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Demonstration of immunochemical identity between the synaptic vesicle proteins synaptin and synaptophysin/p38

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The synaptic vesicle proteins synaptin and synaptophysin/p38 were shown to be immunochemically identical. Western immunoblot analysis of Triton X-100 extracts from rat brain showed that polyclonal polyspecific anti-synaptin antibodies and monoclonal antibody SY38 against synaptophysin both reacted with a band of 38 kDa. In two-dimensional immunoblots of chromaffin granule membranes from bovine adrenal medulla anti-synaptin and anti-synaptophysin antibodies also recognized the same component. Finally, in a Western immunoblotting experiment SY38 reacted with an immunoisolated synaptin antigen.

Synaptin; Synaptophysin/p38; Synaptic vesicle protein; Chromaffin granule; Immunoblotting

1. INTRODUCTION

Synaptin (originally termed the C1 antigen) was initially shown to be present in synaptic plasma membranes and extremely enriched in preparations of rat brain synaptic vesicles [1,2]. Because the C1 antigen was a potential marker protein for nerve endings the name synaptin was introduced [3].

Synaptin was characterized as an acidic and amphiphilic glycoprotein composed of a single polypeptide chain with an apparent M_r of 44000 [4]. Synaptin has been localized to the inside of the synaptosomal plasma membrane, and to the cytoplasmic surface of brain synaptic vesicles and of chromaffin granules of the adrenal medulla [5]. The function of synaptin is unknown, but the membrane topographic localization has led to the suggestion that this protein might be involved in the exo/endocytosis of synaptic vesicles during neurotransmission [5]. Synaptin has been demonstrated in all investigated areas of the central nervous system [6], in subcellular fractions of peripheral nerves, adrenal medulla and the neurohypophysis [5,7,8]. It has been demonstrated in brains from several mammalian species, including man, dog, swine, ox [5] and mouse [9,10]. Synaptin exhibits several features in common with a 38 kDa integral membrane glycoprotein of synaptic vesicles called synaptophysin/p38 [11,12]. Synaptophysin/p38 is found at the terminals of all nerve cells and in a variety of neuroendocrine tissues [13,14]. Synaptophysin/p38 is one of the major integral membrane proteins of the synaptic vesicle [11,12] in which synaptin was also shown to be extremely enriched [2]. Synaptin is localized to chromaffin granule membranes [5] whereas the localization of synaptophysin/p38 to this organelle is disputed [12,14].

In view of the similarities/discrepancies between synaptin and synaptophysin/p38 a comparison of the two molecules seemed warranted. We here report that synaptin and synaptophysin/p38 are immunochemically identical.

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2. MATERIALS AND METHODS

2.1. *Antibodies*

The polyclonal polyspecific antibodies against synaptin were prepared from rabbits injected with rat brain synaptic vesicles [1]. The monoclonal antibody SY38 against synaptophysin was obtained from Boehringer Mannheim. Rabbit antibodies against synaptophysin (kindly provided by R. Jahn, Munich) were generated as described previously [11].

2.2. *Western immunoblot analysis*

Adult rat brain was homogenized in PBS, 1% Triton X-100, 5 mM EDTA, containing 100 U/ml aprotinin, 15 mM NaN_3, 0.1 mM phenylmethylsulfonyl fluoride and solubilized for 30 min at 4°C. The homogenate was centrifuged in a Beckman ultracentrifuge at 140000 × *g* for 1 h at 4°C. The supernatant was used as the source of antigen and submitted to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis in a 12.5% gel according to the method of Laemmli [15]. Proteins were electrophoretically transferred to nitrocellulose paper (Millipore) in a semi-dry blotting apparatus (Kem-En-Tec, Denmark) for 90 min with 48 mM Tris, 39 mM glycine, 38 mM SDS, 20% methanol as the blotting buffer [16]. The nitrocellulose paper was incubated with anti-synaptin or SY38 antibodies and the antigen-antibody complex was visualized using alkaline phosphatase coupled secondary antibodies (DAKO A/S, Denmark) and staining.

2.3. *Two-dimensional immunoblot analysis*

Membranes of chromaffin granules from bovine adrenal medulla were isolated and subjected to SDS-PAGE in a two-dimensional system followed by immunoblotting as described in [17].

2.4. *Crossed immunoelectrophoresis*

Crossed immunoelectrophoresis was performed as described in [1]. Membranes from adult rat brain were solubilized in Triton X-100 and used as an antigen source with anti-synaptin as the precipitating antibody. Synaptin immunoprecipitates were cut out of the gel with a scalpel, dissolved and boiled for 5 min in SDS-sample buffer before SDS-PAGE and immunoblotting analysis.

3. RESULTS

3.1. *Western immunoblot analysis of adult rat brain*

In adult rat brain the antibodies anti-synaptin and anti-synaptophysin (SY38) react wth co-migrating bands of 38 kDa. The polyspecific anti-synaptin antibody also reacts, as expected, with a number of other bands which are faint compared to the main band of 38 kDa. The monoclonal antibody SY38 only reacted with a single band as seen in fig.1.

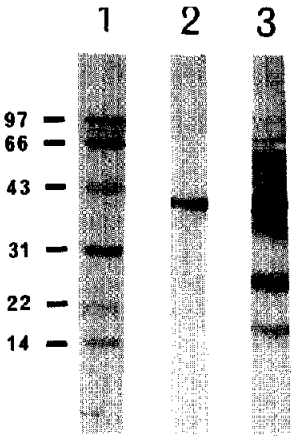

Fig.1. Western immunoblot analysis of adult rat brain. Amidoblack stained molecular mass standards (kDa) in lane 1. Triton X-100 extract of adult rat brain (10 μg protein) was subjected to one-dimensional electrophoresis followed by immunoblotting with monoclonal antibody SY38 (lane 2) and polyspecific anti-synaptin antibody (lane 3).

3.2. *Two-dimensional immunoblot analysis of chromaffin granules*

In membranes of chromaffin granules from bovine adrenal medulla anti-synaptin and anti-synaptophysin antibodies recognized the same 41 kDa identical component. The component is a heterogeneous band ranging in p*I* from 4.8 to 5.8 as seen in fig.2. When two blots corresponding to those shown in fig.2 were incubated first with one antibody and subsequently with the other antibody, i.e. anti-synaptin followed by anti-synaptophysin and vice versa, the same components were more intensively stained (not shown). The anti-synaptin antibody reacts with a number of other spots due to its polyspecificity.

3.3. *Western immunoblot analysis of synaptin immunoprecipitate*

To prove conclusively that anti-synaptin and SY38 antibodies recognize identical molecules a synaptin immunoprecipitate was isolated from a crossed immunoelectrophoresis. The SY38 antibody identified and bound to the synaptin antigen with an M_r of 38000 in a Western immunoblot analysis of the isolated synaptin immunoprecipitate as seen in fig.3. The SY38 antibody did in comparison not react with other different antigen immunoprecipitates used as con-

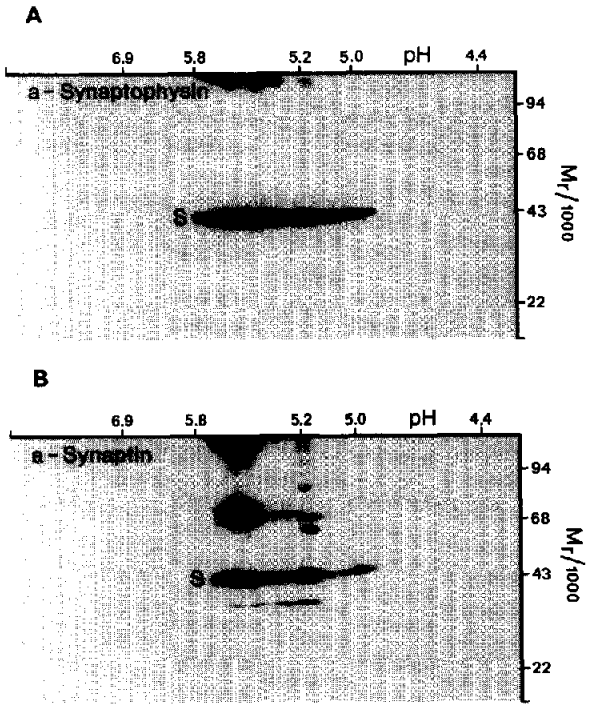

Fig.2. Two-dimensional immunoblot analysis of chromaffin granules. Membranes of chromaffin granules (300 μg protein) were subjected to two-dimensional electrophoresis followed by immunoblotting with anti-synaptophysin (A) or anti-synaptin (B). Synaptin/synaptophysin components are labelled S.

Fig.3. Western immunoblot analysis of synaptin immunoprecipitate. Synaptin immunoprecipitate was subjected to one-dimensional electrophoresis followed by immunoblotting with monoclonal antibody SY38. Synaptin antigen (S) is marked by an arrowhead.

trols (not shown). These results show that anti-synaptin and SY38 antibodies identify the same molecular species.

4. DISCUSSION

Our results show that synaptin and synaptophysin/p38 are immunochemically identical proteins. The discrepancies in the M_r and pI values given in various reports are probably due to variations in glycosylation of the protein [11,12]. The exact protein sequence data are available through the recent cloning and sequencing of synaptophysin/p38 [18–20]. The data indicate a 307 amino acid protein with four transmembrane domains predicted and a single *N*-glycosylation site located inside the vesicle.

Synaptin has previously been shown to be a constituent in membranes of chromaffin granules [5]. In this study also synaptophysin/p38 is shown to be present in membranes of chromaffin granules. Some groups have been unable to demonstrate synaptophysin/p38 in chromaffin granule membranes [12,14]. Other groups have recently shown synaptophysin/p38 to be a constituent of chromaffin granules using more sensitive methods [17,21,22].

It should be noted that a 38 kDa integral membrane protein called SVP38 has been isolated from synaptic vesicles of guinea pig [23]. This protein shows several similarities with synaptin/synaptophysin/p38 and is probably the same.

Since a protein is normally designated by its original name, if it is reasonably descriptive, we suggest that hereafter synaptophysin/p38 (and probably also SVP38) be termed synaptin.

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