CHARGE SHIFT ELECTROPHORESIS OF SYNAPTOSOMAL MEMBRANE ANTIGENS

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1. Introduction

In the characterization of membrane proteins it is important to decide whether the proteins studied possess hydrophobic domains which anchor them to the hydrocarbon interior of the bilayer, as in the case of amphiphilic or intrinsic proteins, or whether they are externally bound to the membrane, as characteristic of hydrophilic or extrinsic membrane proteins. Charge shift electrophoresis introduced by Helenius and Simons [1] explores the detergent binding of amphiphilic proteins as reflected by their changed electrophoretic mobility. The mobility in mixtures of Triton X-100 and different concentrations of a charged detergent is measured and as amphiphilic proteins form detergent–protein complexes containing both the neutral and the charged detergent [2] their electrophoretic mobility will be changed, in contrast to hydrophilic proteins which do not interact with the detergent at nondenaturating conditions.

Previously we have characterized the rat brain synaptosomal membrane proteins D1, D2 and D3 with respect to their brain-specificity [3], their synaptosomal membrane localization [4–6], and the outside localization of D1 and D2 in contrast to the inside localization of D3 on the synaptosomal membrane [7]. As a soluble antigen partially identical to D2 was found in human cerebrospinal fluid [8], D2 might either be an amphiphilic protein protruding into the extracellular space or a hydrophilic membrane protein associated to the outside of the synaptic membrane by ionic interactions. Those possibilities were investigated by charge shift electrophoresis of D2 together with D1 and D3, and it was found that these antigens were all amphiphilic.

2. Materials and methods

The protein extract used as source of antigens during the charge shift electrophoresis was made from forebrains of 35 day-old female Wistar rats. The forebrains were homogenized in 30 vol. (v/w) of 15 g/liter Triton X-100, 100 KIE/liter Aprotenin, 24 mM barbital, 73 mM Tris, 2 mM NaN_3, at pH 8.6. The protein-concentration in the homogenate was 3.9 g/liter [9,10] and the Triton X-100 concentration was high enough as to achieve maximal solubilization [11].

The charge shift electrophoresis was performed as crossed immunoelectrophoresis [12] with different mixtures of charged detergents and Triton X-100 in the first dimensional gels. These first dimensional gels contained 10 g/liter agarose, 10 g/liter Triton X-100, 24 mM barbital, 73 mM Tris, 2 mM NaN_3, at pH 8.6, and further different amount of the anionic detergents sodium deoxycholate or sodium dodecyl sulphate, or the cationic cetyl trimethyl ammonium bromide. Ten microliters of brain homogenate were electrophoresed for 30 min at 10 V/cm measured in that part of the electrophoretic plate not containing ionic detergents. The buffer vessels contained 24 mM barbital, 73 mM Tris, 2 mM NaN_3, at pH 8.6.

By the second-dimensional electrophoresis specific immunoprecipitates were formed orthogonally to the first dimensional separation. The second-dimensional gel contained 10 g/liter agarose, 10 g/liter Triton X-100, 1000 KIE/liter Aprotenin, 24 mM barbital, 73 mM Tris, 2 mM NaN_3, at pH 8.6 and further 30 $\mu l/cm^2$ rabbit antiserum against rat brain synaptic membranes (batch anti SPM 0176). The immunoprecipitates were washed and stained for protein by Coomassie Brilliant Blue [13].

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The distance between the center of the application-well in the first-dimensional gel and the back-projection of the top of the bell-shaped antigen–antibody precipitates into the first-dimensional gel was taken as the mobility of the antigen.

3. Results

The mobilities of the antigens A9, D1, D2 and D3 measured in the presence of different mixtures of 10 g/liter Triton X-100 with cetyl trimethyl ammonium, dodecyl sulphate or deoxycholate are shown on fig.1. The mobilities are expressed relative to the mobility of each antigen in 10 g/liter Triton X-100 buffer without other detergents.

The mobility of the water-soluble antigen A9 [14] was slightly decreased by anionic detergents. Another water-soluble antigen A45 [7] demonstrated a similarly slightly decreased mobility. In contrast to those antigens, the mobilities of D1, D2 and D3 were increased by charge shift electrophoresis with the anionic detergents and decreased by the cationic detergent. The mobilities of D1 and D2 were affected to a similar degree by different concentrations of the charged detergents in contrast to D3 which was more affected than those.

The absolute mobilities of A9, D1, D2 and D3 measured in 10 g/liter Triton X-100 buffer were 18.0, 10.5, 10.2 and 8.0 mm, respectively. Electrophoresed under the same conditions the mobility of the human serum beta-globulin transferrin was 10.0 mm that of alpha$_1$-antitrypsin was 18.0 mm.

Concentrations of charged detergents higher than those shown on fig.1 were also investigated but distortion of the immunoprecipitates of both membrane proteins and water-soluble proteins hindered determinations of mobilities.

4. Discussion

As the mobilities of the synaptosomal membrane antigens D1, D2 and D3 were increased by mixtures of Triton X-100 and the anionic detergents dodecyl sulphate and deoxycholate, or decreased by mixtures of Triton X-100 and the cationic detergent cetyl trimethyl ammonium, interaction was thereby demonstrated between a hydrophobic domain on those antigens and the mixed detergent micelles. Such interactions have been found in proteins with specialized lipid-binding properties as albumin or serum lipoproteins, but much more commonly in amphiphilic membrane proteins [1,2,15]. By analogy, the antigens D1, D2 and D3 must be assumed to be amphiphilic. This is in contrast to the results of Prat et al. [10] which indicated that extrinsic membrane proteins were much more immunogenic than intrinsic amphiphilic proteins.

The antigen A9 is watersoluble and thereby

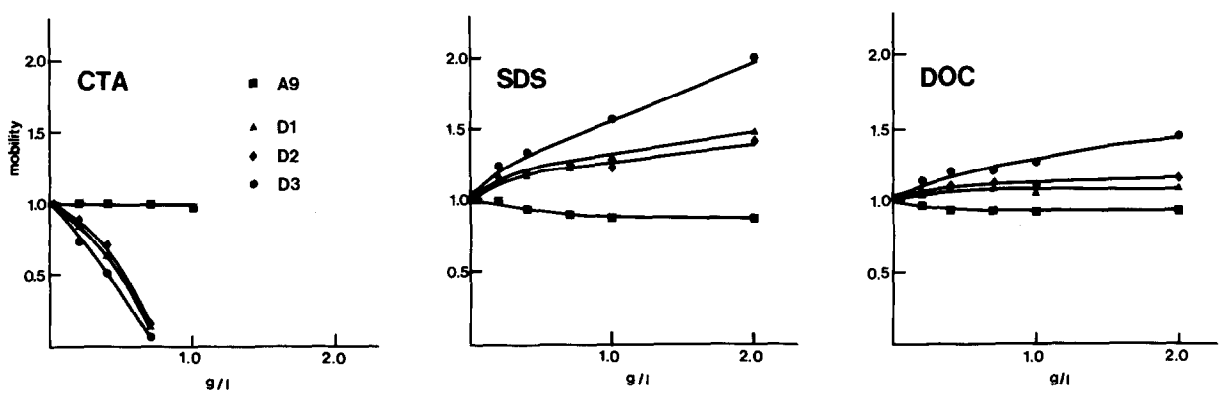

Fig.1. The Triton X-100 extract of the rat brain synaptosomal membrane antigens D1, D2 and D3 and the cytoplasmic antigen A9 were subjected to charge shift electrophoresis in agarose gels containing, besides 10 g/liter Triton X-100, up to 2 g/liter charged detergent. The charged detergents were cetyl trimethyl ammonium bromide (CTA), sodium dodecyl sulphate (SDS) and sodium deoxycholate (DOC). For each antigen are shown the mobilities relative to the mobility without charged detergents in the gels.

hydrophilic. The slight decrease in electrophoretic mobility detected with deoxycholate and with dodecyl sulphate should not be explained by hydrophobic interactions because of the wrong polarity of the effect, but rather by the slightly inhomogeneous electrical field during the electrophoresis caused by the different concentrations of the charged detergents on the electrophoretic plate.

Concentrations of charged detergents higher than those shown on fig.1 gave distorted and diminished immunoprecipitates. Probably two effects were encountered, one effect, especially found with cetyl trimethyl ammonium bromide in the gels, was denaturation, giving no precipitate at all. The other effect, giving diminished precipitates with the expected mobility, might be inhibition of the antigen–antibody reaction in the second dimensional gels [16]. This effect was especially found with sodium dodecyl sulphate in the gels.

The mobility of D3 was more affected by the charged detergents than the mobilities of D1 and D2. As their mobilities in just Triton X-100 buffer were rather close, the effect may be explained by D3 having a relatively larger hydrophobic area than D1 and D2.

The antigens D1 and D2 are localized outside on the synaptosomal membrane [7] but now they have been shown to be anchored to the hydrocarbon interior of the synaptosomal membrane if not protruding. The antigen partially identical to D2 in human cerebrospinal fluid [8] may therefore be either a soluble form of the synaptosomal membrane D2 released by synaptic remodelling or other degenerative processes, or it may be released by extracellular proteases.

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