THE INTERACTION OF BASIC PROTEINS FROM NORMAL AND MULTIPLE SCLEROSIS MYELIN WITH PHOSPHATIDYLGLYCEROL VESICLES

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1. Introduction

In the course of a series of studies on the interaction of myelin basic protein with phospholipid, using X-ray diffraction we discovered that one of the unique roles of basic protein was to promote the organization of phospholipid bilayers into multilayered structures characteristic of native myelin [1]. Since multiple sclerosis is characterized by degeneration of the myelin sheath possibly due to faulty assembly of myelin components, we investigated the ability of the basic protein (BP) isolated from patients who died with multiple sclerosis (MS) to induce the formation of multilayers. The results that we are reporting here were obtained on basic proteins isolated from 5 different normal brains and 3 different multiple sclerosis brains. The results for each concentration of basic protein were reproducible to within 5%.

2. Materials and methods

Myelin was isolated from both normal and diseased white matter as in [2]. Phenylmethylsulphonyl fluoride (PSMF) was present in all solutions during isolation to inhibit protease activity. Basic protein was isolated from myelin by 0.2 N sulphuric acid [2] insuring that protease activity would be totally inhibited.

2.1. *Chemical analysis*

The amino acid compositions of the different basic protein preparations were determined after hydrolysis in 5.7 N HCl under vacuum for 24 h at 110°C. The hydrolysates were run on a Durrum D-500 amino acid analyser. Three analyses were done on each of 3 different normal and 3 different MS BPs, all from different brains. The compositions of all samples agreed well with published data [3].

Phosphorous analyses were done as in [4] after extraction of basic protein with 5 ml chloroform–methanol (C:M, 1:1 + 5% 0.1 N HCl). The means and standard deviations for 24 normals and 17 MS samples were 0.63 ± 0.25 and 0.89 ± 0.32 μmol phosphorous/μmol BP, respectively.

2.2. *Tests for purity and degradation*

Analytical ultracentrifugation was done in a Beckman model E ultracentrifuge equipped with UV optics and scanner, at 60 000 rev./min, 20°C scanned every 8 min. A single boundary was observed for both samples. The sedimentation coefficients were 1.00 for normal BP and 0.99 for MS BP, with 95% confidence limits in both cases. No low molecular weight material was observed.

Polyacrylamide gel electrophoresis was done as in [5]. A single component was observed in all samples.

N-Terminal amino acid analysis was done by the dansyl chloride method [6] in a Beckman 890C protein sequencer with 5 mg each basic protein. No N-terminal amino acids were found by either manual or automated methods consistent with a blocked N-terminus reported [3].

We concluded that our basic protein preparations were high molecular weight, intact with no evidence of fragmentation.

2.3. *Preparation of vesicles*

Egg phosphatidylglycerol (PG) and basic protein were dissolved separately in redistilled 2-chloroethanol and dialysed against Hepes buffer (pH 7.4) [7]. The concentration of lipid was determined by phosphorous analysis [4] and the protein by amino acid analysis as above.

3. Results and discussion

Liquid diffraction X-ray of PG vesicles prepared with increasing concentrations of normal basic protein is shown in fig.1a. Curve 1 is the small angle diffraction pattern of a sample of egg PG. The remaining curves are vesicles of egg PG into which increasing amounts of protein have been incorporated. Focusing on curve 1, we note the presence of a broad maximum centered at $S = 0.127$ corresponding to a correlation distance of 60.8 Å which has been identified as the mean distance between bilayers in a loosely organized diffuse multilayered arrangement of bilayers. As protein is incorporated, the band first increased in magnitude without any apparent narrowing. When the protein concentration reached 30%, the broad maximum sharpened into a definite peak which shifted inwards to $S = 0.10$. A second peak appeared at $S = 0.19$. At a protein concentration of 35 %, the pattern was transformed into a regular repeat pattern with two sharp reflections at $S = 0.09$ and $S = 0.18$. Such a regular pattern is characteristic of myelin in its native form. Thus normal myelin basic protein has ordered the lipids into a 'crystalline' multilayered arrangement.

Fig.1b shows similar curves on samples prepared with basic protein obtained from patients who died with multiple sclerosis. Curve 1 (0% protein) agreed within experimental error (3%) with curve 1, fig.1a. Incorporation of MS BP produced quantitatively different results from those obtained with normal BP. While the initial increase in magnitude of the broad band at $S = 0.127$ is observed again, the increase is only ~50% that obtained with the protein from normal myelin (fig.1a). At 35% concentration, where the normal BP had completely transformed the scattering profile into a sharp repeat pattern (fig.1a, curve 5), the MS BP showed only the sharpened peak at $S = 0.10$ and a faint second peak at $S = 0.19$ which in the normal protein appeared at a significantly lower concentration (30%). Again the intensities of these two lines are significantly lower than with the normal protein. Even at 50% MS BP (not shown) it was not possible to produce the pattern obtained with normal BP (although the peak at $S = 0.10$ did increase in magnitude). We concluded that the MS BP was much less effective in inducing the lipid organization than normal BP. It appears that ~40–50% of the BP isolated from MS material was not participating in the formation or stabilization of the multilayer pattern. The chemical basis of this difference is being investigated.

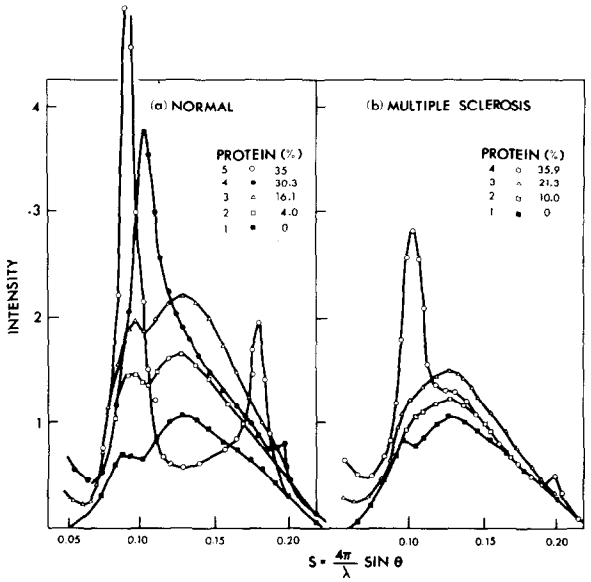

Fig.1. X-ray scattering profiles for myelin basic protein incorporated into phosphatidylglycerol vesicles: (a) normal human myelin basic protein; (b) multiple sclerosis myelin basic protein.

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