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