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MYELOSOMES: THE OSMOTICALLY SENSITIVE MYELIN PARTICLES

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SUMMARY: Osmotic integrity of myelin fraction was critically evaluated by the technique of enzyme osmometry and osmometry linked to turbidimetry. Myelin particles behave as osmometers. Gravity-mediated entry of sucrose across these myelin particles suggests the presence of a naked resealed plasma membrane. Myelin particles, being proximal to synapse, can be used as logical control material for synaptosomal uptake studies.

Myelin constitutes a distinct fraction in the isolation of sub-cellular organelles from brain homogenates. Lack of adequate methodology to establish the structural integrity of these particles has thus far limited the use of this fraction in biochemical studies of organelles and clear definition of what constitutes the myelin fraction has not been achieved (cf. Ref.1). The nerve cell plasma membrane has revealed a dominant tendency to reseal spontaneously, resulting in unique and intact particles, the synaptosomes (2). Similarly, fragmentation of myelinated axons during homogenization could give rise to osmotically intact myelin particles, which may appropriately be referred to as 'myelosomes'. One may visualize 4 types of such structures : A. single plasma membrane vesicles, B. plasma membrane vesicles with an adherent myelin patch, C. plasma membrane vesicles with a complete envelope of resealed myelin membrane and, lastly D. plasma membrane vesicles with a myelin sheath open at both

LDHase, Lactate dehydrogenase;
CNPase, 2',3'-cyclic nucleotide 3'-phosphohydrolase;
B.P., breakpoint.

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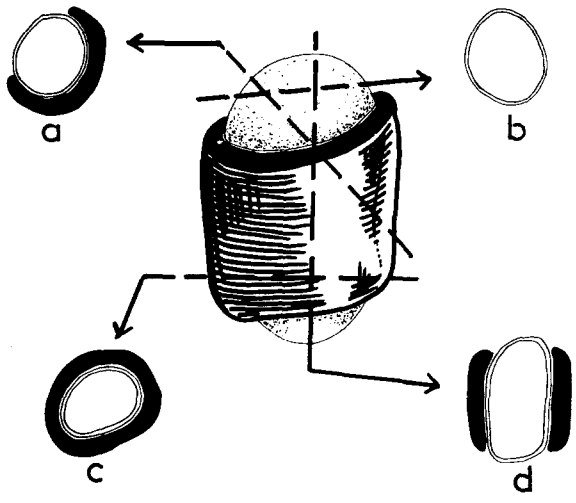

Figure 1: Myelosome-type D: The thin (double) line represents the axonal plasma membrane enclosing the major cytosolic compartment, the axoplasm, and the dark sheath, the multilayered myelin sheath. Note that the plasma membrane is directly exposed to the medium, only partly enveloped by the myelin sheath. Depending on the plane of section (indicated by the dotted lines) this myelosome can give rise to a variety of cross-sections, a-d. The trivial case of a tangential section restricted to myelin sheath is not illustrated.

ends. Figure 1 illustrates typical electron micrographic cross sections that one may encounter and how myelosomes of type D per se could account for all the cross sections (a-d), depending on the plane of section. Since myelosomes would represent structures proximal to the synapse, these would be the logical choice of control material to determine what is specific to the nerve endings, provided that naked axonal plasma membrane exists on myelosomes, across which uptake of molecules can be studied. We report here direct experimental evidence that myelin fraction does indeed represent osmotically intact particles, using the novel technique of enzyme osmometry (3,4) and osmometry linked to turbidimetry (5).

MATERIALS AND METHODS

Isolation of myelin fraction: Rhesus monkeys (Macaca mullata) were killed by air embolism and cortical white matter was rapidly dissected in cold. The tissue was homogenized in

0.32 M sucrose as in the case of synaptosomal isolation (4) and the corresponding crude mitochondrial fraction was obtained. Myelin was further purified in a discontinuous sucrose density gradient by reisolation at the interface of 0.32 M-0.8 M sucrose, representing approx. 95% protein of the crude mitochondrial fraction.

Enzyme osmometry: Activity of an occluded enzyme is monitored under initial velocity conditions, as a function of varied external osmolarity. The rate of reaction of an enzyme occluded by a limiting membrane would be

$$Jr = KA_o/(1+K/P)$$

where K is the kinetic constant of the enzyme, P, permeability of the membrane to the external substrate A_o. When $K \gg P$ and when K and P are both invariant with regard to the osmotic stretch of the membrane, a distinct non-linearity in the activity profile of an occluded enzyme (corresponding to osmolytic release of the occluded enzyme) would be manifest when plotted as a function of external osmolarity. The breakpoint of such an osmometric curve would, ceteris paribus, depend on the osmotically active solute internally (4).

Osmometric behaviour is also manifest for the externally faced membrane-bound enzymes even in the absence of a permeability barrier, e.g., NAD(P)H oxidase of the plasma membrane (6), K^+/H^+ antiporter of mitochondria (7). The kinetic constant of such membrane-bound enzymes would vary with the osmotic stretch of the membrane, resulting in breakpoints larger than that of an osmolysis curve (where the actual release of enzymes/proteins is monitored in the supernatants of osmotically treated particles, e.g., lactate dehydrogenase (LDHase; L-lactate: NAD^+ oxidoreductase, Ec 1.1.1.27) in synaptosomes (4).

Assay of enzymes: LDHase was chosen as a cytosolic marker and assayed as described earlier (4). 2', 3'-cyclic nucleotide 3'-phosphohydrolase (CNPase, EC 3.1.4.16) was chosen as a myelin marker (8) and was assayed according to the method of Sims and Carnegie (9) with two modifications: detergent was omitted in the assay and tonicity was varied by adding sucrose at several concentrations. The solubilized enzymes LDHase and CNPase, had only a monotonic profile of inhibition with added sucrose. Specific activities were plotted as a function of sucrose concentration in the assay medium and were expressed as μmoles of NADH oxidized min^{-1} mg^{-1} protein for LDHase and μmoles of 2', 3'-cAMP hydrolysed min^{-1} mg^{-1} protein for CNPase. Protein was assayed in the presence of sodium dodecyl sulphate by Lowry's method (10).

Turbidimetry: Myelin is characterized by high turbidity and measurably slow changes in turbidity, in response to varied external osmolarity. It can be shown that, while optical density varies inversely with r^2 (r, the radius of the particle) (5), the rate of change of optical density would vary inversely with the volume of the particle, and, is amenable to osmometric analysis. Turbidimetric analyses were carried out with the crude mitochondrial fraction as well as with the purified myelin fraction and initial velocity changes in optical density min^{-1} mg^{-1} protein were obtained, at 520 nm.

Breakpoint analysis: The analytical problem is one of arriving at an objective estimate of two different slopes ($y_1 = m_1 x + c_1$ and $y_2 = m_2 x + c_2$, such that breakpoint (B.P.) = $(c_2-c_1)/(m_1-m_2)$.

A rigorous method is to start at either end of the curve and progressively evaluate the coefficient of correlation of successive data points by least square linear regression. Across the discontinuity, the coefficient of correlation falls indicating the data points that belong to the other slope. Alternatively one can visually discriminate individual slopes and choose the data points relevant to each slope. In practise, the difference in breakpoints obtained by these two methods are indistinguishable. As an additional precaution, the breakpoints were confirmed 2-3 times and were independent of protein concentrations used in the assays. The breakpoints for turbidimetry were independent of the wave length employed as well.

It is instructive to note that the larger negative slope in the osmometry profiles represents a cumulative integral of the frequency distribution of particles in relation to osmotic susceptibility. Breakpoint analysis is therefore relatively free of the variance of the particles as well as secondary solute effects.

RESULTS AND DISCUSSION

Data in Fig.2 confirmed the predictions thus: i. all the three markers exhibited pronounced osmotic curves with distinct breakpoints; ii. all the three markers indicated shifts in the breakpoints of the osmometry curves corresponding to sucrose in the isolation medium: iii. the breakpoints of osmolysis (i.e., that of LDHase as in the case of synaptosomes (cf. Ref.4) were less than those of either turbidimetry or CNPase. Thus myelin fraction consists of osmotically intact particles with, 1. occluded LDHase activity, 2. an osmotically responsive myelin sheath and, 3. a limiting membrane, the permeability characteristics of which change drastically in the presence of gravitational field, leading to equilibration of sucrose in the isolation medium.

The gravity-mediated change in the permeability characteristics of biological membranes appears to be a ubiquitous phenomenon e.g., synaptosomes, peroxisomes, mitochondria (4), lysosomes (11), hepatocytes (12), as also myelin. Thermodynamic considerations would stongly argue against the entry of sucrose into the cytosolic compartment across several bilayers encountered in the myelin sheath. In fact, entry of sucrose across even a single

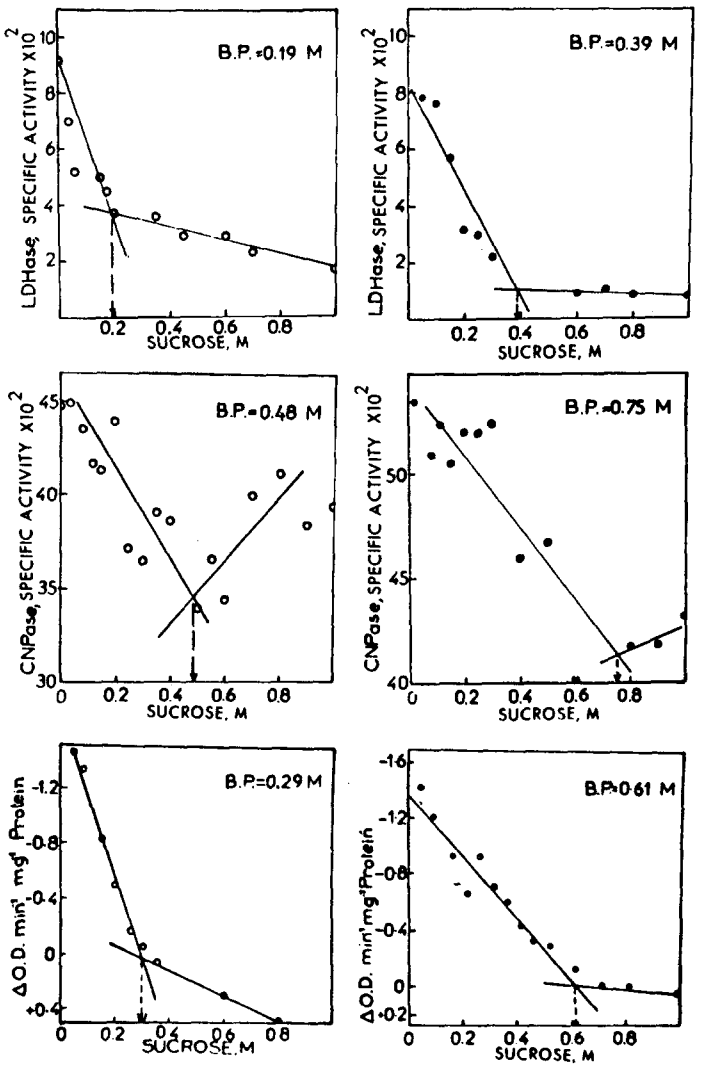

Figure 2: Osmometric analyses on crude mitochondrial fraction (open circles) and purified myelin fraction (closed circles), from white matter of primate brain. Refer to Materials and Methods. B.P. Breakpoint expressed as M sucrose in the assay medium, indicated by a dashed arrow.

bilayer during centrifugation would necessarily invoke heterogeneous membrane models, as we discussed earlier (4). The observed entry of sucrose into the cytosolic compartment (since a shift in the breakpoint of the osmometric profile of an enzyme would mean a shift of corresponding magnitude in the internal solute content of the same compartment as that of the enzyme), may therefore be considered as suggestive of the existence of a

Table. 1. Compatibility of Osmometric data with the predicted structures of myelosomes

Predicted structures of myelosomes	Compatible cross section as in Fig.1	Biochemical Properties			
		LDHase osmometry	CNPase* osmometry	Turbidimetry**	Gravity-mediated entry of sucrose
A. Single plasma membrane vesicles.	only b	+	-	-	+
B. Plasma membrane vesicles with an adherent myelin patch.	a and b	+	-	-	+
C. Plasma membrane vesicles with a complete, resealed myelin sheath.	only c	+	+	+	-
D. Plasma membrane vesicles with myelin envelope open at both ends.	a,b,c and d	+	+	+	+

Logical compatibility is indicated by + or -. Compatibility of biochemical properties is valid for homogeneous suspensions of particles only.

* Myelin should be a continous envelope atleast in one plane circumferentially, to be effectively stretched by osmotic swelling of the plasma membrane vesicles.

** Pertains to osmometric profiles of rate of change of turbidity, which reflects volume changes in myelin. Similar osmometric profiles were observed in other organells such as mitochondria (D.Samabasivarao and V.Sitaramam, unpublished observations); the breakpoint corresponds to an acute deviation of the particles from the ideal Boyle-Van't Hoff pressure-volume relationship in hypertonic solutions owing to limited compressibility of the bilayer domain etc. (15).

plasma membrane (presumably that of the axon) exposed to the medium, in the myelin particles.

Table 1. summarizes the logical compatibility between our experimental data with possible morphological structures. The published electron micrographs of myelin fraction (e.g. Refs. 13,14) indicate the presence of cross sections a,b and c (cf.Fig.1) though methods of preparation of myelin (often involving repeated osmotic shocks and non-adherence to critical isotonic conditions during fixation for electron microscopy) in literature would not allow much certainty. Myelosomes of type 'D' as depicted in Fig.1. offer the simplest explanation for these experimental findings. Since heterogeneity of certain degree is to be expected in myelin preparations, the proof would require electron microscopy under controlled osmotic conditions of fixation.

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