We have purified S-100 protein from bovine brain using Casup+ dependent affinity chromatography on N-(6-aminohexyl)-5-chloro-l-naphthalenesulfonamide (W-7)-Sepharose (Endo, T., Tanaka, T., Isobe, T., Kasai, H., Okuyama, T., and Hidaka, H. (1981) J. Biol. Chem. 256, 12485-12489). By essentially the same procedure, W-7-Sepharose binding protein has been purified to apparent homogeneity from bovine adipose tissue. Electrophoretically, the purified protein from adipose tissue co-migrated with brain S-100 protein both in the presence and absence of sodium dodecyl sulfate and the protein was indistinguishable from brain S-100 region in terms of amino acid composition, two-dimensional tryptic peptide mapping and reactivity with anti-brain S-100b serum. Immunohistochemical analysis confirmed the existence of S-100b protein in the adipose cell where the protein seems to be located in the nucleus and cytoplasm. Thus, the results indicate that the adipose cells contain the protein possibly identical with brain S-100b protein. In addition, the contents of S-100b protein in various rat tissues were measured by enzyme immunoassay method using the anti-bovine brain S-100b serum. Significant amounts of S-100b protein were found not only in the adipose tissue but also in the peripheral tissue such as trachea and skin. These observations suggest that S-100b protein should no longer be considered as a protein specific to nervous tissues.

S-100 protein, first described by Moore (1), is a water-soluble, highly acidic protein with a , ~ 20,000 (2, 3), and has the characteristic feature of an affinity for calcium (4). Although the biological role of S-100 protein remains unclear, it is generally considered to be unique to nervous tissue, where it is found primarily in the cytoplasm and nucleus of glial cells (1, 5). It has been reported that bovine brain S-100 protein is a mixture of two predominant components (S-100a and S-100b), both of which have amino acid sequences that are extensively homologous to the sequence for the calcium-binding sites of parvalbumin, troponin C, and calmodulin (6-8). However, recent findings of S-100 antigen in non-nervous tissue such as adenohypophysis (9), chondrocytes (10), or Langerhans cells of the skin (11) suggested to us the necessity of purifying and characterizing S-100 antigen in non-nervous tissue. We have recently reported that W-7,1 a calmodulin-antagonist (12, 13), coupled to epoxy-activated Sepharose 6B was most useful for purification of bovine brain S-100 protein (S-100a and S-100b) (14). We have also recently described a sensitive enzyme immunoassay method for measurement of S-100 protein in various tissues (15). We now present data showing that significant amounts of S-100b protein do exist in adipose tissue and can be successfully and homogeneously purified by W-7 coupled to epoxy-activated Sepharose 6B column chromatography. In addition, we demonstrate the existence of S-100b protein in various tissues by using a sensitive enzyme immunoassay method for S-100b protein.

**Experimental Procedures**

*Materials—W-7 was synthesized according to the methods of Hidaka et al. (16) or purchased from Rikaken Co., Nagoya, Japan. W-7-coupled epoxy-activated Sepharose 6B (W-7-Sepharose) was prepared by the methods reported previously (14). Na[35I]iodides were obtained from the Radiochemical Centre, Amersham, England. Bovine brain S-100 protein and calmodulin were prepared by Ca++-dependent affinity chromatography on W-7-Sepharose as previously described (14). Bovine brain S-100b protein was separated from S-100a protein by the method of Isobe et al. (6). Antiserum to bovine brain S-100 protein was raised in rabbits by a serial injection of the emulsion of purified S-100b protein and methylated bovine serum albumin (Sigma Chemical Co.) in Freund’s complete adjuvant, as described by Haglid et al. (17). IgG fractions of the rabbit serum were separated by repeated precipitations with ammonium sulfate (50, 40, and 33% saturation). The F(ab’)2 fragments of the antibodies were prepared by digesting the IgG fraction with pepsin (from porcine intestine, Sigma) (18).

*Analytical Procedures—Double immunodiffusion of the purified bovine brain S-100b protein and W-7-Sepharose-binding protein from adipose tissue against anti-bovine brain S-100b serum was performed as previously described (14), on a slide glass covered with 1% agarose containing 0.9% NaCl. Gel electrophoresis was carried out in a 20% polyacrylamide slab gel, in the buffer system of Ornstein and Davis (20), using an electrode buffer containing 0.1% (v/v) thiglycolic acid (6). SDS-polyacrylamide gel electrophoresis was performed in 0.1% SDS, 15% polyacrylamide slab gel as described by Laemmli (21). Tryptic peptide mapping was carried out as described by Tanabe et al. (22). In brief, the stained protein band was cut out from 2% polyacrylamide electrophoresis slab gel and transferred to a glass tube. Then the protein was radioliodinated with [125I] by the chloramine-T method (23). The gel slice was washed and dried, and then 50 μg of trypsin in 0.5 ml of 50 mM NH4HCO3 (pH 8.4) were added to each tube. After incubation for 20 h at 37 °C, the supernatant, which contained most of the tryptic peptide, was lyophilized, and the residue was dissolved in 20 μl of TLE buffer (acetic acid/formic acid/H2O = 15:5:80). A 3-μl portion of this solution was spotted on a silica gel-coated thin layer chromatography plate, and tryptic peptides were resolved by electrophoresis in the first dimension and ascending chromatography in the second dimension. Electrophoresis was carried out at 950 V for 80 min in TLE buffer using a Pharmacia flat bed.
Purification of W-7-Sepharose-binding Protein from Bovine Abdominal Adipose Tissue—W-7-Sepharose-binding protein was purified from 10 kg of bovine abdominal adipose tissue, as was previously described for brain S-100 protein (14) but with the following modifications. After homogenization with a volume of 25 mM Tris-HCl (pH 7.5), 0.15 M NaCl (Buffer B) containing 1 mM EGTA and 0.43 mM phenylmethylsulfonyl fluoride, the homogenate was filtrated through cheese cloth with the aid of a Büchner funnel. The solution was centrifuged at 12,000 × g for 20 min, and the supernatant was applied to a DEAE-Sephadex A-50 column (5 × 6.5 cm) equilibrated with Buffer B containing 1 mM EGTA. After washing the column with 2 liters of Buffer B containing 1 mM EGTA, the elution was carried out with 2 liters of 25 mM Tris-HCl (pH 7.5) containing 1 mM EGTA and 0.5 M NaCl. The eluate was brought to 55% (NH₄)₂SO₄ saturation and the resulting precipitate was removed by centrifugation. The supernatant was then brought to 95% (NH₄)₂SO₄ saturation and the pH was adjusted to 4.2 by slowly adding 2 N HCl. The precipitate obtained by this procedure was collected by centrifugation (crude acidic protein fraction) and dissolved in a small volume of 25 mM Tris-HCl (pH 7.5), 0.15 M NaCl, and 100 mM CaCl₂ (Buffer C). The solution was dialyzed against Buffer C and applied to a W-7-Sepharose column (1.1 × 5 cm) equilibrated with Buffer C. The column was washed with Buffer C until UV absorbance at 280 nm returned to the baseline level. The buffer was then changed to Buffer D (same composition as Buffer C but 2 mM EGTA instead of 100 mM CaCl₂). The typical elution profile of this Ca²⁺-dependent affinity chromatography is shown in Fig. 1. Protein concentration monitored by absorbance at 280 nm and the concentration of S-100b protein measured by enzyme immunoassay in each fraction is also illustrated in Fig. 1. The peak of the S-100b protein coincided with the protein peak. Fractions 54–70 were collected and dialyzed against distilled water. On the other hand, the W-7-Sepharose-binding protein did not bind to a Sepharose column without a coupled drug. Purification procedures of W-7-binding protein from adipose tissue are summarized in Table I. From 10 kg of bovine abdominal adipose tissue, about 2.85 mg of protein were obtained.

Electrophoretic Analyses of the Isolated Protein—Fig. 2A shows a SDS-polyacrylamide slab gel electrophoretic pattern of the W-7-Sepharose-binding protein from adipose tissue, stained with Coomassie brilliant blue. The protein was essentially homogeneous and co-migrated with brain S-100b protein, indicating that the molecular weight of the protein estimated on this electrophoresis was similar to the β subunit (Mᵦ = 10,500) of brain S-100b protein. It was previously demonstrated that bovine brain S-100 protein is a mixture of two closely related components, S-100a and S-100b, that each of the components has the subunit composition α(Mᵦ = 10,400, β(Mᵦ = 10,500)) or (β,β) (7, 8), and that S-100a and S-100b had different electrophoretic mobilities in a 20% polyacrylamide gel, according to the buffer system of Ornstein and Sternberger et al. (21, 27).

Table 1

| Volume | Total protein | S-100b protein |
|--------|---------------|----------------|
| ml     | mg            | mg             |
| Homogenate | 6900          | 6020           | 4.27          |
| DEAE-Sephadex A-50 | 1900          | 782            | 3.60          |
| (NH₄)₂SO₄, 55–90%, pH 4.2 | 70            | 176            | 3.50          |
| W-7-Sepharose affinity | 21            | 2.85           |              |

*Measured by enzyme immunoassay.
compositions of W-7-Sepharose-binding protein from adipose tissue same electrophoretical mobility as bovine brain S-100b protein residue and is quite similar in overall amino acid composition. The W-7-Sepharose-binding protein from adipose tissue characteristically lacks a tryptophan residue and is quite similar in overall amino acid composition to brain S-100b protein (Table 2).

Peptide Analysis—The structural homology of brain S-100b protein and the W-7-Sepharose-binding protein from adipose tissue was examined by tryptic peptide mapping after labeling with 125I. The stained bands of the W-7-Sepharose-binding protein from adipose tissue and brain S-100b protein were cut out from the slab gel of Fig. 2B, the protein in each gel slice was radiodinated and digested with trypsin, and the tryptic peptides were separated two-dimensionally on a silica gel-coated thin layer plate. Fig. 3 shows the autoradiograms of tryptic peptide maps of the W-7-Sepharose-binding protein from adipose tissue and bovine brain S-100b protein. More than 18 spots were detected in each map. Although the difference was detected, most of the spots were common to both maps, indicating that the molecular structures of the W-7-Sepharose-binding protein from adipose tissue are homologous to that of brain S-100b protein.

Immunochemical Analysis—To verify the W-7-Sepharose-binding protein from adipose tissue as S-100b protein, immunochemical analysis was carried out. The anti-bovine brain S-100b sera used in this analysis reacted with S-100b protein but did not cross-react with calmodulin. As shown in Fig. 4, the W-7-Sepharose-binding protein from adipose tissue produced a clear single precipitin line against the anti-brain S-100b serum, and this line fused with that formed between the bovine brain S-100b protein and antiserum.

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Electrophoretic analyses of W-7-Sepharose-binding protein from adipose tissue. A, SDS-polyacrylamide slab gel electrophoresis of W-7-Sepharose-binding protein from adipose tissue. Electrophoresis was performed in 0.1% SDS, 15% polyacrylamide as described by Laemmli (21). Lane 1, bovine brain S-100b protein (2 μg); lane 2, W-7-Sepharose-binding protein from adipose tissue (3 μg). B, 20% polyacrylamide slab gel electrophoresis of W-7-Sepharose-binding protein from adipose tissue. Electrophoresis was performed at 4°C by the discontinuous buffer system of Ornstein (19) and Davis (20) using an electrode buffer containing 0.1% (v/v) thoglycic acid. Lane 1, bovine brain S-100b protein (3 μg); lane 2, W-7-Sepharose-binding protein from adipose tissue (5 μg); lane 3, bovine brain S-100a protein (6 μg).

**Table II**

| Amino acid | W-7-Sepharose-binding protein from adipose tissue | S-100b protein from brain |
|------------|-------------------------------------------------|--------------------------|
| Lys        | 9.7                                             | 8.0                      |
| His        | 6.3                                             | 5.7                      |
| Arg        | 1.5                                             | 1.1                      |
| Asp        | 11.0                                            | 10.3                     |
| Thr        | 3.4                                             | 3.4                      |
| Ser        | 5.5                                             | 5.7                      |
| Glu        | 21.7                                            | 20.7                     |
| Pro        | 0                                               | 0                        |
| Gly        | 4.9                                             | 4.6                      |
| Ala        | 5.4                                             | 5.7                      |
| Cys<sup>a</sup> | 1.8                                            | 2.3                      |
| Val        | 6.7                                             | 6.9                      |
| Met        | 3.4                                             | 3.4                      |
| Ile        | 4.6                                             | 4.6                      |
| Leu        | 7.7                                             | 9.2                      |
| Tyr        | 1.3                                             | 1.1                      |
| Phe        | 7.4                                             | 6.9                      |
| Trp<sup>a</sup> | 0                                               | 0                        |

<sup>a</sup> Taken from Isobe et al. (6).

<sup>a</sup> Determined as cysteic acid after performic acid oxidation (28).

<sup>a</sup> Determined spectrophotometrically (29).

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Autoradiograms of tryptic peptide maps. S-100b protein from bovine brain and W-7-Sepharose-binding protein from adipose tissue in the polyacrylamide gel shown in Fig. 2B were cut out from the gel, and each was radiodinated with <sup>125</sup>I as described under "Materials and Methods." Tryptic peptides were mapped and autoradiography was carried out. A, bovine brain S-100b protein. B, W-7-Sepharose-binding protein from adipose tissue.

![Figure 4](http://www.jbc.org/)

**Fig. 4.** Double immunodiffusion of W-7-Sepharose-binding protein from adipose tissue and bovine brain S-100b protein against anti-brain S-100b serum. Sample wells contain: 1, antiboine brain S-100b serum (5 μl); 2, bovine brain S-100b protein (10 μg); 3, W-7-Sepharose-binding protein from adipose tissue (10 μg); 4, bovine brain S-100b protein (5 μg); 5, W-7-Sepharose-binding protein from adipose tissue (5 μg).
Immunohistochemical Localization of S-100b Protein in Adipose Cells—Immunoperoxidase reaction for S-100b protein was positive in adipose cells, where the reaction product was located in both the nucleus and the narrow cytoplasm (Fig. 5). On the other hand, the surrounding fibrous connective tissue was free from reaction product. Control experiments carried out using anti-brain S-100b serum absorbed with the antigen did not show any reaction product. Thus, the results indicate that the adipose cells contain the protein possibly identical with brain S-100b protein.

Tissue Distribution of S-100b Protein—The tissue distribution of S-100b protein was determined in various rat tissues by means of a sensitive enzyme immunoassay method. The method selectively measured S-100b protein with little cross-reaction of S-100a protein and calmodulin and the dose-response of the bound enzyme activity was observed between 10 and 1000 pg of brain S-100b protein/assay tube (Fig. 6). Table III summarizes the content of S-100b protein in the soluble extract of various tissues from Wistar rats. The contents are expressed as nanograms of bovine brain S-100b protein equivalents/mg of protein. Brain contained extremely high levels of S-100b protein. Among the non-nervous peripheral tissues investigated, pituitary gland and skin contained slightly higher levels of S-100b protein, as was also found by other investigators (9, 11). Of particular note is the finding that the adipose tissue and trachea contained levels of S-100b protein comparable to the levels in the tissue from the central nervous system.

**DISCUSSION**

S-100 protein was considered to be a central nervous tissuespecific protein (1). However, recent findings of S-100 antigen in non-nervous tissue such as adenohypophysis (9), chondrocytes (10), or Langerhans cells of the skin (11) suggested to us the necessity of purifying and characterizing S-100 antigen in non-nervous tissue. The methods previously described (14) for purifying bovine brain S-100 protein were unsuccessful for adipose tissue because this tissue contains large amounts of lipids. Therefore, it was necessary to design a modified procedure for the purification of adipose tissue S-100 protein. We combined the use of DEAE-Sephadex A-50 chromatography, followed by W-7 affinity chromatography and this simple method allows for a good yield of the S-100 antigen which is essentially free from contaminants. The electrophoretic as well as immunochemical analysis together with the coincidence of amino acid analysis and peptide mapping indicated that W-7-Sepharose-binding protein from adipose tissue was possibly identical with brain S-100b protein. It was of particular interest that only S-100b protein could be obtained from adipose tissue by Ca"-dependent affinity chromatography on W-7 coupled to epoxy-activated Sepharose, since we previ-

![Fig. 5. Immunoperoxidase reaction for S-100b protein in human adipose tissue. A, reaction products were observed both in nucleus and cytoplasm of adipose cells. No reaction product was present in extracellular fibrous connective tissue (x 100). B, same section as A (x 400).](image-url)
ously isolated both S-100a and S-100b proteins from central nervous tissue by essentially the same procedure (14). This suggests that the nervous tissue contains both S-100a and S-100b protein, but only S-100b protein distributes in adipose tissue. However, the possibility could not be ruled out that certain tissues contain large amounts of S-100a but show up as containing moderate amounts of S-100b since the antiserum used in this study showed some reactivity with S-100a (Fig. 6). The precise determination of both S-100a and S-100b protein in various tissues is necessary for the elucidation of this point.

In the central nervous tissue, S-100 protein was postulated to play an important role in the control of membrane permeability (30) or in the maturation of glial cells (31). However, our present results, together with recent findings of S-100 antigen in non-nervous tissues suggest that S-100b protein should no longer be considered as a protein specific to nervous tissue. Although the biological significance of non-nervous tissue S-100 protein including that of adipose tissue remains obscure, our results do pave the way for clarification of the physiological role of S-100 protein in both central nervous and peripheral tissues.

Acknowledgments—We thank M. Ohara, Kyushu University, for critical reading of the manuscript and M. Hagiwara for technical assistance.

REFERENCES

1. Moore, B. W. (1965) Biochim. Biophys. Res. Commun. 19, 739–744
2. Uyemura, K., Vincendon, G., Gombos, G., and Handel, P. (1971) J. Neurochem. 13, 823–833
3. Dannies, P. S., and Levine, L. (1971) J. Biol. Chem. 246, 6267–6283
4. Calissano, P., Mercanti, D., and Levi, A. (1976) Eur. J. Biochem. 71, 45–52
5. Eng, L. F., and Bigbee, J. W. (1978) Adv. Neurochem. 3, 43–98
6. Isobe, T., Nakajima, T., and Okuyama, T. (1977) Biochim. Biophys. Acta 494, 222–232
7. Isobe, T., and Okuyama, T. (1978) Eur. J. Biochem. 88, 379–388
8. Moews, P. C., and Kretzinger, R. H. (1975) J. Mol. Biol. 91, 201–228
9. Nakajima, T., Yamaguchi, H., and Takahashi, K. (1980) Brain Res. 191, 523–531
10. Stefansson, K., Wollmann, R. L., Moore, B. W., and Arnason, B. G. W. (1982) Nature (Lond.) 295, 63–64
11. Cocchini, D., Michetti, P., and Donato, R. (1981) Nature (Lond.) 294, 85–87
12. Hidaka, H., Yamaki, T., Totsuka, T., and Asano, M. (1979) Mol. Pharmacol. 15, 49–59
13. Hidaka, H., Yamaki, T., Naka, M., Tanaka, T., Hayashi, H., and Kobayashi, H. (1980) Mol. Pharmacol. 17, 66–72
14. Endo, T., Tanaka, T., Isobe, T., Kasai, H., Okuyama, T., and Hidaka, H. (1981) J. Biol. Chem. 256, 12485–12489
15. Hidaka, H., Endo, T., and Kato, K. (1982) Methods Enzymol., in press
16. Hidaka, H., Asano, M., Iwadare, S., Matsumoto, I., Totsuka, T., and Aoki, N. (1978) J. Pharmacol. Exp. Ther. 207, 8–15
17. Haglid, K. G., Hamberger, A., Hansson, H. A., Hyden, H., Persson, L., and Rommback, L. (1974) Nature (Lond.) 251, 532–534
18. Kato, K., Fukui, H., Hamaguchi, Y., and Ishikawa, E. (1976) J. Immunol. 116, 1554–1560
19. Ornstein, L. (1964) Ann. N. Y. Acad. Sci. 121, 321–349
20. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427
21. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
22. Tanabe, K., Yamaguchi, M., Matsukage, A., and Takahashi, T. (1981) J. Biol. Chem. 256, 3098–3102
23. Greenwood, F. C., Hunter, W. H., and Glover, J. S. (1963) Biochem. J. 89, 114–123
24. Spackman, D. H., Stein, W. H., and Moore, S. (1958) Anal. Chem. 30, 1190–1206
25. Sternberger, L. A., Hardy, P. H., Jr., Cuculias, J. J., and Meyer, H. G. (1970) J. Histochem. Cytochem. 18, 315–333
26. Kaplow, L. S. (1974) Am. J. Clin. Pathol. 63, 451
27. Kato, K., Hamaguchi, Y., Okawa, S., Ishikawa, E., Kobayashi, K., and Katsunuma, N. (1977) J. Biochem. (Tokyo) 81, 1557–1566
28. Hirs, C. H. W. (1967) Methods Enzymol. 11, 197–199
29. Goodwin, T. W., and Morton, R. A. (1946) Biochem. J. 40, 628–632
30. Calissano, P., Alema, S., and Fasella, P. (1974) Biochemistry 13, 4553–4560
31. Labourdette, G., and Handel, P. (1978) Biochem. Biophys. Res. Commun. 85, 1037–1031
Purification and characterization of adipose tissue S-100b protein.
H Hidaka, T Endo, S Kawamoto, E Yamada, H Umekawa, K Tanabe and K Hara

J. Biol. Chem. 1983, 258:2705-2709.

Access the most updated version of this article at http://www.jbc.org/content/258/4/2705

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/4/2705.full.html#ref-list-1