Volume 233, number 1, 22–24 FEB 05904 June 1988

Quantification of p38/synaptophysin in highly purified adrenal medullary chromaffin vesicles

K. Schilling and M. Gratzl

Abteilung Anatomie und Zellbiologie, Universität Ulm, Postfach 4066, D-7900 Ulm, FRG

Received 28 March 1988

Chromaffin vesicles were first purified by differential and density gradient centrifugation in isotonic (Percoll) gradients. In subsequent sucrose gradients p38/synaptophysin exhibited the same distribution as established marker substances of chromaffin vesicles. Quantification of immunoblots revealed that 750 ng p38/synaptophysin per mg of protein were present in the chromaffin vesicles recovered from the sucrose gradient. Thus the amount of p38/synaptophysin per mg protein of chromaffin vesicles is about 100 times lower than that observed in clear (synaptic) vesicles. However, because of the large difference in surface area and protein content, the amount of p38/synaptophysin per single vesicle is the same in both types of organelles.

p38/synaptophysin; Secretory vesicle; Immunoblotting; Quantification; (Adrenal medulla)

1. INTRODUCTION

p38/synaptophysin is a membrane protein present in clear (synaptic) vesicles of neurons and endocrine cells [1–4]. From the amino acid sequence deduced from cDNAs encoding p38/synaptophysin, a model with several membrane spanning polypeptide segments and a carboxy-terminal protein domain exposed to the cytoplasmic surface has been constructed [5–7].

The function of p38/synaptophysin is not known. It has been suggested to form a transmembrane channel for ions, or to interact with cytoplasmic factors via its cytoplasmic domain [7]. Since synaptophysin binds Ca^{2+}, it may also play a role in the release of neurotransmitters stored in clear (synaptic) vesicles [3].

Recently it has been reported [8] that p38/synaptophysin also occurs in hormone containing large dense core vesicles. This would imply that p38/synaptophysin could fulfill similar functions as described above in chromaffin and other secretory cells containing large dense core vesicles. In clear (synaptic) vesicles p38/synaptophysin constitutes 7.5% of the vesicle membrane proteins [1]. The amount of p38/synaptophysin in large dense core vesicles is not known. Here we report on the quantification of p38/synaptophysin in highly purified chromaffin secretory vesicles.

Correspondence address: M. Gratzl, Abteilung Anatomie und Zellbiologie, Universität Ulm, Postfach 4066, D-7900 Ulm, FRG

2. EXPERIMENTAL

The purification of chromaffin vesicles from bovine adrenal medulla was carried out as described previously: the chromaffin vesicles were recovered from the homogenate by differential centrifugation [9] and were further purified on isotonic (Percoll) gradients followed by sucrose gradients [9,10].

Vesicular proteins were separated together with standards on 10% polyacrylamide slab gels in the presence of SDS under reducing conditions [11], transferred to nitrocellulose [12], and analyzed with an antibody to p38 (1 : 15000; kindly provided by Dr R. Jahn, Munich). The immunoreaction was visualized with ^{125}I-labelled protein A (1 μCi/ml) followed by autoradiography. The labelled p38 bands were cut from the nitrocellulose and counted in a gamma-counter. Standards were prepared from adult rat forebrain, which is known to contain 3.2 μg of p38 per mg of protein [1]. The assay was linear in the range of between 1 and 17 ng p38 (see also fig.2). Protein was measured using bovine serum albumin as a standard [13].

Published by Elsevier Science Publishers B.V. (Biomedical Division)

3. RESULTS AND DISCUSSION

Differential centrifugation and subsequent application of isotonic (Percoll) and hypertonic (sucrose) gradients yield highly purified chromaffin vesicles [10]. While mitochondria and microsomes can easily be removed using Percoll gradients [9], further purification of chromaffin vesicles and separation from the remaining lysosomal contamination require an additional sucrose density gradient. When the fractions of the sucrose gradient were assayed for p38 (fig.1), its distribution followed that of the established markers for chromaffin vesicles, e.g., Mg^{2+}-ATPase and cytochrome *b*-561 analysed in the same gradient [10]. In the peak fractions of the sucrose gradient (no.16 and 17), the content of p38 (see fig.2) was 750 ± 70 ng per mg of protein (mean ± SD; $n = 4$). 1 mg of total vesicle protein is equivalent to 5.8×10^{11} chromaffin vesicles (Nordmann, J.J., personal communication). This figure was obtained by determination of the ratio between chromaffin vesicles and a known number of latex particles. Since 20% of the total protein are present in the chromaffin vesicles' membrane (cf. [14]), and p38 is a membrane protein, the concentration of p38 would be 3.75 μg per mg of membrane protein. From these figures and the molecular mass of p38, it can be calculated that each chromaffin vesicle contains 20 molecules of p38.

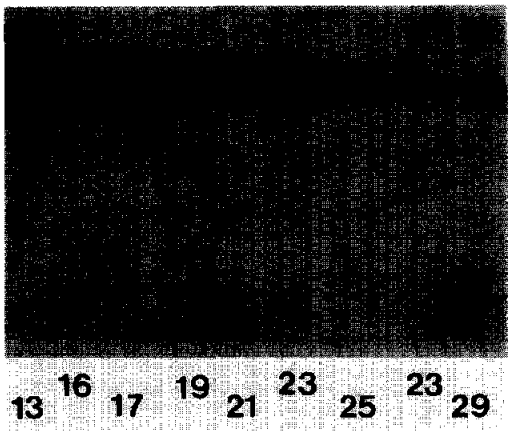

Fig.1. Immunoblot of p38/synaptophysin in fractions of chromaffin vesicles recovered from the sucrose gradient. Equal amounts of each fraction were applied to the gel and assayed for p38 as described in the text.

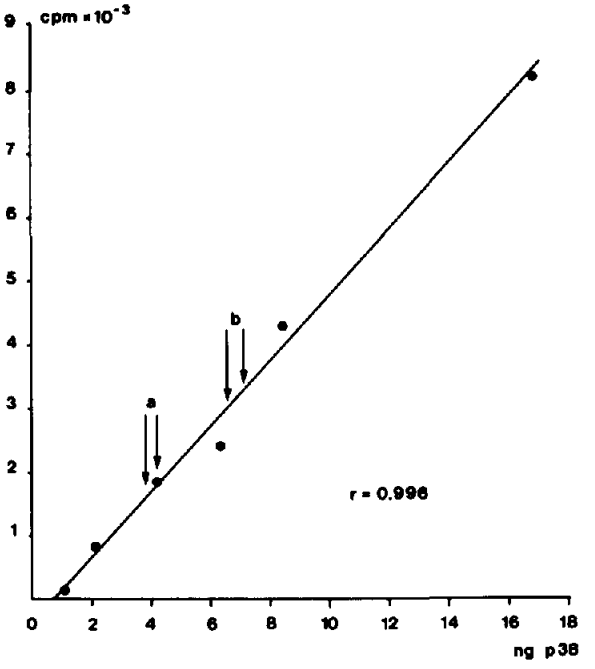

Fig.2. Quantification of p38/synaptophysin. 10 (a) or 20 (b) μl of appropriately diluted fractions 16 and 17 of the sucrose gradient were separated by SDS-PAGE together with standards (from rat forebrain) and assayed for p38/synaptophysin as detailed in the text.

Two characteristic properties of clear (synaptic) and chromaffin vesicles are relevant for the following considerations. Firstly, all protein constituents of clear (synaptic) vesicles appear to be confined to their membrane (cf. [15]). In other words, the reported amount of p38/synaptophysin in these vesicles (75 μg) is the same as the amount per mg of membrane protein. Secondly, clear (synaptic) vesicles have a diameter of 50 nm [4] whereas chromaffin vesicles have a much larger diameter of 275 nm (cf. [14]). Therefore the surface areas of these two types of vesicles differ by a factor of 30. Although chromaffin vesicles contain only 3.75 μg p38/synaptophysin per mg membrane protein which is to be compared to 75 μg in clear (synaptic) vesicles, these calculations lead to the conclusion that the number of protein molecules per vesicle is roughly the same in chromaffin and in clear (synaptic) vesicles and amounts to 20.

In a recent careful immunocytochemical study [4], p38/synaptophysin was detected in clear (synaptic) vesicles but not in chromaffin vesicles

nor in other dense core vesicles. This observation is, however, not at variance with the data presented here since the lower density of p38 per mg of membrane protein (about 20 times) in chromaffin vesicles would reduce the number of gold particles per cross-section of vesicle to background levels. The present results are suited to explain the recent finding that chromaffin vesicles can be bound to p38 antibody-coated beads [8]. Clearly, 20 molecules of p38 per vesicle would be sufficient to bind chromaffin vesicles as well as clear (synaptic) vesicles to such beads.

The fact that the same amount of p38/synaptophysin is present in the two types of intracellular vesicles, irrespective of the large differences in their surface area ($\times$ 30) and volume ($\times$ 166), suggests that the function of the membrane protein is not related to storage or metabolism of transmitters within the secretory vesicles. Such roles are generally catalyzed by several hundreds of biocatalysts (in chromaffin vesicles 350 copies of dopamine β-hydroxylase and 1750 copies of cytochrome *b*-561, for references see [14]). The constant but comparatively low amount of p38/synaptophysin in both types of vesicles rather points to other possible functions of this protein in neural and endocrine cells. For instance, p38/synaptophysin could be necessary for processes such as the 'hooking up' of the vesicles to the microtubule-guided intracellular vesicle transport system or recognition by and fusion with the inner surface of the plasma membrane.

Acknowledgements: We thank Miss S. Keck and Mr D. Rhinehart for expert technical assistance and Mrs B. Mader for typing this manuscript. This work was supported by Deutsche Forschungsgemeinschaft (Grant Gr 681) and by Forschungsschwerpunkt no.24 of the State of Baden-Württemberg.

REFERENCES

[1] Jahn, R., Schiebler, W., Ouimet, C. and Greengard, P. (1985) Proc. Natl. Acad. Sci. USA 82, 4137–4141.
[2] Wiedenmann, B. and Franke, W.W. (1985) Cell 41, 1017–1028.
[3] Rehm, H., Wiedenmann, B. and Betz, H. (1986) EMBO J. 5, 535–541.
[4] Navone, F., Jahn, R., Di Gioia, G., Stukenbrok, H., Greengard, P. and De Camilli, P. (1986) J. Cell Biol. 103, 2511–2527.
[5] Leube, R.E., Kaiser, P., Seiter, A., Zimbelmann, R., Franke, W.W., Rehm, H., Knaus, P., Prior, P., Betz, H., Reinke, H., Beyreuther, K. and Wiedenmann, B. (1987) EMBO J. 6, 3261–3268.
[6] Buckley, K.M., Floor, E. and Kelly, R.B. (1987) J. Cell Biol. 105, 2447–2456.
[7] Südhof, T.C., Lottspeich, F., Greengard, P., Mehl, E. and Jahn, R. (1987) Science 238, 1142–1144.
[8] Lowe, A.W., Madeddu, L. and Kelly, R.B. (1988) J. Cell Biol. 106, 51–59.
[9] Gratzl, M., Krieger-Brauer, H. and Ekerdt, R. (1981) Biochim. Biophys. Acta 649, 355–366.
[10] Gratzl, M. (1984) Anal. Biochem. 142, 148–154.
[11] Weber, K. and Osborn, M. (1975) in: The Proteins (Neurath, H. ed.) 3rd edn, vol.1, pp.179–223, Academic Press, New York, San Francisco, London.
[12] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[13] Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985) Anal. Biochem. 150, 76–85.
[14] Winkler, H., Apps, D.K. and Fischer-Colbrie, R. (1986) Neuroscience 18, 261–290.
[15] Zimmermann, H. (1982) in: Neurotransmitter Vesicles (Klein, R.L. et al. eds) pp.271–304, Academic Press, London.