PHOTORECEPTORS AND PLANT DEVELOPMENT

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PHYTOCHROME AND PHYCOBILIPROTEINS WITH CHEMICALLY MODIFIED CHROMOPHORES

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Phytochrome (P), the photomorphogenetic reaction center pigment, contains a linear tetrapyrrolic chromophore covalently bound to the protein (Grombein et al., 1975 ; Klein et al., 1977). The photochemistry and the subsequent dark reactions of P are governed, however, by **non-covalent protein-chromophore** interactions. We have studied these interactions by chemical modification of the chromophore, using phycocyanine (PC) and allophycocyanine (APC), phycobiliproteins with chromophores structurally nearly identical to P_r, as models. Since there exists no satisfactory method for a reversible cleavage of the chromophores in biliproteins, a selective chromophore modification has been carried out with the chromophores still bound to the protein. In fully denatured biliproteins, e.g. in the presence of 8 M urea, the non-covalent interactions are abolished, and the chromophores then react with suitable reagents similar to free bilins. Sodium dithionite, a reducing agent applied for in the dark reversion of P_{fr} to P_r (Mumford & Jenner, 1971), is such a reagent, known to reduce bilindiones at the central methine bridge to produce bilirubin type pigments (structure C) (Fischer & Plieninger, 1942). The modified pigments can then be renatured, to yield modified native pigments (Kufer & Scheer, 1979a,b). For comparison, the native pigments have been subjected to the same reagent,too.

The reactions carried out with PC are summarized in Fig. 1. One essential result was, that denatured PC could be fully reduced by dithionite, but native PC only partially. When denatured reduced PC was renatured, reoxidation occured even in the presence of dithionite, and the products obtained this way resembled spectroscopically those produced by treatment of native PC with dithionite in the proper concentration. Thus, the protein changes the **thermodynamic properties** of the chromophores, e.g. the stability to dithionite differs in the native and denatured state. This effect is possibly related to the conformational change of the chromophore from a type B to a type A geometry during the folding of the protein to its native state (Scheer & Kufer, 1977).

Likewise, Fig. 2 can be drawn for phytochrome, but renaturation was not possible in this case. A similar change in stability for the P_r chromophore is suggested, however, by analogy from the similar spectroscopic data, and the dithionite

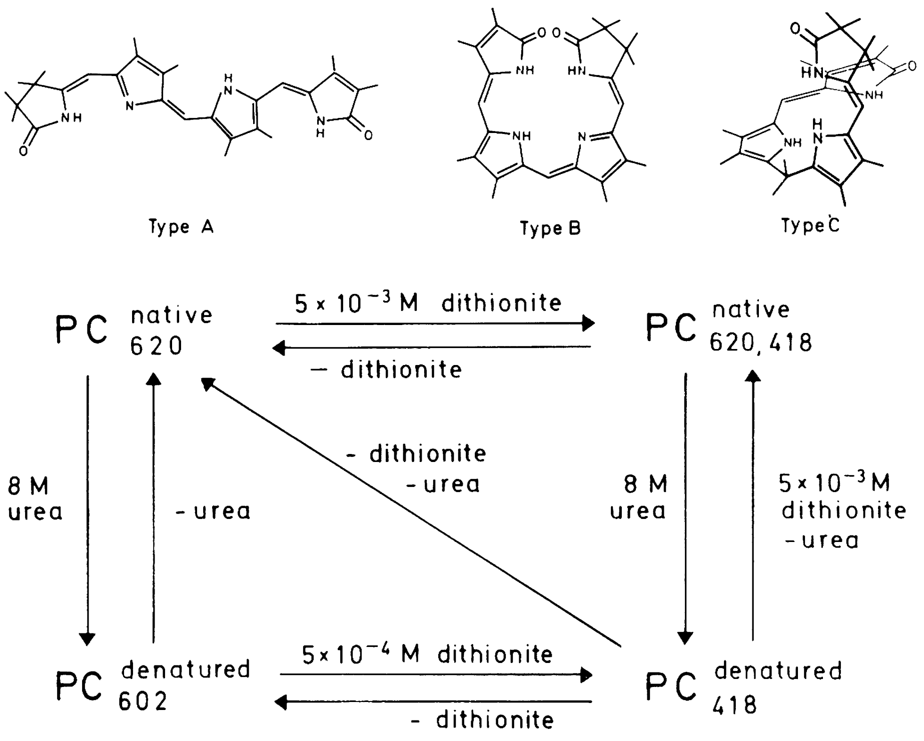

Fig. 1 : Reaction scheme for the denaturation — renaturation and dithionite — reduction — reoxidation studies carried out with C-phycocyanin (PC) from *Spirulina platensis.*

The schematic formulas represent the probable geometries of the tetrapyrrole skeleton in its three different states.

A extended conformation, typical for native PC and P_r (Scheer & Kufer, 1977).

B cyclic-helical conformation, typical for denatured PC and P_r (Scheer & Kufer, 1977) and for free bilindiones (Falk et al., 1978 ; Lehner et al., 1978 ; Sheldrick, 1976).

C ridge-tile conformation, typical for 10,22-dihydrobilindiones ("bilirubins") (Bonnett et al., 1978).

Phycocyanorubin ($PC^{native}_{620,418}$) obtained by saturating reduction of native PC or renaturation of denatured, reduced PC contains both type A and type C chromophores. Similar schemes have been obtained, too, for allophycocyanin isolated from the same organism and phycoerythrin from *Phormidium persicinum.*

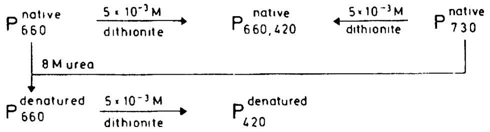

Fig. 2 : **Reaction scheme for the denaturation and dithionite reduction studies carried out with oat phytochrome.**

For P_r, the geometries of the tetrapyrrole skeleton are similar to the ones of PC in the respective states (Fig. 1), the P_{fr} chromophore geometry is unknown.

concentration dependence of the formation of the reduced product. Treatment of native P_{fr} with dithionite results in dark reversion to P_r, as observed earlier (Mumford & Jenner, 1971). In addition, the positive peak at 418 nm, in the difference spectrum (Fig. 3) indicates, that P_r thus obtained is in equilibrium with a species containing reduced chromophores, and is possibly produced via this species. Since the dark reversion of P_{fr} has been suggested to be catalyzed by reducing agents, kinetic studies should give more information on the mechanism of this reaction.

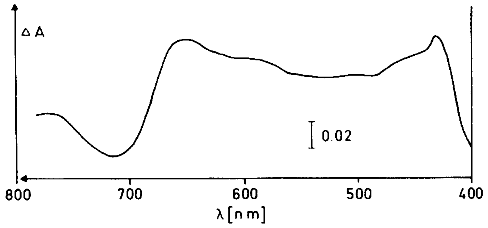

Fig. 3 : **Absorption difference spectrum of oat phytochrome in its P_{fr}-form, treated with sodium dithionite (5×10^{-3} M) vs. the original solution.**

The positive bands correspond to formation of P_r (λ_{max} =660 nm, "reversion") accompanied by the formation of bilirubin-type chromophores (λ_{max} =420 nm).

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