AFFINITY CHROMATOGRAPHY ON CON A-SEPHAROSE OF SYNAPTIC VESICLE MEMBRANE GLYCOPROTEINS

J. P. ZANETTA* and G. GOMBOS**

Centre de Neurochimie du CNRS 11, rue Humann 67085 Strasbourg Cedex, France

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1. Introduction

Dekirmenjian and Brunngraber [1] have reported that adult rat brain synaptic vesicles do not contain protein-bound sialic acid. However our previous studies on highly purified synaptic vesicles (SV) [2] have shown that they contain high levels of protein-bound carbohydrate [3–5] which cannot be explained by the synaptosomal plasma membrane (SPM) contamination of less than 10% determined by chemical and enzymatic markers [2]. In addition, the molar compositions of protein-bound sugars of SV and SPM are too different [4] for the SV sugars to be explained by SPM contamination.

Moreover, the electrophoretic profiles of SV are significantly simpler than those of the SPM [6,7] which is not the case with SV contaminated with SMP [8].

As a first step towards detailed studies of the SV glycoproteins, we have attempted to separate them by affinity chromatography on Sepharose-bound Concanavalin A (Con A-Sepharose) in the presence of sodium dodecyl sulphate (SDS).

2. Material and methods

Highly purified synaptic vesicles were prepared by the method of Morgan et al. [2]. Solubilization and affinity chromatography were as previously described [9]. SV were delipidized with chloroform–methanol, solubilized in a minimum volume of 4% SDS (pH 8.0, 50 mM 2-mercaptoethanol), and alkylated with iodoacetamine. Prolonged dialysis against 49 vol of water brought the SDS concentration of 0.08%.

Con A-Sepharose (Pharmacia, Sweden) was thoroughly washed, then equilibrated with 0.08% SDS in 20 mM Tris–HCl (pH 6.7). Samples of solubilized SV proteins (20 mg protein in 15 ml of 0.08 SDS in 20 mM Tris–HCl [pH 6.7]) were applied to the columns (1 × 30 cm), and after thorough washing with 0.08 SDS in 20 mM Tris–HCl (pH 6.7), bound glycoproteins were eluted with the same solution containing 0.25 M α-methyl–glucoside (α-Mg). Flow rate was 5 ml per hr. Samples of each fraction were electrophoresed on polyacrylamide (12%) gels in the presence of 0.1% SDS, according to the method of Waehneldt and Mandel [10]. Gels were stained for protein (Coomassie Brilliant Blue: CBB) and sugar (periodic acid–Schiff reaction: PAS) [9]. Protein was determined by the method of Lowry et al. [11].

3. Results and discussion

Affinity chromatography on Con A-Sepharose of SV proteins solubilized in 0.08% SDS (over 98% of the total protein) separated them into three fractions (fig. 1). The largest fraction (88%) was composed of the proteins not adsorbed to Con A (fraction CO) whereas the other two fractions: CR (protein retarded and eluted by the solution) and C1 (protein adsorbed and eluted by α-MG) both contained about 6% of the total protein. Upon

* Attaché de Recherche au CNRS.
** Chargé de Recherche au CNRS.

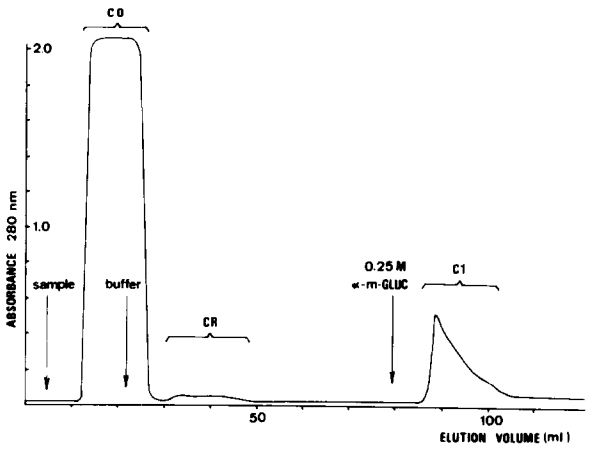

Fig. 1. Affinity chromatography on Con A-Sepharose in the presence of 0.08% SDS, 20 mM Tris–HCl (pH 6.7) of proteins from adult rat brain synaptic vesicles solubilized in 0.08% SDS.

rechromatography, on identical Con A columns, each fraction was eluted as a single peak in the same position as in the first run. This is particularly important in the case of fraction CR, which appears to interact weakly but specifically, with Con A, since, in the presence of α-MG, this fraction was eluted in the same position as a C0 fraction.

The protein and glycoprotein patterns of the 0.08% SDS soluble fraction (fig. 2) were similar to those of

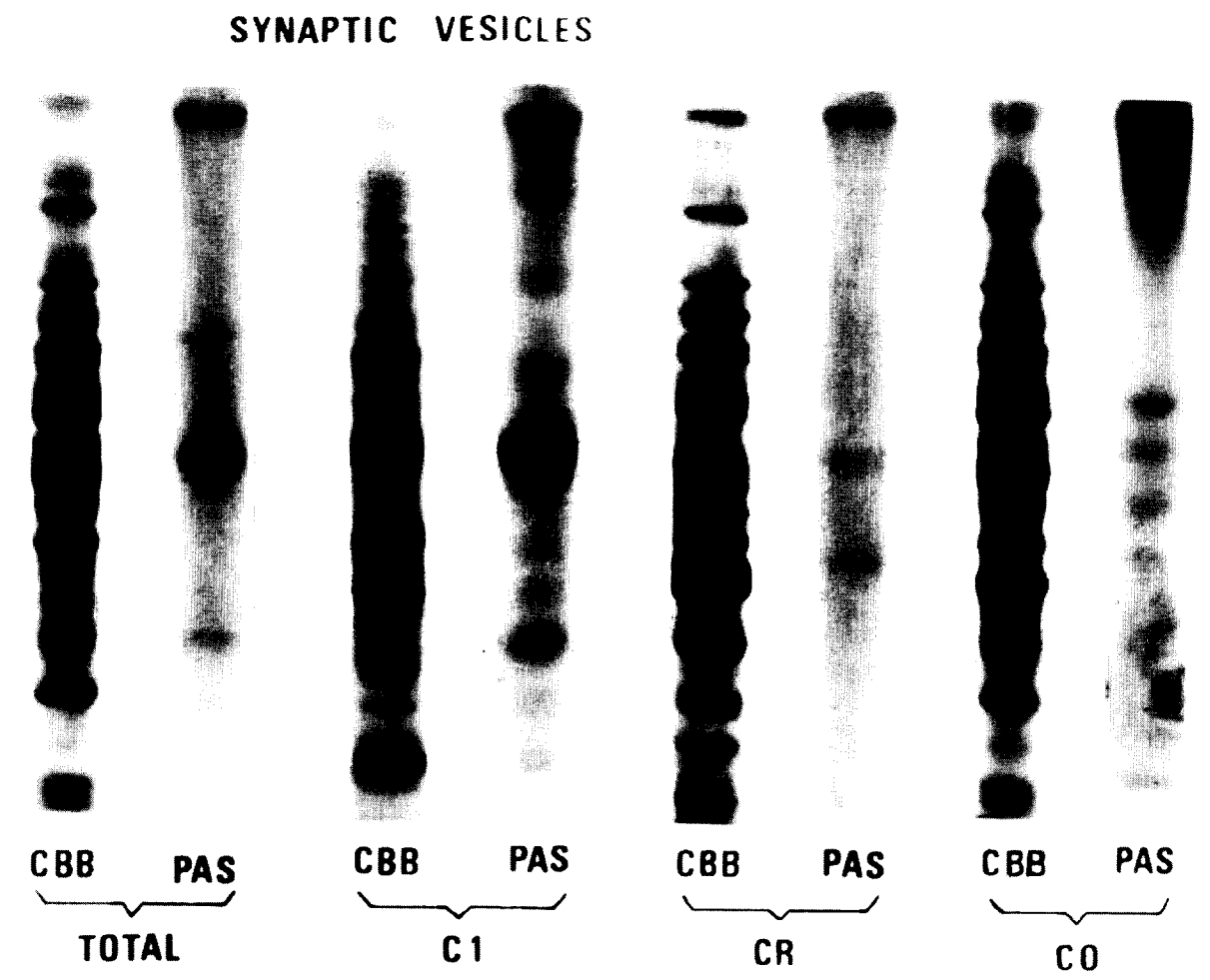

Fig. 2. Polyacrylamide (12%) gel electrophoresis in the presence of 0.1% SDS of fractions isolated as shown in fig. 1. Protein (CBB) and carbohydrate (PAS) stains.

whole SV [4,10]. In addition a low molecular weight glycoprotein, very faintly PAS-positive in our previous experiments, was more coloured by the new PAS procedure.

The three fractions separated by affinity chromatography had protein profiles which differed from each other and from that of the total SV. The glycoprotein profiles of the fractions were much simpler. A few faint bands, and a smear at the start of the gel, were detected in the C0 fraction. Three faintly PAS-positive bands were observed in fraction CR, together with several extremely weak bands which we were unable to reproduce photographically. The major SV glycoproteins were concentrated in fraction C1, although a number of minor bands present in the total SV, but not reproduced photographically, were also seen. Since identical amounts of protein were electrophoresed, fraction C1 must be highly enriched in glycoprotein. On the other hand, C0 might contain large quantities of glycoprotein, diluted in the mass of protein present in this fraction.

The large number of minor glycoprotein bands detected in these enriched fractions might suggest that the SV glycoprotein population is more heterogeneous that was originally believed, but low levels of contamination with other subcellular fractions could account for many of these bands.

Using the technique of affinity chromatography on Sepharose-Con A, we have obtained a fraction (C1) extremely rich in the major SV glycoproteins, free of most of the unglycosylated SV proteins. Separation of individual glycoproteins by other techniques such as preparative gel electrophoresis can now be envisaged. Moreover, these studies will be extended to the glycoproteins of the synaptosomal plasma membrane in order to investigate our suggestion that the SV and the synaptosomal plasma membrane contain some common glycoproteins [4,6,12].

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References

[1] Dekirmenjian, H. and Brunngraber, E. G. (1969) Biochim. Biophys. Acta 177, 1–10.
[2] Morgan, I. G., Gombos, G. and Vincendon, G. (1973) Biochim. Biophys. Acta 320, 671–680.
[3] Breckenridge, W. C., Breckenridge, J. E. and Morgan, I. G. (1972) in: Functional and Structural Proteins of the Nervous System(Davison, A. N., Mandel, P. and Morgan, I. G., eds.), pp. 135–153, Plenum Press, New York.
[4] Vincendon, G., Morgan, I. G., Breckenridge, W. C., Zanetta, J. P. and Gombos, G. (1973) in: Exposés Annuels de Biochimie Medicale (Boulanger, P. Jayle, M. F and Roche, J. eds.) pp. 93–120, Masson, Paris.
[5] Morgan, I. G., Zanetta, J. P., Breckenridge, W. C., Vincendon, G. and Gombos, G. (1974) Biochem. Soc. Trans. 2, 249–252.
[6] Morgan, I. G., Zanetta, J. P., Breckenridge, W. C., Vincendon, G. and Gombos, G. (1973) Brain Research, 62, 405–411.
[7] Morgan, I. G., Breckenridge, W. C., Vincendon, G. and Gombos, G.(1973) in: Proteins of the Nervous System (Johnson, D., Schneider, R., Hogue, R., Angeletti, R. A., Grasso, A. and Moore, B. W. eds.) pp. 171–192, Raven Press, New York.
[8] Bosman, H. B., Case, K. R. and Shea, M. B. (1970) FEBS Letters, 11, 261–264.
[9] Gombos, G., Reeber, A., Zanetta, J. P. and Vincendon, G. (June 1973) Colloque International du CNRS sur les Glycoconjugués, Lille, Editions du CNRS, Paris, in press.
[10] Waehneldt, T. V. and Mandel, P. (1970) FEBS Letters, 9, 209–212.
[11] Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Breckenridge, W. C. and Morgan, I. G. (1972) FEBS Letters, 22, 253–256.