectroscopic Data of the Antibiotic 8-(Dichloroacetyl)-5-hydroxy-2,7-dimethyl-1,4-naphthoquinone from the Fungus *Mollisia* sp.

G. WEBER, T. HÜBNER, A. GIEREN, J. SONNENBICHLER, T. KOWALSKI, and O. HOLDENRIEDER 1

Structure of Azadirachtin B

H. REMBOID, H. FORSTER, and J. SONNENBICHLER 4

n-Alkylphenols from *Schinus terebinthifolius* RADDI (Anacardiaceae) (In German)

G. SKOPP, H.-J. OPFERKUCH, and G. SCHWENKER 7

Methylation of Guanine *in vivo* by the Organophosphorus Insecticide Methamidophos

S. M. A. D. ZAYED and F. M. MAHDI 17

Cytogenetic Effects of the Insecticide Methamidophos in Mouse Bone Marrow and Cultured Mouse Spleen Cells

S. M. AMER and M. A. SAYED 21

Molluscicidal Properties of Quinones (In German)

H. SCHILDKNECHT and J. LUBOSCH 31

Effects of Plant Bioregulators on the Production of Iridoid Derived Terpenoids in *Valeriana wallichii* and *Fedia cornucopiae* Cell Suspension Cultures

W. FÖRSTER and H. BECKER 33

Reactions of Substituted Arenediazonium Chlorides with Methylamine-Formaldehyde Premix Revisited: Reactivity and Transformations of Methylolamine Intermediates and Their Biological Significance

G. F. KOLAR and M. SCHENDZIELORZ 41

Interaction *in vitro* of Non-Epithelial Intermediate Filament Proteins with Histones

P. TRAUB, G. PERIDES, S. KÜHN, and A. SCHERBARTH 47

Transformations of *trans*-2-Hexenal by *Botrytis cinerea* PERS. as Detoxification Mechanisms (In German)

I. URBASCH 64

Alkaloids in Stem Roots of *Nicotiana tabacum* and *Spartium junceum* Transformed by *Agrobacterium rhizogenes*

M. WINK and L. WITTE 69

Flavonoids from the Leaf Resin of Snakeweed, *Gutierrezia sarothrae*

D. HRADETZKY, E. WOLLENWEBER, and J. N. ROITMAN 73

The 14 β -Hydroxylation in the Biosynthesis of Cardenolides in *Digitalis purpurea*. The Role of 3 β -Hydroxy-5 β -pregn-8(14)-en-20-one

M. E. DELUCA, A. M. SELDES, and E. G. GROS 77

Biotransformation of Humulene by Fungi and Enantioselectivity of the Strains Used

W.-R. ABRAHAM and B. STUMPF 79

Enzymatic Synthesis of 1,6-Digalloylglucose from β -Glucogallin by β -Glucogallin: β -Glucogallin 6-O-Galloyltransferase from Oak Leaves

S. W. SCHMIDT, K. DENZEL, G. SCHILLING, and G. G. GROSS 87

PAPS-Reductase from *Escherichia coli*: Characterization of the Enzyme as Probe for Thioredoxins

J. D. SCHWENN and U. SCHRIEK 93

Protein Transport in Chloroplasts: ATP is Prerequisite

C. SCHINDLER, R. HRACKY, and J. SOLL 103

Lipid Influence on the Structure of the Light Harvesting B800–850 Proteins

J. PESCHKE and H. MÖHWALD 109

Daffodil Chromoplast DNA: Comparison with Chloroplast DNA, Physical Map, and Gene Localization

P. HANSMANN 118

Uptake of Protoporphyrin and Violet Light Photo-destruction of <i>Propionibacterium acnes</i> T. B. MELØ	123	Species and Sex Specificity in the Odour Composition of Two Panurgine Bees (Hymenoptera, Andrenidae) W. FRANCKE, W. SCHRÖDER, A. K. BORG-KARLSSON, G. BERGSTRÖM, and J. TENGÖ	169
Evolution of <i>E. coli</i> tRNA ^{lle} : Evidence of Derivation from Other tRNAs M. P. STAVES, D. P. BLOCH, and J. C. LACEY, Jr.	129		
Intermolecular H-Abstraction of Thiyl Radicals from Thiols and the Intramolecular Complexing of the Thiyl Radical with the Thiol Group in 1,4-Dithiothreitol. A Pulse Radiolysis Study M. SH. AKHLAQ and C. VON SONNTAG	134	Contents of Number 3	
The Contents of the Dufour Gland of the Ant <i>Harpagoxenus sublaevis</i> Nyl. (Hymenoptera: Formicidae) D. G. OLLETT, E. D. MORGAN, A. B. ATTYGALLE, and J. P. J. BILLEN	141	Original Communications	
A GC/MS Study of the Propolis Phenolic Constituents V. BANKOVA, A. DYULGEROV, S. POPOV, and N. MAREKOV	147	Analysis of Single Channel Currents with a Microprocessor Based Device W. SCHREIBMAYER, E. HOFER, P. WOLF, A. LUEGER, and H. A. TRITTHART	173
Investigation of the Screening Pigment System in the Compound Eye of the Moth <i>Agrotis segetum</i> (fam. Noctuidae) by Visible Reflectometry T. NORDTUG and T. B. MELØ	152	Natural Waxes Investigated by Soft Ionization Mass Spectrometry H.-R. SCHULTEN, K. E. MURRAY, and N. SIMMLEIT	178
Notes		Epicuticular Leaf Wax of <i>Euphorbia dendroides</i> L., Euphorbiaceae P.-G. GÜLZ, H. HEMMERS, J. BODDEN, and F.-J. MARNER	191
Epicuticular Leaf Wax of <i>Cistus albanicus</i> , Cistaceae T. VOGT and P.-G. GÜLZ	157	Cell-Free Synthesis of the Alkaloids Ammodendrine and Smipine M. WINK and L. WITTE	197
Isopulegol from Liquid Cultures of the Fungus <i>Ceratomyces coerulescens</i> (Ascomycotina) W.-G. KOCH and V. SINNWELL	159	Comparison of the Solid State CPMAS and Solution Carbon-13-NMR Spectra of Humic Acids Extracted from Composted Municipal Refuse R. FRÜND, F. J. GONZALEZ-VILA, H.-D. LÜDEMANN, and F. MARTIN	205
Inhibition of cAMP-Phosphodiesterase by Molybdate B. OFENLOCH-HÄHNLE and K. EISELE	162	Glutamic Acid-1-semialdehyde, a Hypothetical Intermediate in the Biosynthesis of 5-Amino-levulinic Acid A. KAH and D. DÖRNEMANN	209
Polyenic Hydrocarbons as Sex Attractants for Geometrids and Amatids (Lepidoptera) Found by Field Screening in Hungary G. SZŐCS, M. TÓTH, H. J. BESTMANN, O. VOSTROWSKY, R. R. HEATH, and J. H. TUMLINSON	165	Interaction of Sodium, Lithium, Caesium, and Potassium Ions with Ascorbyl Radicals P. WIECZOREK, T. OGOŃSKI, and Z. MACHOY	215
		The Effect of Guanidinium Chloride on the Self-Association of Bovine Liver Glutamate Dehydrogenase: a Gel Filtration Study A. MAZZINI and R. FAVILLA	217

Molecular Mechanics Investigation on Side-Chain Conformations of a 17 α -Ethyl-17 β -hydroxy Steroid with Regard to Receptor Binding M. BOHL	221	Chains: Inhibitory Properties vs Purified <i>E. coli</i> Uridine Phosphorylase A. K. DRABIKOWSKA, L. LISSOWSKA, M. DRAMINSKI, A. ZGIT-WROBLEWSKA, and D. SHUGAR	288
Characterization and Amino Acid Composition of a Hypertrehalosaemic Neuropeptide from the Corpora cardiaca of the Cockroach, <i>Nauphoeta cinerea</i> G. GÄDE	225	A Simple Analysis of Purine and Pyrimidine Nucleotides in Plant Cells by High-Performance Liquid Chromatography H. ASHIHARA, K. MITSUI, and T. UKAJI	297
The Rate of ATP Hydrolysis Catalyzed by Reconstituted CF ₀ F ₁ -Liposomes G. SCHMIDT and P. GRÄBER	231	Magnetotactic Bacteria from Freshwater M. OBERHACK, R. SÜSSMUTH, and H. FRANK	300
Phosphate Accumulation by Muscle <i>in vitro</i> and the Influence of Vitamin D ₃ Metabolites T. BELLIDO and R. BOLAND	237		
A Correlation between Detergent Tolerance and Cell Wall Structure in Green Algae S. BIEDLINGMAIER, G. WANNER, and A. SCHMIDT	245		
Further Characterization of Chickpea Isoflavone 7-O-Glucoside-6"-O-malonate: malonyl-esterase: Evidence for a Highly Specific, Membrane-Bound Enzyme in Roots of <i>Cicer arietinum</i> L. W. HINDERER, J. KÖSTER, and W. BARZ	251		
Chromophore Assignment in C-Phycocyanin from <i>Mastigocladus laminosus</i> S. SIEBZEHRNÜBL, R. FISCHER, and H. SCHEER	258		
The Effect of Phosphinothricin (Glufosinate) on Photosynthesis. I. Inhibition of Photosynthesis and Accumulation of Ammonia A. WILD, H. SAUER, and W. RÜHLE	263		
The Effect of Phosphinothricin (Glufosinate) on Photosynthesis. II. The Causes of Inhibition of Photosynthesis H. SAUER, A. WILD, and W. RÜHLE	270		
Sethoxydim-Uptake by Leaf Slices of Sethoxydim Resistant and Sensitive Grasses I. STRUVE, B. GOLLE, and U. LÜTTGE	279		
Light-Induced Ca ²⁺ Influx into Spinach Protoplasts K. HEIMANN, G. KREIMER, M. MELKONIAN, and E. LATZKO	283		
Acyclonucleoside Analogues Consisting of 5- and 5,6-Substituted Uracils and Different Acyclic			

Contents of Number 4

Original Communications

Stereochemistry and Mechanism of Reactions Catalyzed by Tyrosine Phenol-Lyase from <i>Escherichia intermedia</i> M. M. PALCIC, S.-J. SHEN, E. SCHLEICHER, H. KUMAGAI, S. SAWADA, H. YAMADA, and H. G. FLOSS	307
Distant Precursors of Benzyloquinoline Alkaloids and Their Enzymatic Formation M. RUEFFER and M. H. ZENK	319
Characterization of 2 β (R)-17-O-Acetylajmalan: Acetyl-esterase – a Specific Enzyme Involved in the Biosynthesis of the <i>Rauwolfia</i> Alkaloid Ajmaline L. POLZ, H. SCHÜBEL, and J. STÖCKIGT	333
Induction and Characterization of a NADPH-Dependent Flavone Synthase from Cell Cultures of Soybean G. KOCHS and H. GRISEBACH	343
Proposal for the Mechanism of Action of Urocanase. Inference from the Inhibition by 2-Methylurocanate E. GERLINGER and J. RÉTEY	349
Re-Investigation of the Protein Structure of Coenzyme B ₁₂ -Dependent Diol Dehydrase K. TANIZAWA, N. NAKAJIMA, T. TORAYA, H. TANAKA, and K. SODA	353

Methanogenesis from Acetate by <i>Methanosarcina barkeri</i> : Catalysis of Acetate Formation from Methyl Iodide, CO ₂ , and H ₂ by the Enzyme System Involved K. LAUFER, B. EIKMANN, U. FRIMMER, and R. K. THAUER	360	Functional Group Recognition of Pheromone Molecules by Sensory Cells of <i>Antheraea polyphemus</i> and <i>Antheraea pernyi</i> (Lepidoptera: Saturniidae) H. J. BESTMANN, W. CAI-HONG, B. DÖHLA, LI-KEDONG, and K. E. KAISLING	435
Divergent Evolution of 5S rRNA Genes in <i>Methanococcus</i> G. WICH, L. SIBOLD, and A. BÖCK	373	Building Blocks for Oligonucleotide Syntheses with Uniformly Fragmentable β -Halogenated Protecting Groups (In German) P. LEMMEN, R. KARL, I. UGI, N. BALGOBIN, and J. CHATTOPADHYAYA	442
Characterization of Some <i>Claviceps</i> Strains Derived from Regenerated Protoplasts B. SCHUMANN, W. MAIER, and D. GRÖGER	381	Experiments on the Optical Resolution of Conduramine Analogs by Enzymatic Transesterification in Organic Solvents (In German) G. KRESZE and M. SABUNI	446
Phenylalanine and Tyrosine Biosynthesis in Spore-forming Members of the Order Actinomycetales H.-K. HUND, B. KELLER, and F. LINGENS	387	Steric Course of the Rhodium-Catalyzed Decarbonylation of Chiral 4-Methyl-[1- ³ H,2- ² H ₁]pentanal H. OTSUKA and H. G. FLOSS	449
<i>E. coli</i> Maltodextrin Phosphorylase: Primary Structure and Deletion Mapping of the C-Terminal Site D. PALM, R. GOERL, G. WEIDINGER, R. ZEIER, B. FISCHER, and R. SCHINZEL	394	Synthesis of Immobilized Peptide Fragments on Polystyrene-Polyoxyethylene for Affinity Chromatography (In German) E. BAYER, H. HELLSTERN, and H. ECKSTEIN	455
Fermentation of D-Xylose to Ethanol by <i>Bacillus macerans</i> H.-J. SCHEPERS, ST. BRINGER-MEYER, and H. SAHM	401	Biopterin Synthesis in Mouse Spleen during Bone Marrow Transplantation Correlates with Unimpaired Hemopoietic Engraftment I. ZIEGLER and ST. THIERFELDER	461
Semicontinuous and Continuous Production of Citric Acid with Immobilized Cells of <i>Aspergillus niger</i> H. EIKMEIER and H. J. REHM	408	<i>In vivo</i> Screening of Glutathione Related Detoxification Products in the Early State of Drug Development A. PROX, J. SCHMID, J. NICKL, and G. ENGELHARDT	465
Microbial Hydroxylation of Cedrol and Cedrene W.-R. ABRAHAM, P. WASHAUSEN, and K. KIESLICH	414	Synthesis and Complexing Features of an Artificial Receptor for Biogenic Amines (In German) F. P. SCHMIDTCHEN	476
6-Methylpurine, 6-Methyl-9- β -D-ribofuranosylpurine, and 6-Hydroxymethyl-9- β -D-ribofuranosylpurine as Antiviral Metabolites of <i>Collybia maculata</i> (Basidiomycetes) K. LEONHARDT, T. ANKE, E. HILLEN-MASKE, and W. STEGLICH	420	Metabolism of the Herbicide 2-(2,4-Dichlorophenoxy)-propionic Acid (Dichlorprop) in Barley (<i>Hordeum vulgare</i>) G. BÄRENWALD, B. SCHNEIDER, and H.-R. SCHÜTTE	486
Enzymatic Synthesis of Riboflavin and FMN Specifically Labeled with ¹³ C in the Xylene Ring H. SEDLMAIER, F. MÜLLER, P. J. KELLER, and A. BACHER	425	Site Directed Antisera to the D-2 Polypeptide Subunit of Photosystem II R. GEIGER, R. J. BERZBORN, B. DEPKA, W. OETTMEIER, and A. TREBST	491
A Vitamin D ₃ Steroid Hormone in the Calcinogenic Grass <i>Trisetum flavescens</i> W. A. RAMBECK, H. WEISER, and H. ZUCKER	430		

Contents of Number 5

Original Communications

- Epicuticular Wax Hydrocarbons of Ericaceae in Germany
I. SALASOO 499
- On the Essential Oils from *Chrysanthemum balsamita* L. (In German)
H. STROBEL, K. KNOBLOCH, and E. ZIEGLER 502
- Epoxy-*trans*-isodihydrorhodophytin, a New Metabolite from *Laurencia obtusa* (In German)
S. IMRE, H. LOTTER, H. WAGNER, and R. H. THOMSON 507
- Structure Elucidation of Kwakhurin, a New Prenylated Isoflavone from *Pueraria mirifica* Roots
S. TAHARA, J. L. INGHAM, and S. Z. DZIEDZIC 510
- Biosynthesis of Vitexin and Isovitexin: Enzymatic Synthesis of the C-Glucosylflavones Vitexin and Isovitexin with an Enzyme Preparation from *Fagopyrum esculentum* M. Seedlings
F. KERSCHER and G. FRANZ 519
- Effect of pH on Glycolate and Ammonia Excretion in L-MSO Treated *Chlorella* Cells
Y. SHIRAIWA and G. H. SCHMID 525
- Derepression of Arylsulfatase Activity by Sulfate Starvation in *Chlorella fusca*
I. NIEDERMEYER, S. BIEDLINGMAIER, and A. SCHMIDT 530
- Root Hair Specific Proteins in *Glycine max*
D. WERNER and A. B. WOLFF 537
- Synthesis, Analysis and Characterization of the Coenzyme A Esters of *o*-Succinylbenzoic Acid, an Intermediate in Vitamin K₂ (Menaquinone) Biosynthesis
R. KOLKMANN and E. LEISTNER 542
- Chloride Availability Affects the Malate Content and its Control by the Circadian Clock in Pulvini of *Phaseolus coccineus* L.
W.-E. MAYER, W. A. RUGE, N. STARRACH, and R. HAMPP 553
- Enzymatic Acyloin Condensation of Acyclic Aldehydes
W.-R. ABRAHAM and B. STUMPF 559
- Sn-Glycerol-3-phosphate is a Product of Starch Degradation in Isolated Chloroplasts from *Chlamydomonas reinhardtii*
G. KLÖCK and K. KREUZBERG 567
- Substrate Flow from Photosynthetic Carbon Metabolism to Chloroplast Isoprenoid Synthesis in Spinach Evidence for a Plastidic Phosphoglycerate Mutase
D. SCHULZE-SIEBERT, A. HEINTZE, and G. SCHULTZ 570
- Energy-Dependent Chlorophyll Fluorescence Quenching in Chloroplasts Correlated with Quantum Yield of Photosynthesis
G. H. KRAUSE and H. LAASCH 581
- Spontaneous Release of Malondialdehyde from Ultraviolet Light Exposed Liposomal Membranes
S. AGARWAL, A. GHOSH, and S. N. CHATTERJEE 585
- Pyrimidine Homoribonucleosides: Synthesis, Solution Conformation, and Some Biological Properties
P. LASSOTA, J. T. KUŚMIEREK, R. STOLARSKI, and D. SHUGAR 589
- Reactivation of Streptolysin S by Oligonucleotide
A. TAKETO and Y. TAKETO 599
- Ring Opening Reactions of Bioreactive Lactam Systems (In German)
H. FRISTER and E. SCHLIMME 603
- Embryotoxicity Induced by Alkylating Agents. Some Methodological Aspects of DNA Alkylation Studies in Murine Embryos Using Ethylmethanesulfonate
T. PLATZEK, G. BOCHERT, U. RAHM, and D. NEUBERT 613
- Tetraponerine-8, an Alkaloidal Contact Poison in a Neoginean Pseudomyrmecine Ant, *Tetraponera* sp.
J. C. BRAEKMAN, D. DALOZE, J. M. PASTEELS, P. VAN HECKE, J. P. DECLERCQ, V. SINNWELL, and W. FRANCKE 627

Contents of Number 7/8

Original Communications

- ZZE-Configuration of Chromophore β -153 in C-Phycocyanin from *Mastigocladus laminosus***
G. SCHMIDT, S. SIEBZEHRÜBL, R. FISCHER, W. RÜDIGER, H. SCHEER, T. SCHIRMER, W. BODE, and R. HUBER 845
- α -Diceroptene: A New Dimeric Structure for Isoceroptene**
CH. VILAIN, A. HUBERT, L. DUPONT, K. R. MARKHAM, and E. WOLLENWEBER 849
- Stereochemistry of Two Hydroxybiflavanonols from *Garcinia cola* Nuts**
J. SONNENBICHLER, I. MADUBUNYI, and H. SCHEER 855
- "Epicuticular Waxes" from Exine Material of Pine Pollen**
CH. NIESTER, P.-G. GÜLZ, and R. WIERMANN 858
- Bryoflavone and Heterobryoflavone, Two New Isoflavone-flavone Dimers from *Bryum capillare***
H. GEIGER, W. STEIN, R. MUES, and H. D. ZINSMEISTER 863
- Site of Lupanine and Sparteine Biosynthesis in Intact Plants and *in vitro* Organ Cultures**
M. WINK 868
- Occurrence of Amavadin in Mushrooms of the Genus *Amanita* (In German)**
E. KOCH, H. KNEIFEL, and E. BAYER 873
- Ascorbic Acid and Glutathione Contents of Spruce Needles from Different Locations in Bavaria**
W. F. OSSWALD, H. SENGGER, and F. E. ELSTNER 879
- Acetyl-CoA:4-Hydroxybutinylbithiophene O-Acetyltransferase Isoenzymes from *Tagetes patula* Seedlings**
G. METSCHULAT and R. SÜTFELD 885
- Uptake and Utilization of Sulfonic Acids in the Cyanobacterial Strains *Anabaena variabilis* and *Plectonema* 73110**
S. BIEDLINGMAIER and A. SCHMIDT 891
- Partial Characterization of an Enzyme from the Fungus *Ascochyta rabiei* for the Reductive Cleavage of Pterocarpan Phytoalexins to 2'-Hydroxyisoflavans**
B. HÖHL and W. BARZ 897
- Comparing Short-Term Effects of Ammonia and Methylamine on Nitrogenase Activity in *Anabaena variabilis* (ATCC 29413)**
S. REICH, H. ALMON, and P. BÖGER 902
- Reversible pH-Induced Dissociation of Glucose Dehydrogenase from *Bacillus megaterium*. II. Kinetics and Mechanism**
E. MAURER and G. PFLEIDERER 907
- On the Possible Relation between Morphology and Precursors of the Crystallinities in Calcified Tissues**
F. C. M. DRIESSENS, R. A. TERPSTRA, P. BENNEMA, J. H. M. WÖLTGENS, and R. M. H. VERBEEK 916
- In the Search for New Anticancer Drugs, XXI. Spin Labeled Nitrosoureas**
G. SOSNOVSKY, S. W. LI, and N. U. M. RAO 921
- Transmission of Hormonal Imprinting in *Tetrahymena* Cultures by Intercellular Communication**
G. CSABA and P. KOVÁCS 932
- MTD Calculations on Quantitative Structure-Activity Relationships of Steroids Binding to the Progesterone Receptor**
M. BOHL, Z. SIMON, A. VLAD, G. KAUFMANN, and K. PONSOLD 935
- Synthesis of Heat Shock Proteins during Amino Acid or Oxygen Limitation in *Bacillus subtilis* *relA*⁺ and *relA* (In German)**
M. HECKER, A. RICHTER, A. SCHROETER, L. WÖLFEL, and F. MACH 941
- Sexual Pheromones and Related Egg Secretions in Laminariales (Phaeophyta)**
I. MAIER, D. G. MÜLLER, G. GASSMANN, W. BOLAND, and L. JAENICKE 948
- Comparison of Dufour Gland Secretions of Two Species of *Leptothorax* Ants (Hymenoptera: Formicidae)**
M. F. ALI, E. D. MORGAN, A. B. ATTYGALLE, and J. P. J. BILLEN 955

Comparative Sex Pheromone Biosynthesis in the Obliquebanded Leafroller, <i>Choristoneura rosaceana</i> , and the Redbanded Leafroller, <i>Argyrotaenia velutinana</i> , Moths ST. P. FOSTER and W. L. ROELOFS	961	Are Small RNAs Associated with Crohn's Disease? R. PECHAN, H. KUNERT, and H. J. GROSS	1006
Effect of Hydroxylamine Derivatives on Photorespiration in the Tobacco Aurea Mutant <i>Nicotiana tabacum</i> Su/su G. H. SCHMID, K. P. BADER, A. RADUNZ, C. J. VAN ASSCHE, N. REINIER, and B. COURTIADÉ	965	Inhibition of cAMP Phosphodiesterase by Some Phototherapeutic Agents L. BOVALINI, P. LUSINI, S. SIMONI, D. VEDALDI, L. ANDREASSI, F. DALL'ACQUA, and P. MARTELLI	1009
Estimation of the Extent of DNA Platination after Interaction of <i>cis</i> -DDP with DNA and Chromatin I. KULAMOWICZ and Z. WALTER	970	<i>Report</i>	
Reversed Light Reaction of the Screening Pigment in a Compound Eye Induced by Noradrenaline A. JUSE, G. HÖGLUND, and K. HAMDORF	973	Chimeric Genes – Their Contribution for Molecular Biology and Plant Breeding (In German) H.-H. STEINBISS and J. SCHELL	1011
Light and Electron Microscopic Studies Regarding Cell Contractility and Cell Coupling in Light Sensitive Smooth Muscle Cells from the Isolated Frog Iris Sphincter K. V. WOLF	977	Contents of Number 9/10	
Fluorescence Monitoring of Membrane Potentials: the Spatio-Temporal Resolution in Isolated Neurons of <i>Helix pomatia</i> TH. KNÖPFEL and P. FROMHERZ	986	<i>Original Communications</i>	
Growth Kinetics of the G2-Phase of Ehrlich Ascites Tumor Cells, Separated from Anaerobically Treated Asynchronous Cultures C. KROLL, W. KROLL, and F. SCHNEIDER	991	Terpenoids from a Black Sea Bryozoan <i>Conopeum seuratum</i> P. HADJIEVA, S. POPOV, B. BUDEVSKA, A. DYULGEROV, and S. ANDREEV	1019
Demonstration of Statistically Significant Correlations between 8 and 12 kHz Atmosferics and Sudan Deafness G. RUHENSTROTH-BAUER, K. MEES, R. SANDHAGEN, H. BAUMER, and B. FILIPIAK	999	Thin Layer Chromatographic and Infra Red Spectral Evidence for the Presence of Phosphonolipids in <i>Cicada oni</i> M. C. MOSCHIDIS	1023
<i>Notes</i>		Adnexal Glands Chemistry of <i>Messor ebeninus</i> Forel (Formicidae: Myrmicinae) M. COLL, A. HEFETZ, and H. A. LLOYD	1027
γ -Decalactone, an Odoriferous Compound from the Male Butterfly, <i>Lethe marginalis</i> Motschulsky N. HAYASHI, H. KAWAGUCHI, A. NISHI, and H. KOMAE	1001	A Novel Caffeic Acid Derivative and Other Constituents of <i>Populus</i> Bud Excretion and Propolis (Bee-Glue) E. WOLLENWEBER, Y. ASAKAWA, D. SCHILLO, U. LEHMANN, and H. WEIGEL	1030
The Phosphatidylinositol Species of Suspension Cultured Plant Cells S. HEIM and K. G. WAGNER	1003	Polyunsaturated Pheromones: Semi-Synthesis of (Z,Z)-6,9-Alkadienes and (Z,Z,Z)-3,6,9-Alkatrienes from Naturally Occurring Fatty Acids H. K. MANGOLD, H. BECKER, and E. SCHULTE	1035
		NMR Spectra of Flavone Di-C-glycosides from <i>Apometzgeria pubescens</i> and the Detection of Rotational Isomerism in 8-C-Hexosylflavones K. R. MARKHAM, R. MUES, M. STOLL, and H. D. ZINSMEISTER	1039

Trichothecene Mycotoxins from <i>Fusarium culmorum</i> Cultures N. C. P. BALDWIN, B. W. BYCROFT, P. M. DEWICK, D. C. MARSH, and J. GILBERT	1043	Comparison of Various Strategies Designed to Optimize Indole Alkaloid Accumulation of a Cell Suspension Culture of <i>Catharanthus roseus</i> J. BERLIN, CH. MOLLENSCHOTT, and F. DiCOSMO	1101
Metabolism of the Prenylated Pterocarpan Edunol by <i>Aspergillus flavus</i> S. TAHARA and J. L. INGHAM	1050	Amperometric Titration Largely Overestimates Chloride Concentrations in Chloroplast Extracts G. SCHRÖPPEL-MEIER and W. M. KAISER	1109
Fungal Metabolism of the Prenylated Isoflavone 2,3-Dehydrokievitone S. TAHARA, E. MISUMI, J. MIZUTANI, and J. L. INGHAM	1055	Extrachloroplastic Site of Synthesis of Three Chloroplast Proteins in Maize (<i>Zea mays</i>) L. A. KLECZKOWSKI, C. A. ZEIHNER, and D. D. RANDALL	1113
Novel Flavonoids from the Fern <i>Notholaena sulphurea</i> F. J. ARRIAGA-GINER, M. IINUMA, T. TANAKA, M. MIZUNO, C. SCHEELE, and E. WOLLENWEBER	1063	Organolead Toxicity in Plants: Triethyl Lead (Et_3Pb^+) Acts as a Powerful Transmembrane Cl^-/OH^- Exchanger Dissipating H^+ -Gradients at Nano-Molar Levels A. HAGER, I. MOSER, and W. BERTHOLD	1116
Synthesis of Piperoyl Coenzyme A Thioester U. SEMLER, G. SCHMIDTBERG, and G. G. GROSS	1070	Effect of pH on the Slow Phase Components of Delayed Luminescence in Chloroplasts É. HIDEG and S. DEMETER	1121
Geraniol-10-hydroxylase Activity and Its Relation to Monoterpene Indole Alkaloid Accumulation in Cell Suspension Cultures of <i>Catharanthus roseus</i> O. SCHIEL, L. WITTE, and J. BERLIN	1075	Chemokinesis and Necrotaxis of Human Granulocytes: the Important Cellular Organelles H. GRULER and A. DE BOISFLEURY CHEVANCE	1126
A New Detection Procedure for Aminoacylase Activity of Microorganisms Directly on Plate Culture with <i>o</i> -Phthalaldehyde (In German) Y. YAMAZAKI, W. HUMMEL, and M.-R. KULA	1082	<i>In vitro</i> T_1 and T_2 Relaxation Times of Coagulating Blood and Thrombuses (In German) U. M. LANDLER, K. HERGAN, E. JUSTICH, and H. STERK	1135
Small Angle X-Ray Study on the Structure of Active and Inactive Ribulose-1,5-bisphosphate Carboxylase-Oxygenase from Spinach. Evidence for a Configurational Change I. PILZ, E. SCHWARZ, G. P. PAL, and W. SAENGER	1089	Dependence of a Sleeping Parameter from the N-S or E-W Sleeping Direction G. RUHENSTROTH-BAUER, E. RÜTHER, and TH. REINERTSHOFER	1140
Quantitative Histochemical Analysis of Starch, Malate and K^+ , together with the Activity of Phospho-enolpyruvate Carboxylase along an Elongating Primary Leaf of <i>Hordeum vulgare</i> R. HAMPP, W. H. OUTLAW Jr., and H. ZIEGLER	1092	Notes	
Biotransformation of Citral by <i>Botrytis cinerea</i> P. BRUNERIE, I. BENDA, G. BOCK, and P. SCHREIER	1097	Automatic Turgor Pressure Recording in Plant Cells K.-H. BÜCHNER, G. WEHNER, W. VIRSIK, and U. ZIMMERMANN	1143
		Distinct Substrate Specificity of Dihydroflavonol 4-Reductase from Flowers of <i>Petunia hybrida</i> G. FORKMANN and B. RUHNAU	1146
		Erratum	1149

Contents of Number 11/12

Contents of Nos 1–12 III–XIV

Original Communications

- Identification of Betulin in Archaeological Tar (In German)
F. SAUTER, E. W. H. HAYEK, W. MOCHE, and U. JORDIS 1151
- Chemical Composition and Morphology of Epicuticular Waxes from Leaves of *Solanum tuberosum*
A. SEN 1153
- Asymmetric Reduction of 4(5)-Oxocarboxylic Acids by Baker's Yeast (In German)
M. GESSNER, C. GÜNTHER, and A. MOSANDL 1159
- Partial Lack of N-Acetyl Substitution of Glucosamine in the Peptidoglycan of the Budding Phototrophic *Rhodomicrobium vannielii*
U. J. JÜRGENS, B. RIETH, J. WECKESSER, C. S. DOW, and W. A. KÖNIG 1165
- Accumulation of Phenolic Compounds and Phytoalexins in Sliced and Elicitor-Treated Cotyledons of *Cicer arietinum* L.
U. JAQUES, H. KESSMANN, and W. BARZ 1171
- Natural Inhibitors of Germination and Growth IV Compounds from Fruit and Seeds of Mountain Ash (*Sorbus aucuparia*)
U. OSTER, I. BLOS, and W. RÜDIGER 1179
- The v versus $v[I]$ Plot
A. C. BORSTLAP 1185
- Development of New Plate Tests for the Detection of Microbial Hydrolysis of Esters and Oxidations of 2-Hydroxycarboxylic Acids (In German)
Y. YAMAZAKI and M.-R. KULA 1187
- Enzymatic Synthesis of 4'- and 3',4'-Hydroxylated Flavanones and Flavones with Flower Extracts of *Sinningia cardinalis*
K. STICH and G. FORKMANN 1193
- Purification and Properties of Chalcone Synthase from Cell Suspension Cultures of Soybean
R. WELLE and H. GRISEBACH 1200
- 4-(2'-Carboxyphenyl)-4-oxobutyl Coenzyme A Ester, an Intermediate in Vitamin K₂ (Menaquinone) Biosynthesis
R. KOLKMANN and E. LEISTNER 1207
- Role of Pyrophosphate:Fructose-6-phosphate 1-Phosphotransferase in Glycolysis in Cultured *Catharanthus roseus* Cells
H. ASHIHARA and T. HORIKOSI 1215
- The Separation of Two Different Enzymes Catalyzing the Formation of Hydroxycinnamic Acid Glucosides and Esters
P. A. BÄUMKER, M. JÜTTE, and R. WIERMANN 1223
- Protein Sequence and Structure of N-Terminal Amino Acids of Subunit Delta of Spinach Photosynthetic ATP-Synthase CF₁
R. J. BERZBORN, W. FINKE, J. OTTO, and H. E. MEYER 1231
- Isolation and Characterization of a Supramolecular Complex of Subunit III of the ATP-Synthase from Chloroplasts
P. FROMME, E. J. BOEKEMA, and P. GRÄBER 1239
- The Polyphasic Rise of Chlorophyll Fluorescence upon Onset of Strong Continuous Illumination: I. Saturation Characteristics and Partial Control by the Photosystem II Acceptor Side
CH. NEUBAUER and U. SCHREIBER 1246
- The Polyphasic Rise of Chlorophyll Fluorescence upon Onset of Strong Continuous Illumination: II. Partial Control by the Photosystem II Donor Side and Possible Ways of Interpretation
U. SCHREIBER and CH. NEUBAUER 1255
- Benzofuroxan as Electron Acceptor at Photosystem I
B. LOTINA-HENNSSEN, A. GARCIA, M. AGUILAR, and M. ALBORES 1265
- CARS Investigation of Changes in Chromophore Geometry of C-Phycocyanin from *Mastigocladus laminosus* Induced by Titration with *p*-Chloromercuribenzenesulfonate
S. SCHNEIDER, F. BAUMANN, and U. KLÜTER 1269
- Inhibition by Sethoxydim of Pigment Accumulation and Fatty Acid Biosynthesis in Chloroplasts of *Avena* Seedlings
H. K. LICHTENTHALER, K. KOBEK, and K. ISHII 1275

Bioenergetics Studies of the Cyanobacterium <i>Anabaena variabilis</i> S. SCHERER, H. SADOWSKI, and P. BÖGER	1280	Thermal Lability of Membrane Proteins of Age Separated Erythrocytes as Studied by Electron Spin Resonance Spin Label Technique G. BARTOSZ, G. CHRIST, H. BOSSE, R. STEPHAN, and H. GÄRTNER	1343
Radiochemical Methods for Studying Lipase-Catalyzed Interesterification of Lipids R. SCHUCH and K. D. MUKHERJEE	1285	Lipids in the Gular Gland Secretion of the American Alligator (<i>Alligator mississippiensis</i>) P. J. WELDON, A. SHAFAGATI, and J. W. WHEELER	1345
cAMP-Dependent Protein Kinase Activity in Yeast Mitochondria G. MÜLLER and W. BANDLOW	1291	Identification of the Sex Pheromone of Eggplant Borer <i>Leucinodes orbonalis</i> Guenée (Lepidoptera: Pyralidae) ZHU PINGCHOU, KONG FANLEI, YU SHENGDI, YU YONGQING, JIN SHUPING, HU XINHUA, and YU JIANWEI	1347
Twisted Fibrils are a Structural Principle in the Assembly of Interstitial Collagens, Chordae Tendineae Included W. FOLKHARD, D. CHRISTMANN, W. GEERCKEN, E. KNÖRZER, M. H. J. KOCH, E. MOSLER, H. NEMETSCHKE-GANSLER, and T. NEMETSCHKE	1303	(Z)-5-Dodecen-1-ol, Another Inhibitor of Pheromonal Attraction in <i>Coleophora laricella</i> E. PRIESNER	1349
Lysine Decarboxylase from <i>Hafnia alvei</i> : Purification, Molecular Data and Preparation of Polyclonal Antibodies H. BEIER, L. F. FECKER, and J. BERLIN	1307	(Z)-3-Tetradecenyl Acetate as a Sex-Attractant Component in Gelechiinae and Anomologinae (Lepidoptera: Gelechiidae) E. PRIESNER	1352
Recognition of HLA Class II Molecules by Antipeptide Antibodies Elicited by Synthetic Peptides Selected from Regions of HLA-DP Antigens A. CHERSI, R. A. HOUGHTEN, M. C. MORGANTI, and E. MURATTI	1313	Mammalian Pheromone Studies. VI. Compounds from the Preorbital Gland of the Blue Duiker, <i>Cephalophus monticola</i> B. V. BURGER and P. J. PRETORIUS	1355
Active Cyanogenesis – in Zygaenids and Other Lepidoptera K. WITTHOHN and C. M. NAUMANN	1319	Structural Features and Biological Functions in Blue Copper Proteins Y. NISHIDA	1358
Efficacy of Sustained-Release Radioprotective Drugs <i>in vivo</i> J. SHANI, S. BENITA, M. ABDULRAZIK, and A. YERUSHALMI	1323	Inhibition of the Acetyl-CoA Carboxylase of Barley Chloroplasts by Cycloxydim and Sethoxydim M. FOCKE and H. K. LICHTENTHALER	1361
MHC-Antigens: Constituents of the Envelopes of Human and Simian Immunodeficiency Viruses H. GELDERBLOM, H. REUPKE, T. WINKEL, R. KUNZE, and G. PAULI	1328	Formation of Large Thioredoxin <i>f</i> Accompanies Chloroplast Development in <i>Scenedesmus obliquus</i> P. LANGLOTZ and H. FOLLMANN	1364
Evidences for Circadian Rhythmicity in the <i>per</i> ⁰ Mutant of <i>Drosophila melanogaster</i> CH. HELFRICH and W. ENGELMANN	1335	Cellular Spin Resonance of Yeast in a Frequency Range up to 140 MHz R. HÖLZEL and I. LAMPRECHT	1367
Notes		Melting Pressure, Volume and Stability of Blood at High Pressure (In German) A. KLUGE and H. LENTZ	1370
Conformational Changes in Proteins Induced by Low Temperatures: an Infrared Study H. L. CASAL, U. KÖHLER, H. H. MANTSCH, F. M. GOÑI, and J. L. R. ARRONDO	1339	Subjekt Index	1373
		Authors Index	1403

Protein Transport in Chloroplasts: ATP is Prerequisite

Christine Schindler, Ruth Hracky, and Jürgen Soll

Botanisches Institut der Universität München, Menzinger Straße 67,
D-8000 München 19, Bundesrepublik Deutschland

Z. Naturforsch. **42c**, 103–108 (1987); received August 26, 1986

Protein Transport, ATP-Hydrolysis, Chloroplasts, Envelope, *Pisum sativum*

The energy requirement for protein transport into chloroplast was assayed under conditions that permit to distinguish whether the posttranslational translocation is dependent on ATP or whether a membrane potential across the chloroplast envelope can drive this transport event.

A membrane potential is not required for translocation. ATP can support protein transport in the presence of protonophores and ionophores. Non-hydrolyzable ATP analogues and GTP, CTP, UTP cannot serve as ATP substitutes. Translocation could be observed when an ATP generating system was used to supply ATP. In contrast ATP degrading systems completely abolished translocation.

The inner envelope membrane localized ATP-ase is probably not involved in the transport event. The results suggest that ATP is needed at the outer chloroplast envelope.

Inhibition of protein transport by ADP, pyrophosphate and NaF is studied and its consequences discussed.

Introduction

Mitochondria and chloroplasts contain proteins of dual genetic origin. Some are coded for and made in the organell while others are coded for in the nucleus and made on cytosolic ribosomes as larger precursor proteins. These precursor proteins are then post-translationally taken up into the organell in an energy-dependent step [1].

In mitochondria it was shown that a membrane potential across the inner mitochondrial membrane was able to drive protein translocation. The energy requirement in chloroplasts was much less well documented and subject to debate [2, 3]. The present report is aimed to resolve this problem. We used the precursor form of the small subunit (pSSu) of ribulose-1,5-bisphosphate carboxylase (E.C. 4.1.1.39) to elucidate this question with pea chloroplasts.

While this work was in progress several papers appeared describing the ATP-dependent protein translocation into *E. coli* membrane vesicles [4] and across yeast microsomal membranes [5, 6]. In

accordance with these reports we present the following evidence that posttranslational protein uptake into chloroplasts is completely dependent on the hydrolysis of ATP and cannot be substituted for by a membrane potential.

Materials and Methods

Materials

[³⁵S]methionine (sp. act. 800 Ci/mmol), was from Amersham-Buchler (Braunschweig, FRG). Valinomycin, A 23187, carbonylcyanide *m*-chlorophenylhydrazine (CCCP), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the ATP analogues 5-adenylylimidodiphosphate (AMP-PNP) and β , γ -methyleneadenosine-5-triphosphate (AMP-PCP), pyruvate kinase (E.C. 2.7.1.40) and inorg. pyrophosphatase (E.C. 3.6.1.1) were from Sigma (München, FRG). Hexokinase (E.C. 2.7.1.1) was from Boehringer (Mannheim, FRG). All other chemicals were purchased from commercial sources and of reagent grade.

Plant material and growth conditions

Pea plants (*Pisum sativum*, c.v. Rosa Krone) were grown on vermiculite in the greenhouse for 12–14 days.

Chloroplast isolation

Chloroplasts were isolated in low ionic strength medium, 330 mM sorbitol, 20 mM morpholino-

Abbreviations: SSu, small subunit of ribulose-1,5-bisphosphate carboxylase; pSSu, precursor form of SSu; PEP, phosphoenolpyruvate; OAA, oxalacetate; DHAP, dihydroxyacetonephosphate; AMP-PNP, 5-adenylylimidodiphosphate; AMP-PCP, β , γ -adenylylmethylenediphosphonate; CCCP, carbonylcyanid *m*-chlorophenylhydrazine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Reprint requests to Dr. J. Soll.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0100–0103 \$ 01.30/0

propane sulfonic acid-Tris (pH 7.3), 0.4 mM MgCl_2 [7]. The crude plastid pellet was resuspended and chloroplasts further purified through a step silica sol gradient (40% v:v/80% v:v Percoll, Pharmacia, Freiburg) [8]. Intact, purified chloroplasts were recovered from the gradient and washed free of Percoll by repeated centrifugation. A dense organell suspension (5 mg chlorophyll/ml) was used as stock solution for transport assays. Etioplasts were isolated as in [9]. Chlorophyll was determined by the method in [10].

Isolation of poly(A)RNA

Total RNA was extracted from 4–5 days old pea plants. RNA was purified by centrifugation through a CsCl gradient containing guanidinium rhodanide as described in [11]. Oligo-dT-cellulose was used to select for poly(A)RNA. Poly(A)RNA was precipitated in ethanol and stored in H_2O at -196°C [11].

In vitro translation and uptake experiments

Poly(A)RNA was translated in a cell-free wheat germ system (prepared as in [12]), containing 20 mM Hepes-KOH (pH 7.6), 100 mM KCl, 3.5 mM Mg-acetate, 1 mM ATP, 20 μM GTP, 2 mM DTT, 8 mM creatine phosphate, 25 μM each of the amino acids (minus methionine), 4 μg creatine kinase, 100 μCi [^{35}S]methionine and 4 to 5 μg of poly(A)RNA in final volume of 100 μl . Translation mixtures were incubated at 25°C for 1.5 h and then centrifuged at $140,000\times g$ for 1 h. The supernatant was carefully removed and was used for uptake experiments. To remove ATP, GTP and creatinphosphate the translation products were precipitated by adding solid $(\text{NH}_4)_2\text{SO}_4$ (52 mg/100 μl), precipitation was allowed to complete for 30 min at 4°C . The pellet recovered after centrifugation at $140,000\times g$ for 30 min was dissolved in 50 mM Hepes-NaOH (pH 8) and dialyzed against the same buffer for 3 h.

The uptake mixture (final volume 300 μl) contained 3 mM MgSO_4 , 10 mM methionine, 26 mM Na-gluconate, 2% bovine serum albumine, 330 mM sorbitol, 10 mM NaHCO_3 , 6.6 mM ATP (unless otherwise indicated), 50 mM Hepes-NaOH (pH 8), 30 μl of postribosomal supernatant derived from the translation mixture and intact chloroplasts (equivalent to 100 μg chlorophyll). Experiments were carried out either in the dark or in the light at 25°C for 30 min. Whenever uncouplers, ionophores and inhibitors

were tested, a stock solution in ethanol was made. The chloroplasts were incubated 10 min with these inhibitors before starting the transport reaction by adding the translation mixture. Final ethanol concentration was less than 1% in the uptake experiment.

Following the incubation, the transport was stopped by centrifugation. To remove those plastids that had become lysed during incubation period, intact chloroplasts were reisolated by centrifugation through a 40% v:v Percoll cushion, resuspended in 50 mM Hepes-NaOH (pH 8) and treated with thermolysin (100 $\mu\text{g}/\text{ml}$) [9] in order to show protease resistant protein transport into the organell. After thermolysin treatment for 30 min on ice, the reaction was stopped by the addition of 10 mM EDTA, centrifuged for 4 min at $12,000\times g$ and the pellet was washed with 50 mM Hepes-NaOH (pH 8), 330 mM sorbitol, 5 mM EDTA. The chloroplasts were then lysed on ice by resuspending them in 10 mM Tricine-KOH (pH 7.9), 4 mM MgCl_2 . The broken plastids were separated into a soluble extract fraction and a membrane fraction, containing both thylakoid and envelope membranes, by centrifugation for 5 min at $12,000\times g$. The fractions were then analyzed by SDS-PAGE.

Polyacrylamide gel electrophoresis and autoradiography

All samples were analyzed by SDS-PAGE containing a 10–15% linear acrylamide gradient and 1% bisacrylamide. Proteins were solubilized in 2.5% LDS, 12% sucrose, 70 mM DTT and 60 mM Na_2CO_3 . Electrophoresis was done essentially as described by Delepelaire *et al.* [13]. Stained polyacrylamide gels were fluorographed according to Bonner and Laskey [14] and autoradiographed using an intensifying screen (Agfa-Gevaert MR 600) at -80°C .

Transport experiments were quantified by SDS-PAGE

Aliquots of posttranslational supernatant and from the transport experiments were electrophoresed on the same polyacrylamide gel. The pSSu and SSu were exactly located on the gel by autoradiography and the spots were excised from the gel, rehydrated in H_2O and the radioactivity recovered by treatment of the gel slices with 0.2 M NH_3 in 30% H_2O_2 for 48 h at 37°C . By then the gel pieces were completely dis-

solved and scintillation fluid (Rotizint 22, Zinsser, FRG) was added. The radioactivity was quantitatively determined using a scintillation counter (Kontron, Betamatic). Specific activity of SSu was calculated from the published SSu-sequence in [15] and was not corrected for unlabelled methionine endogenously present in the wheat germ system.

Results

Hydrolysis of ATP is required to drive pSSu transport into pea chloroplasts. Normal transport efficiency was 20–30% uptake of the total pSSu in the transport mixture which is equivalent to about 10⁵ molecules pSSu uptake per µg protein · h. Substitution of ATP by the nonhydrolyzable analogues AMP-PNP and AMP-PCP cannot restore protein transport. Re-addition of ATP to the posttranslational translocation reaction resulted in pSSu transport. In the presence of higher AMP-PNP concentrations (2–6.6 mM) ATP (6.6 mM) could only partially reconstitute pSSu transport. At lower AMP-PNP concentrations (up to 2 mM) pSSu translocation was fully restorable (data not shown). AMP-PCP did not show such an effect and using up to 6.6 mM AMP-PCP did not result in any decrease of protein transport in the presence of ATP (Fig. 1). The binding of pSSu did not change in the presence of AMP-PNP and AMP-PCP, respectively. Binding was determined in transport experiments which were not treated with thermolysin. GTP, CTP and UTP (6.6 mM) could also not restore pSSu translocation into chloroplasts and etioplasts from pea (data not shown). Exogenously added ATP could be replaced by the inclusion of ATP-synthesizing systems like pyruvate kinase in the presence of phosphoenolpyruvate (PEP) and ADP. PEP could not drive protein translocation, which was demonstrated when ADP was

ATP	-	6.6	-	6.6	-	6.6
AMP-PNP	-	-	6.6	6.6	-	-
AMP-PCP	-	-	-	-	6.6	6.6



Fig. 1. Protein translocation in chloroplasts requires ATP hydrolysis. ATP was removed by (NH₄)₂SO₄ precipitation of the posttranslational supernatant. ATP or ATP analogues are supplied at the indicated final concentrations (mM).

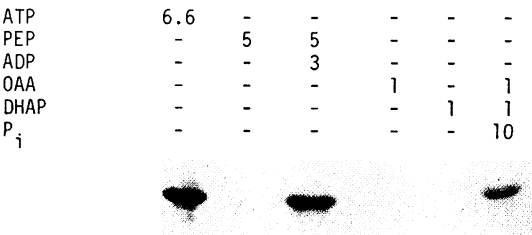


Fig. 2. ATP dependent protein uptake is driven by ATP synthesized inside or outside of the chloroplast. Pyruvate kinase (1.5 units) was added exogenously. All other concentrations (mM).

omitted from the uptake mixture (Fig. 2). The system described above yields ATP outside the chloroplast.

In order to see whether ATP synthesized inside the chloroplast can also support protein uptake, we incubated the protein transport system in the presence of oxalacetate (OAA), dihydroxyacetone phosphate (DHAP) and P_i. These substrates are transported *via* specific carriers into the chloroplast [16] and then can be used inside the chloroplast to yield ATP. The ATP made inside the chloroplast can also stimulate protein translocation (Fig. 2).

The role of an inner envelope bound ATP-ase in the energy requiring step in the protein translocation has been discussed [17]. We used specific inhibitors of this ATP-ase *e.g.* Na₃VO₄, LaCl₃ and SbCl₃. At concentrations known to inhibit the ATP-ase protein transport was also influenced. But Na₃VO₄ the most potent ATP-ase inhibitor [17, 18] had the least effect (Table I). On the other hand the envelope bound ATP-ase can also hydrolyze GTP and CTP at rates similar to ATP [18] which are ineffective in supporting protein uptake (see above).

Chloroplasts are surrounded by the outer envelope membrane in contact with the cytosol and responsi-

Table I. Influence of chloroplast envelope bound ATP-ase inhibitors on protein uptake into chloroplasts.

Inhibitor	mm	Protein uptake	Inhibition [%]	ATP-ase*
Na ₃ VO ₄	0.1	38		98
SbCl ₃	1	51		50
LaCl ₃	1	20		50

* Data taken from [17].

ble for pSSu recognition and transport initiation [19] and the inner envelope membrane forming the border for the soluble chloroplast proteins. The results above demonstrated that the hydrolysis of ATP supports protein translocation across the envelope membranes. It does however not allow to conclude at which membrane the ATP is used. We tried the following approach to elucidate this question.

ATP was made inside the chloroplast *via* the OAA-DHAP system, simultaneously glucose and increasing concentrations of hexokinase were added. Hexokinase should degrade the ATP which has left the chloroplast to the outside of the outer envelope and therefore hexokinase should influence the ATP dependent transport, if ATP is needed at the outer leaflet of the outer membrane. In fact, increasing concentrations of hexokinase finally completely abolished pSSu translocation (Fig. 3), thus indicating that ATP is needed at the outer envelope membrane. If the postribosomal supernatant was incubated with 6 mM ATP, glucose and hexokinase 5 min prior to the transport experiment, almost no SSu appeared in the stroma, but when glucose-6-P was included into the assay system to inhibit the hexokinase protein transport was completely restored (not shown). It is evident that ATP synthesized inside the chloroplast can support protein uptake. The chloroplast is able to supply ATP via a multitude of pathways, i) in the light *via* photophosphorylation, ii) in the dark *via* starch degradation and glycolysis. ATP made from photosynthetic electron transport can also drive protein translocation [2] (Table II). Using specific energytransfer inhibitors it was possible to demonstrate that ATP derived either from cyclic or non-cyclic electron transport is able to deliver the energy required in pSSu uptake. DCMU and antimycin A were used as inhibitors of non-cyclic and cyclic electron transport, respectively (Fig. 4, Table II).

In order to exclude a membrane potential as driving force in pSSu translocation, different classes of

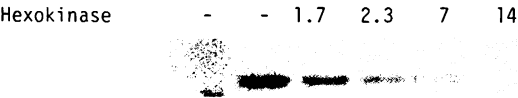


Fig. 3. ATP synthesized inside the chloroplast *via* the OAA-DHAP system is degraded by exogenously added hexokinase and thus cannot drive ATP dependent protein uptake anymore. Final concentration are OAA (1 mM), DHAP (1 mM), P_i (10 mM) and hexokinase (units) as indicated above, except in the left lane, no additions.

Table II. pSSu Translocation in chloroplasts in the presence of CCCP, antimycin A and nigericin in the light or in the dark. Final ATP concentrations were 6.6 mM, where used. CCCP, antimycin A and nigericin concentrations are indicated in the table [μM].

Treatment	Transport [molecules/μg protein · h]
dark + ATP	2.4 × 10 ⁴
dark + ATP + CCCP 30	2.8 × 10 ⁴
dark + ATP + antimycin A 8	2.0 × 10 ⁴
light	1.0 × 10 ⁴
light + CCCP 2	7.4 × 10 ³
light + CCCP 6	5.2 × 10 ³
light + CCCP 10	3.4 × 10 ³
light + CCCP 30	3.4 × 10 ³
light + CCCP 30 + ATP	1.8 × 10 ⁴
light + antimycin A 2	1.2 × 10 ⁴
light + antimycin A 8	1.1 × 10 ⁴
light + antimycin A 8 + ATP	2.4 × 10 ⁴
dark + ATP	7 × 10 ⁴
dark + ATP + nigericin 0.4	6.8 × 10 ⁴
light	1.7 × 10 ⁴
light + ATP + nigericin 0.4	8.8 × 10 ³

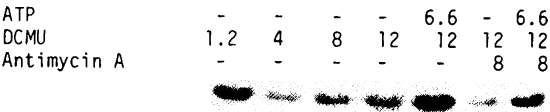


Fig. 4. Effect of cyclic and non-cyclic electron transport inhibitors on pSSu translocation. Transport was assayed in the light in the presence of DCMU and antimycin A (concentrations μM), (ATP, mM).

uncouplers were used. A potassium gradient (KCl 0–100 mM) could neither support by itself nor stimulate in the presence of ATP protein uptake into chloroplasts (Fig. 5). Valinomycin (a K specific ionophore) had no effect on pSSu uptake in the presence of varying ATP concentrations (0.6–6.6 mM)

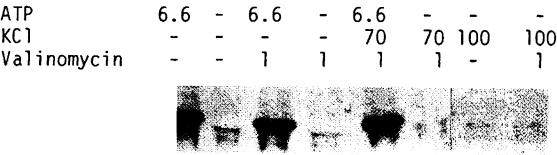


Fig. 5. Effect of the K-ionophore valinomycin on protein import in chloroplasts. Valinomycin (μM), ATP and KCl (mM) concentrations are indicated in the top panel.

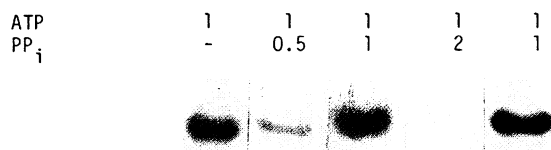


Fig. 6. Protein uptake in pea etioplasts was studied in the presence of sodium pyrophosphate and its reversibility assayed by the addition of pyrophosphatase (10 units, right lane) (concentrations in mM).

(Fig. 5). Nigericin [3] was also ineffective in protein transport inhibition in the presence of ATP (Table II). The ionophore A 23187 specific for Ca^{2+} and divalent cations had no effect on pSSu uptake in the presence of ATP (A 23187 1–10 μM , Mg 10 mM).

Protonophores like CCCP do inhibit protein uptake in the light, by uncoupling photosynthetic electron transport, but CCCP does not influence pSSu translocation in the presence of exogenous ATP (Table II).

Finally the influence of several other substrates on protein translocation into plastids was determined which were thought to alter ATP hydrolysis. Sodiumpyrophosphate inhibited pSSu transport into etioplasts and chloroplasts (Fig. 6). Simultaneous inclusion of pyrophosphatase completely reversed this effect. At lower ATP concentrations ADP is also able to inhibit transport into plastids (data not shown).

NaF, a phosphatase inhibitor leads to a strong decrease in transport while binding is increased (Fig. 7). Similar data have been obtained with etioplasts [9].

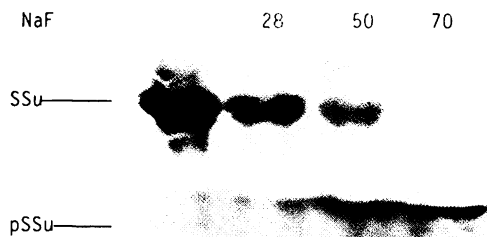


Fig. 7. pSSu translocation across chloroplast envelope membranes is influenced by NaF. After completion of the uptake experiment, chloroplasts were not treated as usually by thermolysin but were separated into a membrane and a soluble fraction which contains pSSu and SSu, respectively (see also Materials and Methods).

Discussion

Posttranslational protein transport into mitochondria, chloroplasts and other organelles and membranes is an energy dependent process [1, 2]. In mitochondria it was clearly demonstrated that a membrane potential across the inner mitochondrial membrane can drive the translocation [1]. The data presented above however show that in chloroplasts and etioplasts [9] the hydrolysis of ATP is necessary to stimulate this process.

Uncouplers such as valinomycin, CCCP or A 23187 had no effect. But when ATP was depleted *via* an enzymatic reaction, transport was completely abolished. Moreover the energy-rich nucleosidetriphosphates GTP, CTP and UTP were unable to support pSSu transport. Hydrolysis of ATP is prerequisite for translocation. The nonhydrolyzable ATP analogues AMP-PNP and AMP-PCP did not stimulate protein transport, readdition of ATP however resulted in a restoration of translocation.

The ATP required in the pSSu translocation event can be generated inside and outside of the chloroplast. The manipulation we used to generate ATP inside the chloroplasts *via* the OAA-DHAP system or by light was as successful in driving protein translocation as the PEP-pyruvatekinase system outside the chloroplast. ATP can derive either from cyclic or non-cyclic electron transport as was demonstrated using DCMU and antimycin A as specific inhibitors. This demonstrates that plastids have a very versatile system to provide ATP for protein uptake and are not fixed to one ATP source. This is especially important in plastid development, when for example in etioplasts and proplastids ATP cannot derive from photosynthetic electron flow. While the outer envelope membrane is probably permeable to ATP the inner envelope membrane has a specific carrier system, which is more active in pea than in spinach chloroplasts. This would allow newly synthesized ATP to move to the place of utilization. We have obtained some evidence that the ATP is needed at the outer envelope or at least at the outside of the inner envelope membrane by using hexokinase as an external ATP depleting system. Hexokinase which cannot penetrate the envelope membranes, abolishes protein transport when the internal chloroplast ATP synthesizing system *via* OAA-DHAP is used. The inner envelope membrane-bound ATP-ase is probably not involved in the hydrolysis of ATP necessary

for pSSu translocation as was demonstrated using specific inhibitors. And also by the fact that this ATP-ase can hydrolyze GTP and CTP to a similar extent as ATP while GTP and CTP are not able to support pSSu uptake. The question now arises which is the ATP requiring step and what is the mechanistic background. The data presented in this paper and in [9] agree with the idea that ATP is needed to phosphorylate a protein which is required for translocation [6, 20].

An outer envelope membrane-bound protein kinase has been described [20, 21] which shows similar inhibition responses to ADP and PP_i as does the

pSSu transport. In addition NaF, a known phosphatase inhibitor effectively blocks protein import, while binding is concomitantly increased. Though this data are not direct evidence, they second a very interesting model which should be elucidated in more detail.

Acknowledgements

We like to thank Dr. U. J. Flügge, University of Göttingen, for his continuous support and critical discussion. This work was in part supported by the Deutsche Forschungsgemeinschaft, Bonn.

- [1] R. Hay, P. Böhni, and S. Gasser, *Biochim. Biophys. Acta* **779**, 65–87 (1984).
- [2] A. Grossman, S. Bartlett, and N. H. Chua, *Nature* **285**, 625–628 (1980).
- [3] K. Cline, M. Werner-Washburne, T. H. Lubben, and K. Keegstra, *J. Biol. Chem.* **260**, 3691–3696 (1985).
- [4] L. Chen and P. C. Tai, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4384–4388 (1985).
- [5] J. A. Rothblatt and D. J. Meyer, *Embo J.* **5**, 1031–1036 (1986).
- [6] M. G. Waters and G. Blobel, *J. Cell Biology* **102**, 1534–1540 (1986).
- [7] H. Y. Nakatani and J. Barber, *Biochim. Biophys. Acta* **461**, 510–512 (1977).
- [8] G. Mourioux and R. Douce, *Plant Physiol.* **67**, 470–473 (1981).
- [9] C. Schindler and J. Soll, *Arch. Biochem. Biophys.* **247**, 211–220 (1986).
- [10] D. J. Arnon, *Plant Physiol.* **24**, 1–15 (1949).
- [11] T. Maniatis, E. F. Fritsch, and J. Sambrook, *Molecular cloning*, pp. 196–197, Cold Spring Harbor, N.Y. 1982.
- [12] B. E. Roberts and B. C. Paterson, *Proc. Natl. Acad. Sci.* **70**, 2330–2334 (1973).
- [13] P. Delepelaire and N. H. Chua, *Proc. Natl. Acad. Sci.* **76**, 111–115 (1979).
- [14] W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83–88 (1974).
- [15] G. Coruzzi, R. Broglie, A. Cashmore, and N. H. Chua, *J. Biol. Chem.* **258**, 1399–1402 (1983).
- [16] M. D. Hatch, L. Dröschner, U. J. Flügge, and H. W. Heldt, *FEBS Letters* **178**, 15–19 (1984).
- [17] T. D. Nguyen and P. A. Siegenthaler, *FEBS Letters* **164**, 211–220 (1986).
- [18] D. R. McCarthy and B. R. Selman, *Plant Physiol.* **80**, 908–912 (1986).
- [19] A. Bitsch and K. Klopstech, *Eur. J. Cell Biol.* **40**, 160–166 (1986).
- [20] J. Soll and B. B. Buchanan, *J. Biol. Chem.* **258**, 6686–6689 (1983).
- [21] J. Soll, *Planta* **166**, 394–400 (1985).