RESISTANCE OF THE BASIC MEMBRANE PROTEINS OF MYELIN AND BACTEROPHAGE PM2 TO PROTEOLYTIC ENZYMES

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1. Introduction

Basic protein(s) comprise about 30% of the proteins of both peripheral and central nervous system (PNS and CNS) myelin [cf.1]. Although the amino acid sequence of several myelin basic proteins has been determined, the secondary structure is not well understood [1,2]. Through our studies on another basic membrane protein, protein II of the lipid-containing bacteriophage PM2 [3], we became interested in a comparison of the properties of the myelin and PM2 basic proteins [4]. For this comparison we have used the basic protein from human or bovine CNS myelin, designated as protein Al [1]. After determining the isoelectric point of human Al by a simple micromethod [3], we studied the susceptibility of Al and PM2 protein II to proteolytic degradation, both in situ and after purification. In the latter case the effect of added acidic lipids on the susceptibility to proteolysis was also investigated. In the case of Al it is known that the acidic lipids form distinct lipoprotein phases with the protein. In the case of the PM2 protein, binding studies have been used to demonstrate a specific interaction between the basic protein and acidic lipids [6].

2. Experimental

Myelin, as well as protein Al, was isolated from human or calf brain white matter by the methods of Norton [7] and Banik and Davison [8] using the following modification: during the entire procedure we used solutions containing 20 mM Tris–HCl, pH 7.6 and 10 mM β-mercaptoethanol instead of water as described by the authors in the original publications. Published methods were also used to prepare the acidic lipids from CNS myelin [9] and for preparing phases containing Al with the total mixture of acidic lipids or with the sulfolipids alone [5]. The determination of the isoelectric point of Al was carried out by extrapolation of the (pH dependent) mobilities on analytical cellulose acetate sheets to zero [3]. Electrophoresis was on analytical cellulose acetate sheets in the presence of 20 mM H_3BO_4 + 20 mM H_3PO_4, adjusted with NaOH to the desired pH values.
The purification of bacteriophage PM2 was described earlier [10]. Protein II was purified from PM2 as described by Hinnen et al. [11]. The acidic lipid phases, containing protein II and phosphatidylglycerol, were prepared as follows: 100 μg of protein II in 0.5 ml buffer (20 mM Tris–HCl; pH 7.5; 10 mM β-mercaptoethanol; 1 M urea) was shaken vigorously for 4 hours with 0.5 ml chloroform containing 500 μg PG. The aqueous phase was then used for the assay.

3. Results

The isoelectric point of the purified basic myelin protein was 10.8. In the presence of bovine α-chymotrypsin, trypsin, bromelin, and thermolysin, myelin protein was hydrolyzed completely within 30 min. The basic protein in intact myelin or in the lipoprotein phases containing acidic lipids, on the other hand, was not hydrolyzed in the presence of trypsin, chymotrypsin (fig.1), bromelin, or thermolysin, even after

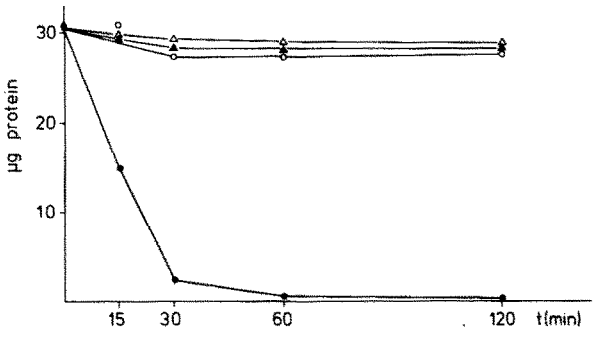

Fig.1. 5 µg of bovine α-chymotrypsin was added to 0.5 ml of Tris-buffer (30 mM Tris–HCl pH 7.6) containing either the purified human CNS myelin basic protein or intact myelin or Al lipoprotein phase; in each case with a protein content of 350 µg/ml. The mixtures were incubated at 37°C for different times. At the end of the incubation peroids, 2 µl of 1 M phenylmethanesulfonylfluoride (PMSF) in dimethylsulfoxide and 5 µl of an aqueous solution containing 10% SDS were added to the mixtures in order to inactivate the chymotrypsin and to prepare the proteins for gel electrophoresis. Aliquots of 100 µl of the mixtures were analyzed by polyacrylamide gel electrophoresis in the presence of SDS. The gels were stained and scanned [10] and the stained areas of the Al protein were integrated. The values represent the amount of intact basic protein remaining after the different periods of hydrolysis in the presence of chymotrypsin. (–●–) Purified myelin basic protein; (–△–) myelin; (–○–) basic protein + acidic lipids; (–▲–) basic protein + sulfolipid.

prolonged incubation times (table 1a). The basic structural protein in intact bacteriophage PM2 or in a lipoprotein phase with phosphatidylglycerol was also resistant to the proteolytic enzyme thermolysin whereas protein II alone was degraded by this enzyme (table 1b).

Table 1

	Buffer	Enzyme	% Degradation after 60 min
a) myelin basic protein (Al)	30 mM Tris–HCl pH 7.6	chymotrypsin	98
myelin basic protein (Al)	30 mM Tris–HCl pH 7.6	trypsin	97
myelin basic protein (Al)	30 mM Tris–HCl pH 7.6	bromelin	98
myelin basic protein (Al)	20 mM Tris–HCl pH 7.6; 10 mM $CaCl_2$	thermolysin	96
Al + sulfolipid	30 mM Tris–HCl pH 7.6	chymotrypsin	4
Al + sulfolipid		trypsin	2
Al + sulfolipid		bromelin	2
Al + sulfolipid	20 mM Tris–HCl pH 7.6; 10 mM $CaCl_2$	thermolysin	1
b) PM2 protein II (isolated)	25 mM Tris–HCl pH 7.5; 0.1 M NaCl; 10 mM $CaCl_2$; 2 M urea	thermolysin	92
protein II in intact PM2	25 mM Tris–HCl pH 7.5; 0.1 M NaCl; 10 mM $CaCl_2$	thermolysin	< 1
protein II + PG	25 mM Tris–HCl pH 7.5; 0.1 M NaCl; 10 mM $CaCl_2$; 2 M urea	thermolysin	1

(a) Hydrolysis of myelin protein Al in the presence and absence of sulfolipid. The conditions of the assays are described in the legend to fig.1. In each case, 5 µg of proteolytic enzyme was added to 0.5 ml solutions containing 350 mg/ml of protein Al. The proteolytic activity of chymotrypsin was stopped by the addition of PMSF and SDS, that of trypsin and bromelin by SDS and that of thermolysin by EDTA (see fig.1 and below).
(b) 100 µg of purified protein II [11], 200 µg of bacteriophage PM2, or 100 µg of protein II as a lipoprotein complex (see Experimental), in each case in a total volume of 0.5 ml was incubated at 30°C in the presence of 2 µg of thermolysin. The proteolytic activity was stopped by the addition of EDTA to the final concentration of 20 mM. The samples were prepared for polyacrylamide gel electrophoresis as described in the legend to fig.1.

4. Discussion

In most of the published experiments the basic protein of myelin has been reported to be susceptible to digestion with trypsin [cf. 12,13] Wood, Davison, and Hauser [12] isolated myelin in 0.32 M sucrose and then treated it with either 10 μg/ml trypsin or 50 μg/ml of acetylated trypsin at room temperature. But at 37°C the basic protein was degraded even in the absence of added trypsin, which suggests that the myelin preparations might have been contaminated with endogenous proteolytic enzymes. Banik and Davidson [13] prepared myelin by the method of Norton [7], yielding a purer preparation than that used by Wood, Davison, and Hauser [12]. The repeated osmotic shock treatments in distilled water used in this isolation procedure could lead to possible damage of the myelin membranes. We modified this procedure to prevent membrane damage. Banik and Davison [13] used 10 or 250 μg of acetylated trypsin in 3 ml of suspended myelin containing about 7 mg of myelin protein and incubated at 37°C. We used considerably less proteolytic enzyme, employing enzyme/protein ratios similar to those used in studies on amino acid sequences. It may well be that a combination of damaged or impure myelin preparations with excess enzyme concentrations might lead to degradation of Al in situ, thus explaining the loss of 15% of the total myelin protein after 30 min in the case of Banik and Davison [13]. In the present work protein Al in the form of lipoprotein complexes is also resistant to proteolytic digestion. Even in this case both Banik and Davison [13] and London and Vossenberg [14] were able to degrade Al, probably due to the more severe conditions used for proteolysis. There are some interesting parallels in the chemical properties of myelin and bacteriophage PM2, and these have a basis in the structures of the corresponding bilayers. Bacteriophage PM2 has four structural proteins. Proteins III and IV plus DNA form the nucleo-capsid with protein III, a proteolipid soluble in chloroform: methanol being the capsid protein [15,16]. Protein II forms the outer protein shell and protein I forms the spikes. An asymmetric lipid bilayer is disposed between the outer protein shell and the nucleo-capsid; most of the phosphatidylglycerol residues are in the outer lamella, most of the phosphatidylethanolamine residues are in the inner lamella [3], as is to be expected on the basis of the proposed ionic interactions with protein II. Protein III on the other hand, appears to interact hydrophobically with the lipids of the bilayer. The implication of the above observation for the structure of PM2 and myelin are interesting. In PM2, protein II may penetrate into the bilayer much deeper than we originally supposed and this may partly explain the large amount [30 to 50%] of protein in the bilayer [cf. 4,17]. The model proposed for the disposition of protein Al in the myelin bilayer, with strong electrostatic interactions with the acidic lipids of the bilayer and with hydrophobic sequences penetrating into the bilayer [1] is in agreement with the experiments reported here and may also serve as a working model for the disposition of protein II in the PM2 bilayer. At any rate, it is clear that electrostatic interactions between a basic membrane protein and acidic lipids can result in conformational changes resulting in an increased stability with respect to proteolytic enzymes. The resistance of membrane protein to proteolysis is not, however, a fundamental property. As one example, the envelope proteins of Semliki forest virus, which interact hydrophobically with the viral lipid bilayer, can be almost completely hydrolyzed by proteolytic enzymes, leaving a small hydrophobic polypeptide fragment which is the part of the protein embedded in the bilayer [18,19]. Furthermore, in certain pathological conditions, such as multiple sclerosis, the basic CNS myelin protein Al is susceptible to proteolytic attact, at least with cerebral acid proteinase [20], suggesting some alteration in the myelin structure.

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