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ON THE BINDING OF BRAIN MYELIN BASIC PROTEIN TO CHROMATOGRAPHIC RESINS

P.Riccio, A.De Santis , A.Bobba and E.Quagliariello

Istituto di Chimica Biologica,
and Centro di Studio sui Mitocondri e Metabolismo Energetico,
CNR, Università di Bari, 70126 Bari, Italy

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Summary: Myelin basic protein, only in association with certain detergents, is able to bind irreversibly to the usual gel filtration media. While this binding is greatly advantageous in purification of proteolipid (the other major myelin protein), the question arises of the uncommon, nonphysiological behaviour of the basic protein. A relationship between binding property and basic protein structure is suggested.

Although the basic protein, a major protein of the brain myelin membrane, has been largely studied because of its implied role in neurological diseases (1,2), its functional role remains to be established. In addition, very little is known about the interaction of detergents with the myelin membrane and/or basic protein (3-6).

During our studies on the fractionation of myelin by detergents (7), we observed that in association with some of the detergents used, the basic protein is able to bind irreversibly to resins normally used for molecular sieving chromatography. The unexpected anomalous chromatographic behaviour is absent when the basic protein is extracted in the conventional way without detergents (8,9). In addition the binding can be prevented, but not removed. This paper outlines some salient features of the observed binding and discusses possible implications for the structure and function of the basic protein.

MATERIALS AND METHODS: Urea, SDS, Triton X-100 and Ultrogel AcA 34 were purchased from LKB, CTAB from SERVA, sodium cholate from Merck, Sepharose CL-6B and Sephadex G-25 from Pharmacia, Bio Gel P-150 and Bio Gel P-6DG from Bio-Rad. Deoxycholic acid (Koch-Light Lab.) was further purified. Octyl-POE

Abbreviations: MBP, myelin basic protein; CTAB, cethyltrimethylammonium bromide; octyl-POE, n-octylpolydisperse oligooxyethylene; SDS-PAGE, polyacrylamide gel electrophoresis in sodium dodecylsulfate.

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was a gift of Prof. J.P. Rosenbusch (Heidelberg, Basel).
Myelin was prepared from bovine brain white matter as described by W.T.Norton (10).
Protein content was determined with a modified Lowry method in the presence of 0.5% sodium dodecylsulfate (SDS) (11). Where indicated the Bio-Rad micro-assay procedure was used. BSA was used as a standard.
A discontinuous gel electrophoresis system on 15% acrylamide gels was adapted from the U.K. Laemmli procedure (12). Runs were carried out using 4.5% spacer gels. The ammonium persulfate and TEMED content was 3 times higher than in the standard procedure.
Preparation of the protein samples for the binding studies:
CTAB-EXTRACT: myelin was resuspended in a medium containing 0.25±0.02% CTAB, 50mM Tris-HCl, 0.1mM phenylmethylsulfonylfluoride (PMSF) pH 8.5 at a protein concentration of 3.6±0.7mg protein/ml (0.69±0.1mg CTAB/mg protein). After 30 min incubation in ice, the suspension was centrifuged for 40 min at 40000 rpm in the 50 Ti Beckman rotor. Solubility percentage was 9.5±1.0%. MBP was 90% pure.
CHOLATE-EXTRACT: myelin was resuspended in a medium containing 1% cholate, 250mM NaCl, 10mM Tris-HCl pH 8.5 at a protein concentration of 3.65mg protein/ml (2.7mg cholate/mg protein). Incubation and centrifugation were carried out as above. Solubility percentage was 20.3%. MBP was 85% pure.
TRITON-EXTRACT: myelin was resuspended in a medium containing 2.5% Triton X-100, 500mM NaCl, 20mM Tris-HCl, 0.1mM PMSF pH 8.5 at a protein concentration of 2.9mg protein/ml (8.6mg Triton/mg protein). After 30 min incubation in ice the suspension was centrifuged for 40 min at 40000 rpm in the 42.1 Beckman rotor. Solubility percentage was 58%. The Triton-extract was applied for a negative adsorption chromatography on hydroxyapatite (HTP). The non-adsorbed fractions, containing both proteolipid (45%) and basic protein (55%) were eluted with 0.1% Triton, 20mM Tris-HCl pH 8.5. These fractions, pooled and concentrated on Amicon PM 10 membrane were used for the binding studies. For the purification of proteolipid protein the concentrated HTP pass-through was then applied on a 120ml column of Sepharose CL-6B equilibrated with 0.1% Triton, 300mM NaCl, 20mM Tris-HCl, 0.05% NaN_3 pH 8.5. The proteolipid was eluted with the same equilibrating medium.

RESULTS

Study of myelin basic protein binding to gel filtration resins was performed either by mixing basic protein extracts with gels at fixed ratios and subsequent phases separation by centrifugation, or by chromatography on small columns.

In the experiment shown in fig. 1 the centrifugation method was used. A definite amount of the gel chosen, Ultrogel AcA 34, containing both acrylamide and agarose, was added to the basic protein extracted with different detergents from bovine brain myelin. After mixing, centrifugation and washing, protein content was determined in the supernatants. The results of fig. 1 clearly demonstrate that the basic protein selectively extracted, as previ-

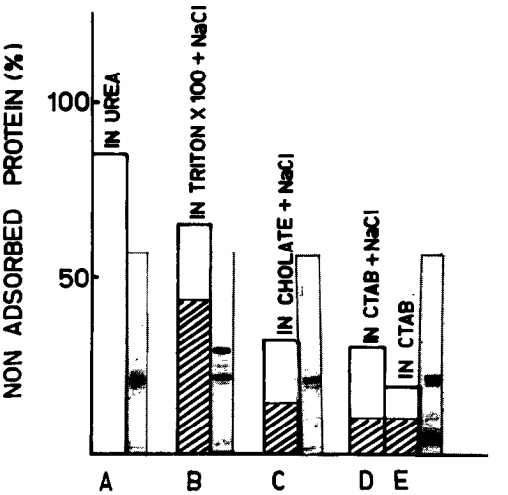


Fig. 1: Demonstration of the binding of myelin basic protein to the filtration gel Ultrogel AcA 34. Centrifugation method. The gel was added in increasing amount to different extracts of the basic protein. After incubation in Eppendorf cups in ice for 30 min, the samples were centrifuged at about 13000g for 10 min. The sediments were washed once with buffers containing 0.35% Triton X-100, 0.25% cholate, 0.1% CTAB respectively. Protein content was determined in both supernatants by the Lowry method. In the figure the results are shown for a gel/protein ratio of 0.7 (w/w). On the right of the columns, SDS-PAGE's of the extracts prior to mixing are respectively shown. Extracts were obtained as described in the methods section. Where indicated, NaCl concentration was 0.5M.

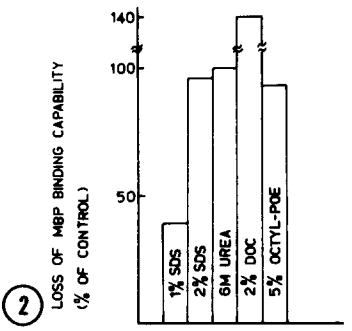
ously described (7), either by cethyltrimethylammonium bromide (CTAB), or by cholate, remains strongly bound to the gel (columns C-E) regardless of ionic strength, whereas the protein present in the partially purified Triton extract is only apparently poorly bound (column B). In effect, as established by SDS-PAGE, the Triton extract contains both the proteolipid and the basic protein of myelin. In this Triton extract the proteolipid fraction amounts to about 45% of the total. If this value is subtracted (dashed part of the column B), protein strong binding in Triton is also evident. On the other hand the basic protein prepared by the traditional procedure in the hydrophylic form (9) is unable to bind the gel (column A). Nor is binding observed when basic protein is extracted by SDS, urea, deoxycholate or octyl-POE.

Different types of gel (not shown) were tested using the same procedure as above with a single Triton extract. Strong binding was observed both with Sepharose CL-6B, containing agarose, and Bio Gel P-6DG, containing acrylamide. The observed affinity scale: Sepharose CL-6B = Bio Gel P-6DG > AcA

34 > Sephadex G-200 > Bio Gel P-150 seems to exclude a preferential gel component for the binding. Chromatographic behaviour on hydroxyapatite is normal.

The question now arises as to whether the binding is effective, since, if binding exists, it should be possible to remove it and recover the bound basic protein, or else prevent it.
For this study Triton extracts, partially purified by negative adsorption on hydroxyapatite, were applied to small columns containing Sepharose CL-6B. Once bound to the resin the basic protein cannot be removed from the column. Elution with 0.1% Triton in the presence of either 1% SDS, or 2% octyl-POE, or 0.5M galactose, or 0.05M N-acetyl-D-galactosamine, or 2% deoxycholate, or finally with the same Triton but at pH 5, gives no positive results.
On the other hand, binding of the basic protein to the columns may be prevented when the detergents, in which the basic protein does not bind to the resin, are each added to the basic protein in Triton prior to the application to the Sepharose CL-6B columns, which are then eluted by Triton buffer only (results shown in fig. 2). Urea and all detergents tested are effective in preventing binding. This effect cannot be reversed. In particular, dependence on SDS concentration demonstrates that the observed effect of binding prevention can be ascribed to the detergent used. Addition of either 0.6M galactose or 0.67M N-acetyl-D-galactosamine does not affect MBP binding.

The presence of the basic protein in the eluates upon addition of the detergents, which are able to avoid binding, is visualized by the SDS-PAGE shown in fig. 3. In control, lane B, basic protein is absent in the eluate but present when 2% SDS (lane C), 6M urea (lane E), or 2% deoxycholate (lane F) were incubated with the Triton sample prior application.
Results shown in fig. 3, lane B, also demonstrate how easily the proteolipid can be purified in Triton X-100. In the first step, negative adsorption chromatography on HTP, the proteolipid is separated from the high MW proteins (fig. 3, lane A), in the second and final step only a small gel filtration column is required because of the binding of the basic protein to the gel (lane B). Thus the purification of the proteolipid can be achieved in few hours. The closely related (13) DM 20 fraction was also present, although in low amount, after chromatography on larger columns.



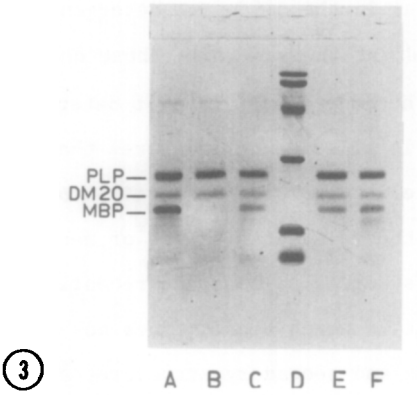


Fig. 2: Prevention of the basic protein binding to Sepharose CL-6B columns. Fractions (350 µl) of a single Triton extract (cf methods section) containing 5 mg proteins (Bio-Rad titer) were applied to 15 ml columns of Sepharose CL-6B, equilibrated with 0.1% Triton X-100, 0.3M NaCl, 0.01-0.05M Tris-HCl and 0.02% NaN_3 at pH 8.5, and eluted with the same medium. Triton sample was incubated with the chosen detergent at room temperature and for 60 min at the concentration shown in the figure prior to application to the column. Loss of binding capability was related to protein binding in controls taken as 100%. DOC is an abbreviation for deoxycholate.

Fig. 3: SDS polyacrylamide gel electrophoresis (SDS-PAGE) of the eluates of the experiment of fig. 2. (A) Triton extract, as obtained after negative adsorption on hydroxyapatite and applied to gel filtration; (B) control: eluate of 15 ml Sepharose CL-6B column after incubation of the sample to be applied for 60 min and room temperature with 0.1% Triton X-100, 10mM Tris-HCl, 0.1mM PMSF at pH 8.5; (C,E,F): column eluates of Triton samples treated prior to application with 2% SDS, 6M urea or 2% deoxycholate respectively; (D): standard proteins: ovotransferrin, 77 KDa; BSA, 66.3 KDa; ovoalbumin, 45 KDa; carbonic anhydrase, 30 KDa; myoglobin, 17.2 KDa; cytochrome c, 12.3 KDa. PLP is an abbreviation for proteolipid protein.

DISCUSSION

Evidence has here been presented on binding of the basic protein to chromatographic resins. The binding, which occurs in few detergents only and which can be prevented, appears to be a new property of the basic protein, never described before.

The question now arises as to whether this quite uncommon and not physiological property, besides facilitating purification of the proteolipid protein, could be significantly helpful in understanding structure and function of the basic protein.

Notably, detergents in which binding occurrs differ greatly: CTAB is cationic and often considered as denaturing, while cholate is anionic and Triton, mild and nonionic (14). Nevertheless, if the observed binding is a real property of the basic protein, this protein should possess some common

features in the different detergents to which binding capability could be related. At the same time these characteristics should be absent in the other heterogeneous class of detergents where binding does not occur.

Initial evidence indicates that basic protein purified by hydroxyapatite in CTAB or cholate (15) contains high amounts of myelin lipids and shows the relevant presence of secondary structure (16). However, similar results were also observed recently in protein purified in octyl-POE, a detergent in which binding does not occurr (17). Thus, these properties, lipid binding and secondary structure, although completely or nearly absent in SDS (18), or in deoxycholate (6), or after acid extraction (19) could be considered necessary but not enough for the binding.

Our present working hypothesis is that to bind to the large gel surfaces, the basic protein should be in an oligomeric, at least dimeric, state, besides being in a lipid bound form and possessing a relevant β-structure. In fact whether in SDS, deoxycholate (6), octyl-POE (20) or extracted by acid (19) the non-binding basic protein is always present in the monomeric form. In addition, evidence has shown that the basic protein may act as a dimer (21), linked to its hypothetical function of biological glue to maintain the multilamellar myelin structure.

Further studies will explain whether our hypothesis is correct and whether the observed binding to the gel has a physiological reply in biological systems.

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