Resolution of Protein Components in Lysophosphatidylcholine-Solubilized Rat Brain Myelin

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Analysis by dextran-gel filtration and moving-boundary electrophoresis revealed the presence of at least two protein components in lysophosphatidylcholine-solubilized myelin (Gent & Gregson, 1966). This solubilized material has been further investigated by column chromatography with agarose gels under various conditions of pH, ionic strength and lysophosphatidylcholine/myelin solubilization ratio. Two essentially lipid and two distinct protein-rich components were evident on analysis with 4% agarose gel in phosphate buffer, pH 7.8 and *I* 0.1. The major protein-rich component contained cholesterol and most of the lysophosphatidylcholine. The apparent molecular size decreased with rise in the lysophosphatidylcholine solubilization ratio and was approx. 65Å Stokes radius on solubilization with four times the amount of lysophosphatidylcholine required for clearing ($4.0 \times W$ min; Gent, Gregson, Gammack & Raper, 1964). Elution profiles and lysophosphatidylcholine/protein ratios indicated that this material consisted of similar but not identical units. The second protein component was of greater molecular size, but column elution was retarded owing to interaction with the agarose gel. This material was insoluble at high ionic strength, one of the properties of myelin basic protein (Gonzalez-Sastre, 1970).

Further analyses were performed in tris buffer, pH 9.0 and *I* 0.1, and two major protein components were again demonstrated. The leading component, probably containing basic protein, was excluded on columns of 6% gel but was seen to consist of at least two protein-containing components by analysis with 4% gel. Elution positions of the major protein-rich component were again consistent with a Stokes radius of approx. 65Å at $4.0 \times W$ min. Preliminary analyses of freeze-dried material and flotation-density determinations by sucrose-density-gradient centrifugation indicated that the major component at $4.0 \times W$ min is a substantially hydrated lipid-protein unit of molecular weight approx. 650 000 containing 8–10% dry weight of protein. The other component, which probably involves basic protein, is less hydrated, has a molecular weight in excess of 10^6 and is apparently heterogeneous.

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Interaction of Lysophosphatidylcholine with Central-Nervous-System Myelin

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The solubilization of brain myelin by lysophosphatidylcholine exhibits a marked stoichiometry and does not appear to be dependent on the absolute detergent concentration. In a previous brief discussion of the solubilization process it was considered in terms of the attrition of the large particles and the release of protein-lipid-lysophosphatidylcholine complexes (Gent, Gregson, Gammack & Raper, 1964). We have since investigated the process more closely and conclude that it is a two-stage process.

The turbidity of the myelin suspension as a function of added lysophosphatidylcholine and the release of protein and phospholipid all indicate an initial stage, involving approx. 40% of the stoichiometric amount of lysophosphatidylcholine required for full solubilization. This first phase is defined by a gradual release of protein and phospholipid with little fall in turbidity. The use of [^3H]stearoyl-labelled lysophosphatidylcholine further indicates that in this first stage the major part of the added lysophosphatidylcholine is adsorbed on the particulate myelin.

The second stage of the solubilization process occurs rapidly and is indicated by the rapid fall in turbidity and release of protein and phospholipid on further additions of lysophosphatidylcholine. This material released in the second phase is predominantly low-density material and includes the major part of the 2':3'-cyclic AMP 3'-phosphohydrolase activity and the complement-fixing antigen (Gregson, Kennedy & Leibowitz, 1971).

It is therefore suggested that the initial action of the lysophosphatidylcholine is to substitute for a protein-lipid component of the myelin sheath. Examination of the material released in the early stages of solubilization by density-gradient centrifugation and gel chromatography indicates that it consists primarily of the high-density large-particle-size component (W. L. G. Gent, N. A. Gregson & C. A. Lovelidge, unpublished work). This component appears to contain primarily the basic proteins, and thus the protein released during the first phase is also predominantly basic protein.

After incubation of myelin with various amounts

of lysophosphatidylcholine the remaining particulate material was rapidly fixed in glutaraldehyde. Electron microscopy indicates that the two phases of the solubilization process involve a structural transition of the myelin membrane.

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Amino Acid Sequence of the Basic Protein of Human Brain Myelin

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The central nervous system contains an unusual basic protein that comprises 30% of the total protein in myelin (Eng, Chao, Gerstl, Pratt & Tavaststjerna, 1968). The sequence of the 170 amino acids in the protein has been determined (Carnegie, 1971). Micro-heterogeneity occurs in the protein as a result of methylation of a single arginine residue. A cytoplasmic enzyme from brain transfers methyl groups to arginine-107 with the formation of ω -N-monomethyl- and ω -NN'-dimethyl-arginine (Baldwin & Carnegie 1971). This methylated arginine occurs adjacent to a non-polar region in the molecule that has been isolated and shown to induce an autoimmune attack by lymphocytes on the central nervous system (Lennon, Wilks & Carnegie, 1970).

The basic protein has been shown to be present in the intraperiod line in the lamellar structure of myelin (Dickinson, Jones, Aparicio & Lumsden, 1970). As the protein has numerous aspartic acid, histidine and serine residues interspersed along the molecule it is speculated that in the non-polar environment within myelin they may be participating in a 'charge-relay system' (cf. Blow, Birktoft & Hartley, 1969). Fernandez-Moran (1959) has suggested that membranes with a lamellar structure may be functioning as semi-conductors.

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Trypan Blue: Reaction with Myelin

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A simpler and more sensitive method for estimating the basic encephalitogenic protein of myelin in myelin fractions than is afforded by conventional extraction and ion-exchange purification techniques was required. It had been claimed (Adams & Bayliss, 1968) that the tetra-azo-tetrasulphonic acid dye, Trypan Blue, in certain conditions combines specifically with this protein and with the nucleohistones in frozen sections of central-nervous-system tissue. Reaction of central-nervous-system myelin, purified in the ultracentrifuge and dissolved in dry chloroform-methanol (2:1, v/v), with the dye dissolved in methanol (to give chloroform-methanol, 1:1, v/v, final composition) leads to a precipitate, which, after the washing away of excess of dye, is dissolved in 0.1 M-sodium dodecyl sulphate solution, and the precipitated dye is determined from the extinction at 585 nm. The method satisfies criteria for quantitative use.

Different myelin preparations have different capacities for reacting with the dye: correlation plots with all lipid components show, as expected, weak negative regressions. Only plots against total nitrogen and total protein show strong positive regressions, and only that against protein gives a regression line that has an intercept not significantly different from zero ($0.7 > P > 0.6$) as well as a strong correlation ($P < 0.001$). [Total protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) on solutions of myelin in 0.1 M-sodium dodecyl sulphate; the values obtained are always equal to, or greater than, those obtained after alkali digestion with or without prior lipid extraction, presumably owing to reduced handling losses and degradation.]

The weight fraction of myelin precipitated with dye is very similar to the total protein content, and the amino acid compositions of the precipitate and the parent myelin are closely similar, even though these may differ considerably for different myelins. The sum of the uncorrected amino acid residue weights accounts for more than 90% of the non-dye part of the precipitates.

Thus, in these restricted conditions, Trypan Blue combines with all proteins and not specifically with basic protein, and the dye-combining power in dry organic solvents may be used as a measure of protein content in proteolipids (that is protein and lipid complexes soluble in chloroform-methanol, 2:1, v/v). The method is reproducible, readily standardized and has a slightly greater sensitivity (E units/mg of protein per test) than the standard method of Lowry *et al.* (1951).