A SOLUBLE PROTEIN CHARACTERISTIC OF THE NERVOUS SYSTEM*

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"Protein maps" were made of the soluble proteins extracted from brain and liver in order to find any proteins unique to the nervous system. The maps, which were prepared by chromatographing the proteins on DEAE-cellulose columns followed by starch gel electrophoresis of the individual fractions, gave 70 to 100 separate protein bands on the stained gels for brain or liver (Moore and McGregor, 1965). One protein band in particular, which moved faster on starch gel electrophoresis than any of the other proteins, was found in brain patterns but was absent in those of liver. This report describes the preparation of this protein in pure form from three species and some of its properties. Because it was soluble in saturated ammonium sulfate solution, it was named the "S-100" protein.

Methods

Homogenates of brain and other organs were prepared in 5m M tris phosphate buffer, pH 7.2. DEAE-cellulose columns were run, and starch gel electrophoresis was done as already described (Moore and McGregor, 1965). The G-200 Sephadex column (2.2 X 200cm.) used during the purification (Table I) was eluted with 0.1M potassium phosphate pH 7.1, by pumping

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at a rate of 8 ml per hour with a peristaltic pump. Columns of DEAE-Sephadex (Grade A-50) were eluted with a parabolic (3 chambered) gradient from 0.1M potassium phosphate pH 7.1, as the starting buffer to 2M sodium chloride, 0.1M potassium phosphate pH 7.1 as the limit.

The "S-100" protein was quantitated in extracts and in fractions during purification by the following method based on starch gel electrophoresis, since it moved well ahead of other proteins on the gels with no overlap. After electrophoresis the gels were stained with Amido Black and destained in an electrophoretic destainer in the usual manner, the "S-100" band was cut out, and then the dye was eluted with 0.2N NaOH in a tube for 20 hours. The amount of "S-100" protein in the fraction was estimated by comparing the absorbance of the extracted dye solution from it, with that from standards of pure "S-100" run on the same gel. There was a linear relation between amount of dye and amount of "S-100" protein.

Results and Discussion

The general method of purification is shown in Table I. The protein was partially soluble in saturated ammonium sulfate at pH 7.1 but it was essentially completely precipitated by saturated ammonium sulfate at pH 4.2. Recoveries during purification are shown also in Table I. About 0.6 percent of the total soluble protein of beef brain was "S-100" protein. The same method was used to prepare the protein from hog or rabbit brains. All three preparations were pure as judged by starch gel electrophoresis at several pH values between 6.5 and 9, and by appearance of single peaks when chromatographed on DEAE-Sephadex, G-100 Sephadex, and hydroxylapatite columns.

The "S-100" protein could be seen on starch gel patterns when brain extracts were concentrated to 20-30 mg per ml and run directly without previous chromatography (Fig. 1). By starch gel electrophoresis the "S-100" protein was found in brains of all species examined: beef, hog,

TABLE I

Preparation of S-100 Protein
from 1500 g. Wet Weight of Beef Brain

	Grams Protein		
	Total Protein*	S-100+	% recovery
Soluble fraction	25.3	0.153	100
Sat'd $(NH_4)_2$ SO_4, pH7 + 4.2	4.8	0.139	91
DEAE-cellulose chromatography	0.36	0.098	64
G-200 Sephadex chromatography	0.167	0.092	60
DEAE-Sephadex chromatography	0.090	0.090	59

* Assayed by the Lowry method
\+ Assayed by elution from starch gel as described in Methods.

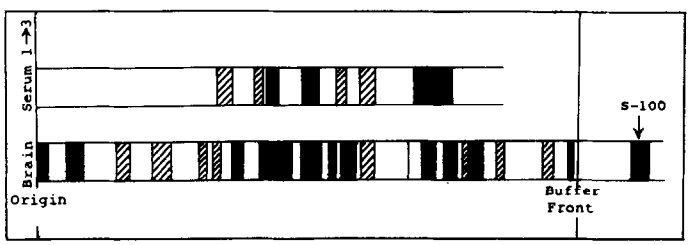

Figure 1. Starch gel electrophoretic patterns of rabbit brain extract and rabbit serum. The electrophoresis was done by the discontinuous method of Poulik (1957). The S-100 protein moves ahead of the borate buffer front in this system.

rat, rabbit, hamster, guinea pig, mouse, dog, monkey, human, turkey, eagle, alligator, black snake, turtle, pompano, and red snapper. The mobilities varied slightly but significantly from species to species. It was not present in liver of any species examined, specifically beef, rabbit, rat, monkey, and mouse; and it was not found in any other than

nervous tissue in the rat, specifically liver, kidney, heart, skeletal muscle, lung, serum, and red cells. When the pure protein from brain was mixed with extracts from these organs it was quantitatively recovered from starch gel electrophoresis, indicating that it was not destroyed or bound by some component there. It was found in several parts of the nervous system of the rabbit: whole brain, both grey and white matter, cerebrum, cerebellum, brain stem, spinal cord, sciatic nerve, and vagus. It was also found in retina from beef and in sciatic nerve and spinal cord of rat.

The molecular weight of the protein was about 30,000 as estimated by the method of Whitaker (1963) when it was chromatographed on a 1 X 200 cm G-100 Sephadex column. The protein contained no phosphorus, carbohydrate, or lipid. Samples of the pure beef and rabbit proteins were hydrolyzed 24 hours in 6N HCl at 112° under N_2 and the hydrolyzates subjected to amino acid analysis. The results are shown in Table II[1]. The protein was also assayed for amide nitrogen by the

TABLE II

Amino Acid Analyses of S-100 Proteins
Moles/30,000g*

	Beef	Rabbit		Beef	Rabbit
Ala	15	15	Lys	20	23
Arg	3	4	Phe	15	18
Asp	26	24	Pro	3	1
Cys	5	4	Ser	8	7
Glu	44	50	Thr	7	7
Gly	13	14	Try+	0	0
His	9	10	Tyr++	5	3
Isoleu	8	8	Val	33	34
Leu	21	20	Amide	13	not det.

* to the nearest integer.
\+ measured by method of Spies and Chambers (1949).
++ measured by spectrophotometry

1 The author is indebted to Dr. A.L. Rubin of the Cornell Medical School, New York City, for performing the amino acid analyses.

method of Leach and Parkhill (1955). As shown in Table II the "S-100" protein contained a high content of free carboxyl groups, a fact which would explain its high mobility by starch gel electrophoresis. There was no tryptophan by the method of Spies and Chambers (1949). Titration of sulfhydryl groups in the beef protein by the method of Boyer (1954) gave a value of one - SH per 11,000 molecular weight both in the absence and presence of 8M urea.

Antiserum to the beef "S-100" protein was prepared using rabbits[2]. The rabbit anti-beef "S-100" cross-reacted with the pure protein from rabbit and hog, by the precipitin method and by micro complement fixation (Wasserman and Levine, 1961), and with rat brain extract by complement fixation. Extracts of various organs of the rat were made as described under Methods and tested for "S-100" protein by complement fixation as shown in Table III. It can be seen that brain extract

TABLE III

C'-fixation by Dilutions of Rat Organ Extracts plus Rabbit Anti-Beef-S-100*

Dil'ns	Brain	Liver	Kidney	Heart	Spleen	Muscle	Lung
undil.	+	+	+	-	-	+	-
10	++	-	-	-	-	±	-
30	++	-	-	-	-	-	-
100	++	-	-	-	-	-	-
300	++	-	-	-	-	-	-
1000	++	-	-	-	-	-	-
3000	++	-	-	-	-	-	-
10,000	±	-	-	-	-	-	-
30,000	-	-	-	-	-	-	-

* Method of Wasserman and Levine (1961)

[2] The author is indebted to Dr. L. Levin of Brandeis University, Boston, for preparing the antiserum to the beef brain protein.

contained 1000 to 10,000 times as much "S-100" as other organ extracts except for muscle, whose slight "S-100" content could be explained by its innervation.

The following facts suggested that the "S-100" protein may have a role in some of the unique functions of the nervous system: (1) it was markedly different from other soluble proteins extracted from tissues in its highly polar and acidic nature, its high electrophoretic mobility on starch gel, and its solubility in strong salt solutions; (2) it was found in all parts of the nervous system, peripheral and central; (3) it was found in brains of all species examined; (4) it was absent from any organs other than the nervous system. On the basis of its distribution it seems probable that it is a neuronal protein, and that it is not part of the myelin sheath structure.

REFERENCES

Boyer, P.D., J. Am. Chem. Soc., 76, 4331 (1954).
Leach, S.J. and Parkhill, E.M.J., Proc. Intern. Wool Text. Research Conf. Australia, C 92 (1955).
Moore, B.W. and McGregor, D., J. Biol. Chem., 240, 1647 (1965).
Poulik, M.D., Nature, 180, 1477 (1957).
Spies, J.R. and Chambers, D.C., Anal. Chem., 21, 1249 (1949).
Wasserman, E. and Levine, L., J. Immunol., 87, 290 (1961).
Whitaker, J.R., Anal. Chem., 35, 1950 (1963).