Incorporation of Labelled Fucose and Glucosamine into Glycoproteins in vivo from Cerebellar Neuronal Perikarya and Nerve Endings

GARY R. DUTTON and JAMES COHEN

Brain Research Group, Open University, Milton Keynes MK7 6AA, Bucks., U.K.

and GRAHAM WILKIN

Medical Research Council Neuropsychiatry Unit, Woodmansterne Road, Carshalton, Surrey, U.K.

Adult Rat Brain Synaptic Vesicles: Protein and Glycoprotein Composition

I. G. MORGAN, J. P. ZANETTA, W. C. BRECKENRIDGE, G. VINCENTON and G. GOMBOS

Centre de Neurochimie du Centre National de la Recherche Scientifique, Institut de Chimie Biologique de la Faculté de Médecine, Université Louis Pasteur, 11 rue Humann, 67085 Strasbourg Cedex, France

Several studies of the protein and glycoprotein composition of synaptic vesicles have been reported (Mehl, 1967; Cotman & Mahler, 1967; Bosmann et al., 1970; McBride & van Tassel, 1972), but have all been hampered by the impurity of the preparations, and the limited amounts of material available. Thus complex profiles from 16 up to 22 protein bands have been reported, and only Bosmann et al. (1970) were able to detect glycoproteins. However, the protein and glycoprotein profiles of the synaptic vesicles of Bosmann et al. (1970) were very similar to those of their synaptosomal plasma membranes, suggesting that their fractions were markedly cross-contaminated. Chemical analyses carried out on similarly impure fractions were unable to detect intrinsic protein-bound sialic acid in synaptic vesicles (Dekirmenjian & Brunngeraber, 1969), although their studies were carefully controlled.

Morgan et al. (1973b) developed a method for the large-scale preparation of synaptic vesicles from adult rat brain by a modification of the method of Whittaker et al. (1964). These preparations were shown by chemical and enzymic assay to be over 90% pure, the only appreciable contaminant being fragments of the synaptosomal plasma membrane (Morgan et al., 1973b).

When these synaptic vesicles were analysed for protein-bound carbohydrate by the method of Zanetta et al. (1972), 19.4 nmol of sialic acid/mg of protein was detected. This amount could not be explained by the contamination detected, and suggested that synaptic vesicles did contain glycoproteins. This observation agrees with the labelling of synaptic vesicles with glucosamine and fucose in both biochemical (Marinari et al., 1972) and radioautographic (Bennet & Koenig, 1972) experiments.

For electrophoresis, the synaptic vesicles were dissolved in 1% (w/v) sodium dodecyl sulphate, 1% (w/v) 2-mercaptoethanol, and 2M-urea in 50mM-Tris-HCl (pH 7.0) and
Fig. 1. Line drawings of 12 × 2.5% (w/v) polyacrylamide gels stained for (a) protein (Amido Black) or (b) carbohydrate (periodic acid–Schiff reaction)

Synaptic vesicles were dissolved in 1% (w/v) sodium dodecyl sulphate, 1% (w/v) 2-mercaptoethanol, 2M-urea, in 5mM-Tris–HCl (pH 7.0) then dialysed against 0.2% sodium dodecyl sulphate, 30mM-iodoacetamide in 5mM-Tris–HCl (pH 7.0). Approx. 200μg of protein was applied per gel, □ and ■ show relative density of straining of different bands.

dialysed overnight against 0.2% sodium dodecyl sulphate and 30mM-iodoacetamide in 5mM-Tris–HCl (pH 7.0). Proteins and glycoproteins were completely solubilized by this procedure. Electrophoreses were performed in 12 × 2.5% (w/v) polyacrylamide gels as described by Waehneldt & Mandel (1970). The gels were stained for protein with Amido Black and with a periodic acid–Schiff reaction (Gombos et al., 1974) for carbohydrate.

- As shown in Fig. 1, the glycoprotein profile of the synaptic vesicles was very simple. One major band and several minor periodic acid–Schiff-positive bands were detected. These bands were not due to micelles of lipids or gangliosides (Dutton & Barondes, 1972) since they were still detected in samples electrophoresed after extraction with chloroform–methanol. The protein profile was slightly more complex, in that seven major bands were detected. In some experiments an extra band of molecular weight around 90000 was detected. This band corresponded to the major band of the synaptoosomal plasma membranes (Waehneldt et al., 1971; Banker et al., 1972; Morgan et al., 1973a) and is believed to be the large, phosphorylated subunit of the Na⁺+K⁺-stimulated ATPase (adenosine triphosphatase) (Kyte, 1971; Uesugi et al., 1971). This band is
Table 1. Molecular weights of synaptic vesicle proteins

These were determined in 12 × 2.5% (w/v) polyacrylamide gels in 0.2% sodium dodecyl sulphate. For details see the text.

<table>
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<th>Mol. wt.</th>
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<td>Proteins</td>
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<td>128 000</td>
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<tr>
<td>96 000</td>
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<td>64 000</td>
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<tr>
<td>13 800</td>
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<td>12 000</td>
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believed to be a sensitive index of the contamination of preparations of synaptic vesicles with fragments of the synaptosomal plasma membrane, a contamination previously detected by enzymic and chemical assay (Morgan et al., 1973b).

The molecular weights of the proteins and glycoproteins (Table 1) of the synaptic vesicles were determined by calibration against a standard curve of molecular weight against migration (Weber & Osborn, 1969) for a series of standard proteins (phosphorylase A, bovine serum albumin, catalase, aldolase, trypsin, ribonuclease and cytochrome c) and glycoproteins (ovalbumin, ovomucoid, avidin and pancreatic trypsin inhibitor). All the standards fell on a straight line in a semi-logarithmic plot, and thus the molecular weights of glycoproteins determined by this method are not necessarily wrong. It may be that only those glycoproteins which have a very high carbohydrate content migrate anomalously (Bretscher, 1971; Marchesi et al., 1972).

An interesting problem is raised by the observation of simple protein and glycoprotein profiles. Synaptic vesicles prepared from total brain would seem likely to be a mixture of vesicles containing acetylcholine, noradrenaline, dopamine (3,4-dihydroxyphenylalanine), 5-hydroxytryptamine, γ-aminobutyric acid, etc. The simplicity of their profile suggests either that synaptic vesicles which contain different transmitters are composed of common protein and glycoprotein species, or that the preparations of synaptic vesicles which we have studied are composed of only a limited number of vesicle types. In this respect it must be remembered that although synaptic vesicles have been shown to contain acetylcholine (De Robertis et al., 1963; Whittaker et al., 1964), and perhaps catecholamine (Michaelson et al., 1963; Maynert et al., 1964; De Robertis et al., 1965), they do not appear to bind the putative transmitter amino acids (Rassin, 1972; De Belleroche & Bradford, 1973).

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The Role of Lipid in the Structure of Central-Nervous-System Myelin

D. H. ADAMS

Medical Research Council Demyelinating Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, U.K.

All membranes contain lipids of various types as part of their structure, but myelin appears to be unique in containing the highest known proportion of lipid to protein, a ratio of approximately 4:1. Opinion in general also regards myelin as having an unusual rigidity in that it is specifically excluded from 'fluid mosaic' models of membrane structure (see for example Singer & Nicholson, 1972).

In view of this exceptional lipid content it would seem reasonable to suppose that the proteins of myelin are sheathed and protected by the lipids, and as a result normally exist in a lipid rather than an aqueous environment.

Studies by Adams & Fox (1969) and Adams & Osborne (1973) have indicated that when rat brain myelin in particular was prepared as described, and subjected to polyacrylamide-gel (12%) electrophoresis in phenol–formic acid–water (14:3:3, w/v/v), virtually all the protein was solubilized and ran as discrete bands on the gel. Only very occasionally was any detectable amount of protein found to remain at the origin, and since (Adams, 1972) the exclusion limit for such gels is approximately $4 \times 10^4$ daltons it seems reasonable to suppose that myelin contains no protein exceeding this size. Also, many workers in the field state that myelin should be soluble in chloroform–methanol (2:1, v/v), and this property seems to have been commonly accepted as a criterion of purity. However, Adams & Fox (1969) and Adams & Osborne (1973) showed that the chloroform–methanol-insoluble fraction of myelin (comprising about 20% of the total...