S-100 Protein in Cerebral Cortex Synaptosomes

S-100 protein has been shown to be specific to the nervous system[1] and to conserve its immunological identity throughout phylogenesis[2-4]. It is generally assumed to be a soluble protein of the glia[5-8]. Evidence is now available that the protein is also a neuronal one, and that it flows along the axon[9].

In order to gain further information on neuronal S-100, the experiments to be described deal with the localization of S-100 protein in the synaptosomal subfractions of guinea-pig cerebral cortex. The results indicate that synaptosomes do contain S-100 protein, especially in their soluble fraction.

Materials and methods. Synaptosomal subfractions from the cerebral cortex of guinea-pig, weighing 200–250 g, were obtained according to the methods of WHITTAKER et al.[10] and WHITTAKER and SHERIDAN[11], except that the crude mitochondrial fraction (pellet P_2) was washed twice before hypo-osmotic shock.

S-100 protein was measured immunologically by complement fixation assay by the method of MOORE and PEREZ[12], with an incubation time of 19–20 h at 2–4 °C. Samples with complement alone, and with complement and antiserum, were assayed to test the anticomplementary activity of the antigens and antiserum. S-100 protein was obtained from ox brain using the method of MOORE[1]. The antiserum was obtained and characterized according to the method of ZUCKERMAN et al.[13].

The soluble proteins of synaptosomes were separated by disc electrophoresis by the methods of ORNSTEIN[14] and DAVIS[15]. The S-100 in the gel was immunologically recognized, as indicated in Figure 1. When required, ^{125}I-S-100 was used. The iodination of S-100 was carried out electrochemically using the method of ROSA et al.[16]. ^{125}I-S-100 conserves the immunological and electrophoretic properties of the native S-100[17]. Protein was determined by the method of LOWRY et al.[18].

Results and discussion. S-100 protein is present in all the subfractions of synaptosomes: as shown in Table I,

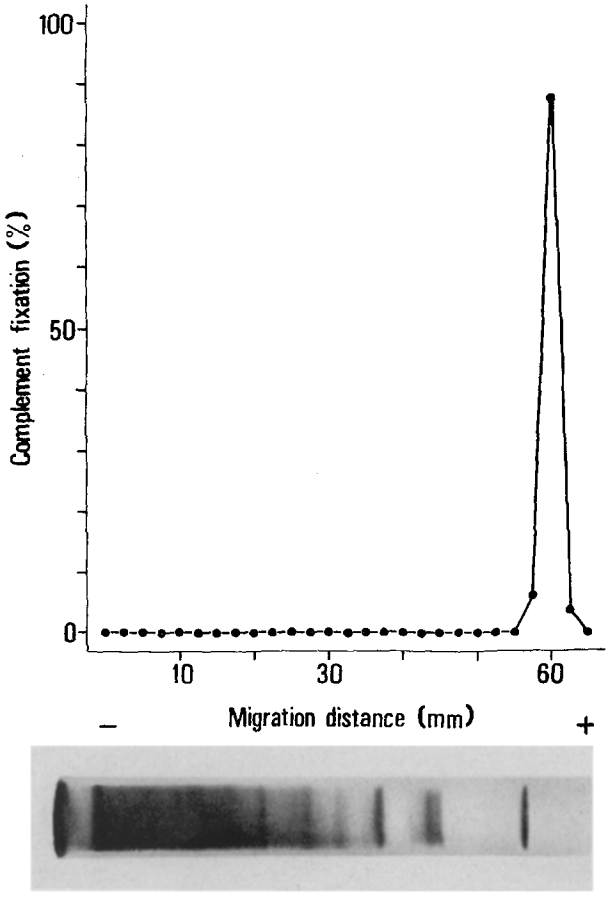

Fig. 1. Polyacrylamide-gel (7.5%) electrophoretic pattern of the soluble fraction of synaptosomes from guinea-pig cerebral cortex (500 µg protein). An unstained gel was sliced into 2 mm sections. Each gel slice was homogenized in a hand-operated homogenizer in 0.5 ml distilled water, and the proteins were allowed to diffuse by gently stirring overnight at 2–4 °C. An aliquot of each solution was taken for complement fixation against anti-S-100 antiserum. The graph shows the positive immunological reaction in the band moving with the front (S-100 protein).

Table I. Distribution of S-100 protein in subfractions of synaptosomes and in cytosol (150,000 g supernatant) from guinea-pig cerebral cortex

Fraction	S-100/g cerebral cortex (µg)	S-100/mg protein (µg)
O (soluble)	24.89 ± 5.79	8.14 ± 0.85
D (vesicular)	1.93 ± 0.43	2.64 ± 0.61
E (microsomal)	0.71 ± 0.19	1.36 ± 0.25
F (membranous)	0.34 ± 0.17	0.97 ± 0.37
G (membranous)	0.48 ± 0.17	0.80 ± 0.29
H (heterogeneous)	0.64 ± 0.18	1.30 ± 0.54
cytosol	177.97 ± 45.79	5.39 ± 1.18

For identification of subfractions (O, D, E, F, G, H) see WHITTAKER et al.[10]. The values are the mean of 6 experiments in triplicate ± S.D.

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the specific activity as well as the relative amount of S-100 is maximal in the soluble fraction of synaptosomes (O) followed by the vesicular (D), microsomal (E), and membranous fractions (F and G) in that order. The relatively high values of S-100 in fraction H are probably accounted for by the heterogeneity of the fraction (membranous fragments, intact and partially disrupted synaptosomes).

The presence of S-100 protein in fraction O is confirmed by gel-acrylamide electrophoresis (Figure 1). The fast-moving band of the gel cross-reacts by complement fixation with the anti-S-100 antiserum.

Table II. Contamination of the synaptosomal subfractions from the cytosol-S-100 protein during the preparative procedure as revealed by adding ^{125}I-S-100 to the homogenizing medium, and percentage distribution of native S-100 in the same subfractions.

Fraction	Percentage distribution	
	^{125}I-S-100	Native S-100
cytosol	100[a]	100 ± 25.73
O	0.35	13.98 ± 3.24
D	0.05	1.08 ± 0.24
E	0.03	0.40 ± 0.10
F	0.04	0.19 ± 0.09
G	0.04	0.27 ± 0.09
H	0.04	0.39 ± 0.10

The figures of column 2 were calculated on figures of column 1, Table I. [a] ^{125}I-S-100 in the homogenizing medium was 1.28×10^6 cpm/g guinea-pig cerebral cortex.

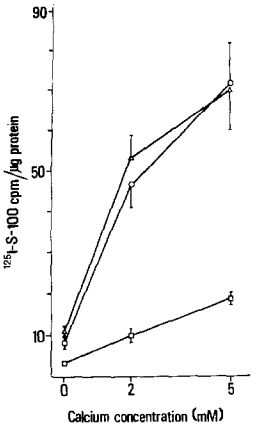

Fig. 2. In vitro interaction of ^{125}I-S-100 protein with vesicular (○, △) and membranous (□) fractions of synaptosomes from guinea-pig cerebral cortex in absence or in presence of $CaCl_2$. For identification of the subfractions see WHITTAKER and SHERIDAN[11]. Aliquots (1 ml) of resuspended particulates in 0.32 *M* sucrose were incubated at 37 °C for 30 min in a Dubnoff incubator at moderate shaking in a final volume of 2 ml containing 0.32 *M* sucrose, 5 m*M* *Tris*-HCl, various concentrations of $CaCl_2$, pH 7.4 (reaction medium), and ^{125}I-S-100 (about 270,000 cpm, specific activity 1 ± 0.3 mCi/mg). About 175 µg protein of intact (○—○) or sonicated (△—△) synaptic vesicles (MSE, Ultrasonic Power Unit; sonication time: 2 min at 1.0 Amp at 0–2°) and 650 µg protein of the membranous fraction (□—□) were used. The reaction was stopped in an ice-bath by adding 5 ml of the reaction medium and centrifuging at 150,000 *g* for 30 min. The pellets were washed twice and the radioactivity was measured by a Nuclear Chicago gamma scintillation counter. Controls without synaptosomal particulates were run: no precipitation of ^{125}I-S-100 was observed in the experimental conditions.

To make sure that the S-100 protein recovered in the synaptosomal fractions is not the result of contamination from the cerebral cortex cytosol during the preparative procedure, ^{125}I-S-100 protein was used as a marker of the cytosol. When ^{125}I-S-100 was added to the homogenizing medium, insignificant amounts of it were recovered in the synaptosomal fractions (Table II).

Two data in Table I seem to be worth consideration and comment: 1. the specific activity of fraction O is significantly higher ($2\ P < 0.001$) than that of the cerebral cortex cytosol (150,000 *g* supernatant); 2. the vesicular fraction, among the synaptosomal particulates, shows the maximal specific activity. Point 1. strongly suggests that synaptosomes represent a compartment where S-100 is concentrated. This storage could result from the arrival of the protein from the perikaryon by means of axonal transport[9], although a transfer of S-100 from the perisynaptic glial cells or an in loco synthesis cannot be excluded.

As to the S-100 protein recovered in the vesicular fraction, the risk of contamination from the soluble fraction O is reasonable. This notwithstanding, the possibility that S-100 protein may interact with synaptic vesicles and other synaptosomal particulates has been tested in vitro by means of radioiodinated S-100. Figure 2 shows that binding occurs with synaptic vesicles to a greater extent than with synaptosomal membranes. Ca^{2+} or Mg^{2+} are required for the interaction to take place at a significant rate. On the other hand, EDTA (0.5 m*M*) further lowers the values for each fraction below those observed in absence of Ca^{2+} or Mg^{2+}. Since both the intact and sonicated vesicles interact with the protein to the same extent, the integrity of the vesicles is not a condition necessary to the binding.

The possibility of a membrane-bound fraction of S-100 protein has recently been investigated by RUSCA et al.[19] and HAGLID and STAVROU[20]. In particular, RUSCA et al. found that S-100 protein is n-pentanol extractable from rat brain synaptosomes previously submitted to hypo-osmotic shock. The relatively high affinity of ^{125}I-S-100 for synaptic vesicles in our experiments suggests that among the synaptosomal particulates the vesicles could play an important role in the biological function of neuronal S-100 protein. Analyses in this direction are in progress in our laboratory.

Riassunto. I sinaptosomi di corteccia cerebrale di cavia contengono una notevole quantità di S-100 (proteina acida specifica del sistema nervoso). La S-100 sinaptosomale è più concentrata nella frazione solubile del sinaptosoma che nel neuroplasma della corteccia. Le vescicole sinaptiche e le membrane sinaptosomali legano ^{125}I-S-100 in opportune condizioni. È possibile che il terminale sinaptico abbia importanza ai fini della funzione biologica della S-100.

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