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S-100 PROTEIN SYNTHESIS BY ISOLATED RAT BRAIN POLYRIBOSOMES

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**S-100 Protein Synthesis by Isolated Rat
Brain Polyribosomes.**

Abstract

The radioactive proteins synthesized in a cell-free system and released from rat brain polyribosomes were analyzed for the presence of a brain specific protein. Rat liver polysomes were used to demonstrate the organ specificity of the radioactive product synthesized in vitro. S-100 protein was 0.15% of the radioactive proteins released from brain polyribosomes.

S-100 protein, an acidic soluble protein is unique to the nervous system (1). Although its role in neural function is not yet clear, it provides an organ-specific marker to study macromolecular synthesis in in vivo and in vitro neural systems. Early in vivo labeling studies of rat brain suggested that S-100 has very high rates of synthesis and degradation (2). However, a careful reexamination of S-100 protein turnover rates in whole rat brain indicated that the degradation of this protein in vivo occurs at about the same rate as total soluble protein (3). The degradation rates of S-100 and total protein were also quite similar in clonal lines of glial cells in culture. The differential rate of S-100 synthesis in culture was only a fraction of one percent of total protein synthesis (4). Earlier studies using microsomal or ribosomal preparations from rabbit brain indicated that 15% of the labeled, released protein could be precipitated with anti-S-100 antiserum (5). Because of these conflicting reports concerning synthesis and degradation, we decided to reinvestigate S-100 synthesis in cell-free preparations using rat brain polysomes. Our results indicate that only a small percentage of the ribosomal released protein is S-100 protein.

Brains and livers from Sprague-Dawley rats were removed into ice-cold 0.25 M sucrose - NH_4Cl buffer (0.1 M NH_4Cl , 0.03 M Tris-HCl, pH 7.4, 0.005 M MgCl_2 , 0.005 M B-ME (6). Both tissues were minced, rinsed several times, and homogenized with 8-10 strokes of a teflon-glass homogenizer. The homogenate was centrifuged for 10 minutes at 27,000 x g. The resulting solution was centrifuged for 90 minutes at 195,000 x g. The supernatant solution from the second centrifugation was passed over a G-25 Sephadex column equilibrated with NH_4Cl buffer. This High Speed Supernatant (HSS) was used as a source of enzymes and t-RNA. To prepare polysomes, the 27,000 x g pellet was rehomogenized

and centrifuged. The resulting supernatant solution was combined with the pelleted polysomes from the 195,000 x g centrifugation. After resuspension the polysome solutions were treated with a mixture of DOC-Tween 40: final concentration was 1.0% DOC - 1% Tween for brain, and 1.3% DOC-1% Tween for liver. The detergent treated polysomes were kept at 4°C for 10 minutes, and then centrifuged at 27,000 x g for 10 min. The supernatant solutions were then layered over 0.5M - 2.0M discontinuous sucrose gradients (in NH₄Cl buffer) and centrifuged at 4°C for 24 hrs at 130,000 x g. After centrifugation the polysome pellets were rinsed with 0.25 M sucrose - NH₄Cl buffer, resuspended by light homogenization in the same buffer, and centrifuged at 1,000 x g for 10 minutes. The supernatant polysomes were stored at -70°C until use.

The cell-free protein synthesis reaction (1.0 ml) contained: 100 μ moles NH₄Cl, 30 μ moles Tris-HCl, pH 7.4, 5 μ moles MgCl₂, 5 μ moles B-ME, 1 μ mole ATP, 1 μ mole GTP, 10 μ moles creatine phosphate, 4 enzyme units creatine phosphokinase (Sigma Chemical Co.), 20 mM moles each of a mixture of 15 amino acids and 20 μc each (7) of ³H leucine, ³H lysine, ³H phenylalanine, and ³H valine. In addition, each reaction contained 6 OD₂₆₀ units of polyribosomes and 1.2 OD₂₈₀ units of HSS. Reactions were incubated for 60 minutes at 37°C. After incubation the reactions were chilled to 4°C and centrifuged for 2 hours at 280,000 x g to separate the released proteins from the polyribosomes.

The amount of S-100 released from polyribosomes was determined by a modification of the method of Herschman (4). Suitable aliquots of released protein were passed over Sephadex G-15 columns (equilibrated in PBS - 0.01 M B-ME) to remove unincorporated amino acids and NH₄Cl. The excluded volume,

as determined by dextran blue, was concentrated to 2.0 ml. with a Diaflow UM-2 membrane (MW exclusion - 1,000; Amicon Corp). Samples were heated to 60°C for 10 minutes, then centrifuged for 10 minutes at 27,000 x g. The supernatants were passed over Sephadex G-25 in PBS. The antibody precipitation and SDS polyacrylamide gel separation of S-100 protein has been described (4). Gels were cut into 1 mm slices and counted in a Nuclear-Chicago Iso-cap 300 counter.

A profile of the radioactivity in the polyacrylamide gels of S-100-anti S-100 precipitates prepared from the protein released from rat brain and liver polyribosomes is shown in Figure 1. The fastest moving protein in the brain sample contained the labeled S-100 protein. The liver sample also contained a small amount of radioactivity in the fastest migrating peak. This radioactivity probably represents proteins trapped in the antibody-antigen complex, and released during treatment with SDS. The fact that the liver sample did contain some radioactivity in the area of the S-100 protein emphasizes the importance of using non-neural tissues as controls. The radioactive profile of brain polyribosomal proteins precipitated with anti-S-100 antibody is quite similar to those obtained from rat glial tissue culture cells (4).

Table 1 shows the results of two experiments designed to measure polyribosomal released S-100 protein. Both experiments started with 5-10 ml of polyribosomal released protein containing about 800,000 CPM. After Sephadex G-15 chromatography and Diaflow concentration, there were about 300,000 CPM per sample. Heating the samples to 60°C removed about one-third of the brain protein and two-thirds of the liver protein. S-100 protein, in the presence of B-ME, is not denatured by the heat step. In these experiments

0.15% of the polysomal released protein S-100.

In whole rat brain S-100 reaches adult levels by 25 days of age (8). Using complement fixation we have found that in adult rats about 0.5-0.8% of the soluble protein of brain is S-100 protein. A relatively low rate of S-100 synthesis is suggested by the amount of this protein present in rat brain and the modest degradation rate recently reported (3). Unlike previous reports which proposed that a large percentage of brain polyribosomal synthesized protein is S-100 (5), our data indicates that S-100 synthesis comprises only a small percentage of the total released protein synthesized on rat brain polyribosomes.

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6. The abbreviations used are: B-ME, B-mercaptoethanol; HSS, High Speed Supernatant; DOC, sodium deoxycholate; PBS, phosphate buffered saline; 0.15 NaCl, 0.01 M PO_4 , pH 7.0; SDS, sodium lauryl sulfate; CPM, counts per minute; S.A., Specific activity.
7. The tritiated amino acids, obtained from Schwarz Bioreserach were: (4,5- ^3H) leucine, S.A., 51 c/m mole; (2,3- ^3H) lysine, S.A., 42 c/m mole; (2,3- ^3H) valine, S.A. 17 c/m mole; (^3H) phenylalanine, S.A., 6.5 c/m mole.
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11. Supported by NSF grant GB-30518 and in part by contract AT (04-1) GEN 12 between the U.S. Atomic Energy Commission and the University of California. MPL was supported in part by NIH postdoctoral fellowship 1 F02 NS 45571-02.

Table 1. S-100 in rat brain and liver polysome released protein. Polyribosomes were isolated from 35 day old animals, and the amount of S-100 was determined as described in the text. The radioactivity in proteins was determined by the method of Mans and Novelli (9). Counting efficiencies ranged between 33-40% for all samples.

<u>Sample</u>	<u>Experiment 1-CPM</u>		<u>Experiment 2-CPM</u>	
	<u>Brain</u>	<u>Liver</u>	<u>Brain</u>	<u>Liver</u>
Polysome-free supernatant	840,000	860,000	700,000	680,000
After Sephadex G-15	595,000	580,000	560,000	540,000
After Diaflow Concentration	350,000	350,000	325,000	300,000
After heating and centrifuging	220,000	95,000	250,000	100,000
After Sephadex G-25	215,000	95,000	220,000	100,000
S-100 gel fractions	1,890	600	1,170	130
Brain Specific S-100	1,290	--	1,040	--
(Brain CPM-Liver CPM)				
Percent S-100 ($\frac{\text{CPM S-100}}{\text{CPM polysome-free supernatant}}$)	0.15	--	0.15	--

Figure Legend

Fig. 1. Polyacrylamide gel patterns of S-100 anti-S-100 precipitates prepared from proteins released from rat brain and liver polyribosomes.

The proteins released from polyribosomes from 35 day old animals were analysed as described in the text and as shown in Experiment 1, Table 1.

The solublized antigen-antibody precipitates were applied at slice number 1.

Slices 23-32 contain S-100 protein as determined by Comassie brilliant blue staining. Open circles, brain; solid circles, liver.