DEMONSTRATION OF SUBUNITS IN BEEF BRAIN ACIDIC PROTEIN (S-100)*

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Received August 26, 1969

SUMMARY

S-100 protein, purified from beef brain, has a molecular weight of 21,300 when measured by high speed sedimentation equilibrium and 7,000 when measured by acrylamide electrophoresis in SDS. Gel filtration through Sepharose 6B in the presence of 6 M guanidine hydrochloride and 0.1 M β-mercaptoethanol also demonstrated the presence of subunits in S-100 protein.

The biological function for the S-100 protein, first detected and isolated by Moore and McGregor (1965), is not known. However, the protein has created interest because of the following unusual properties: 1) The protein is unique to brain when measured by electrophoretic and serologic techniques (Moore and McGregor, 1965; Moore and Perez, 1968); 2) Its conformation, when measured by serologic methods, has been conserved during evolution; i.e., aqueous extracts of mammalian, avian, amphibian, and even piscine brains react effectively with antibody to bovine S-100 protein (Moore and Perez, 1968; Kessler et al., 1968); 3) Its appearance in the

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*Publication No._____from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. Supported in part by research grants from the National Institutes of Health (AI-01940) and the American Cancer Society (E-222). L.L. is an American Cancer Society Professor of Biochemistry (Award No. PRP-21)

†P. S. Dannies is a predoctoral trainee supported by Training Grant No. 5241 from the National Institute of Neurological Diseases and Blindness.

frontal cortex of the developing human brain parallels the onset of electrical activity in the cortex (Zuckerman et al; 1969); 4) S-100 protein is highly acidic: 30% of its total amino acid content is glutamic and aspartic acid residues (Moore and McGregor, 1965).

It is present in glial cells (Benda et al, 1968; Hyden and McEwen, 1965) and also in neurons (Hyden and McEwen, 1965; Moore and Perez, 1968). Antiserum to S-100 protein affects the morphology of mollusc neurons as well as transmission of nerve impulses (DeRobertis, 1967). S-100 protein has some unusual conformational properties; it is thermolabile, but its conformation is stabilized by EDTA, Ca^{++}, or β-mercaptoethanol (Kessler et al., 1968). S-100 is a relatively small protein of 20,000-25,000 molecular weight as measured by gel filtration (Moore and Perez, 1968). We present data demonstrating that it is composed of subunits, most probably three.

S-100 protein was purified from beef brain by a modification of the method of Moore (1965). After precipitation at pH 4 from a neutral saturated ammonium sulfate solution, the protein was eluted from a DEAE Sephadex column with a linear gradient of 0.05 M NaCl to 0.6 M NaCl in 0.01 M potassium phosphate buffer, at pH 7.0 with 0.001 M EDTA and 0.001 M β-mercaptoethanol (ME). The presence of S-100 protein was determined serologically by gel diffusion and the concentrated fractions containing S-100 were applied to a Sephadex G-100 column and eluted with 0.1 M potassium phosphate buffer, pH 7.0, 0.4 M $(NH_4)_2SO_4$, 0.001 M EDTA and 0.001 M ME.

S-100 protein purified by this method gives one band when analyzed by the disc gel electrophoresis technique of Davis (1964). Equilibrium sedimentation also indicates that the

protein is homogeneous. High speed equilibrium centrifugation (Yphantis, 1964) of 0.5 mg of S-100 in 0.1 M potassium phosphate, pH 7.0, with 0.001 M ME was used to determine the molecular weight of the native protein. The ME prevents aggregation that occurs with this protein. A least squares analysis of the data (Fig. 1) gives a molecular weight of 21,300. We have found by gel filtration through Sephadex G-100 that S-100 protein elutes before ribonuclease and after egg albumin.

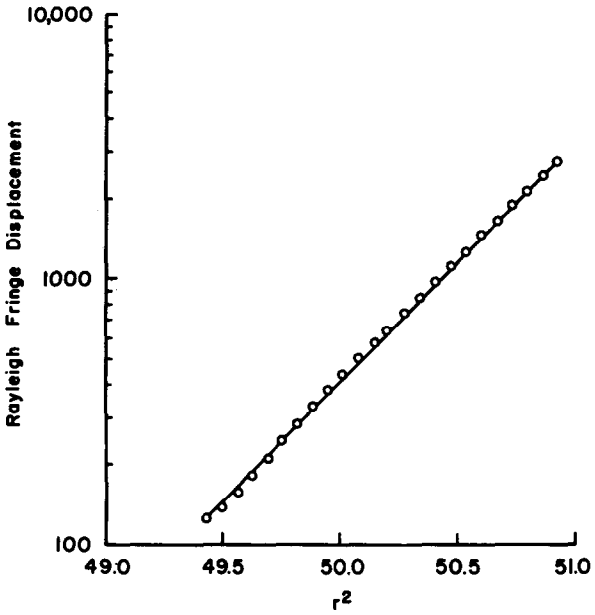

Figure 1. High speed sedimentation equilibrium of 0.5 mg S-100 protein in 0.1 M KHP, pH 7.0, and 0.001 M ME at 40,000 rpm and at 25°C. A 77A Watten filter and GII spectroscopic plates were used with the camera focused on the midplane of the cell.

Electrophoresis with polyacrylamide gels in the presence of SDS which has been used to determine molecular weights of polypeptide chains or subunits of proteins (Shapiro et al, (1967), was used to determine the molecular weight of S-100. The data in Fig. 2 show the relationship of molecular weight

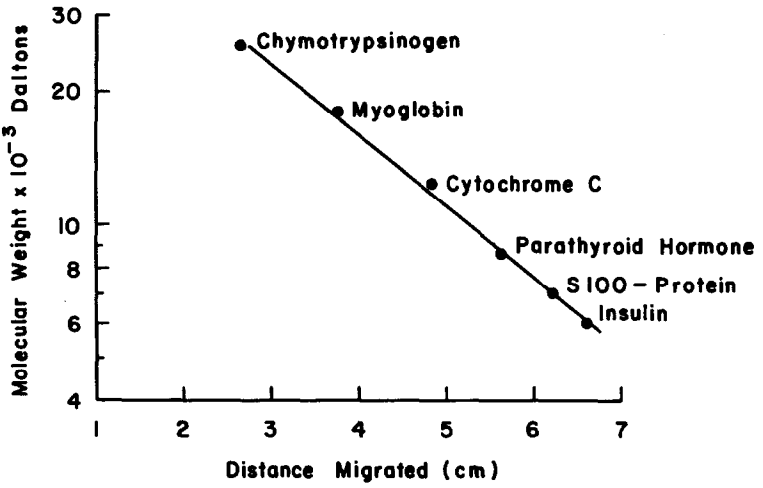

Figure 2. Estimation of molecular weight of S-100 protein in 0.1% SDS polyacrylamide gels. Ten mgs/ml of each protein were incubated in 0.1 M sodium phosphate buffer, pH 7.0, with 2% SDS and 0.1% ME. After incubation overnight 2λ of the solution were layered on to 12½% acrylamide gels, with bromphenol blue as the marker dye. The molecular weight marker proteins were beef pancreas chymotrypsinogen, horse heart cytochrome C, sperm whale myoglobin, beef insulin, and parathyroid hormone. The insulin was incubated with SDS but in the absence of ME since ME would have cleaved the disulfide bridge. The tray buffer was 0.1 M sodium phosphate buffer, pH 7.0, and 0.1% SDS.

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to distance traveled. S-100 protein migrates to a distance corresponding to a molecular weight of 7,000. These results suggest that S-100 protein has a native form with a molecular weight of about 21,000 and is composed of 3 subunits of 7,000 molecular weight.

Molecular weight estimation of denatured polypeptides by agarose filtration in 6 M guanidine hydrochloride and 0.1 M ME (Fish, et al, 1969), was used to confirm the presence of subunits in S-100. The S-100 protein was eluted from the column (Fig. 3) in a position between ribonuclease (13,500) and insulin (3,000).

When measured by gel filtration using 6 M guanidine hy-

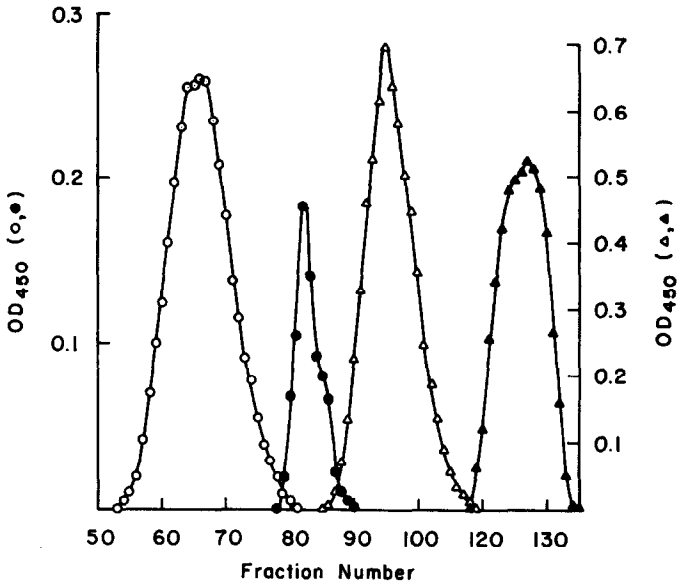

Figure 3. Elution of proteins from a Sepharose 6B column equilibrated with 6 M guanidine hydrochloride and 0.1 M ME. Ten mgs of protein were incubated overnight in 0.5 ml 6 M guanidine hydrochloride and 0.1 M ME (pH 8.6) and then applied to the column, and eluted with 6 M guanidine hydrochloride and 0.1 M ME. Egg albumin, ribonuclease and insulin were used as marker proteins. The insulin in this case was reduced to the two polypeptide chains. The fractions were assayed for protein by measuring turbidity as described by Fish et al (1969). The column was 48 cm x 1.5 cm; the flow rate was 12 ml/hr; the fraction volume was 0.8 ml. (○) ovalbumin; (●) ribonuclease; (△)S-100 protein; (▲)insulin polypeptide chains.

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drochloride and ME and by SDS-acrylamide electrophoresis, S-100 protein appears to consist of subunits. Noncovalent bonds appear to stabilize the 21,000 molecular weight protein, since the migration of S-100 protein when subjected to SDS acrylamide electrophoresis without ME also showed the presence of subunits.

Acknowledgements

We wish to thank Dr. Armen Tashjian for the parathyroid hormone and Dr. Richard Weisenberg for advice in the measurement of the molecular weight by high speed sedimentation equilibrium.

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