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SELENIUM BINDING PROTEINS IN RAT TISSUES

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The 100,000 x g extracts of rat intestine and colon were incubated in vitro with $Na_2[^{75}Se]O_3$. Chromatography of this material on a Sephadex G-100 column produced three radioactive peaks corresponding to molecular weights of 17,000, 68,000 and >90,000. The 17,000 peak corresponded to a protein which sedimented in the 2S region of a 5-20% (w/v) linear sucrose density gradient. Selenium binding to this protein was specific, stable and sensitive to thiol inhibitors such as p-chloromercuriphenylsulfonic acid (1 mM) and iodoacetamide (2 mM). Chromatography of rat serum - [^{75}Se] complex on Sephadex G-100 yielded only two radioactive peaks that corresponded to molecular weights of 68,000 and >90,000. The 2S selenium binding protein of intestine and colon may mediate the biological functions of selenium in those tissues.

The role of selenium as a micronutrient has been well established (1). Its deficiency has been associated with reduced life span, anemia, liver necrosis and immunologic disorders. Deficiency of selenium also causes structural deformation of sperm tail in rats (2) and muscular dystrophy in lambs (3). The anticarcinogenic property of selenium has been documented in various test systems; its mechanism of action, however, is not known. Dietary supplementation of selenium has been shown to reduce the incidence of cancer of colon (4), liver (5), intestine (6), skin (7), and of mammary glands (8). Epidemiologic observations also point to a reverse correlation between the availability of selenium and the incidence of cancer (7).

The metabolism of selenium and the biochemical mechanism of its action are not well understood. The biological activity of several enzymes, namely mammalian and avian glutathione peroxidase, clostridial glycine reductase and formate dehydrogenase of E. coli, depends on the presence of selenium covalently bound to the protein (9). Presumably selenium participates in the oxidation-reduction step of the reactions catalyzed by these selenoproteins. An immunological role has also been postulated for selenium (10). The variety of functions carried out by selenium suggest multiple modes of biochemical action. In an attempt to further elucidate mechanisms by which selenium

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may act, we have studied the in vitro interaction of selenium with extracts from rat tissues. We report here the presence of a 2S protein in the cytosols of rat intestine and colon which specifically binds selenium. The binding activities of selenium to two proteins in rat serum are also briefly described.

MATERIALS AND METHODS

Sodium selenite ($Na_2[^{75}Se]O_3$, 7.9 mCi/mg) was purchased from Amersham Corporation, Arlington Heights, IL. Dithiothreitol, iodoacetamide, p-chloromercuriphenylsulfonic acid (CMPS) and pronase were purchased from Sigma Chemical Company, St. Louis, MO.

Preparation of Tissue Extracts and Detection of Binding Proteins

Intestine and colon isolated from 4-weeks old Sprague Dawley rats were cut open and rinsed with 50 mM Tris-HCl, pH 7.4 containing 0.1 mM dithiothreitol (standard buffer). They were minced and homogenized in a VirTis teflon homogenizer in 3 volumes of standard buffer and centrifuged at 20,000 x g for 30 min. The supernatants were further clarified by filtration through 3 layers of cheese cloth followed by centrifugation at 100,000 x g for 1 h and stored frozen at -60°. The serum prepared from the rat blood also was frozen at -60°.

Selenium binding proteins were detected by sucrose density gradient centrifugation. Tissue extracts containing 1 mg protein were incubated with 300 pmoles of sodium selenite ($Na_2[^{75}Se]O_3$) at room temperature for three hours. In competitive displacement experiments a 200-fold molar excess of sodium selenite was added along with the radioactive ligand. Unbound selenium was removed by dialysis against two changes of one liter of standard buffer for 18 h. The dialyzed material was layered on linear 5-20% (w/v) sucrose density gradients in standard buffer, centrifuged for 18 h at 180,000 x g in a Spinco 50.1 rotor at 4°. Fractions (0.2 ml) were collected from the top of the developed gradients by using an Auto Densi-Flow pump and the radioactivity was determined using a Packard gamma counter. Bovine serum albumin, β-lactoglobin, and myoglobulin were included as external standards.

Sephadex Gel Filtration

Tissue extracts (15 mg of protein) were incubated with 5 nmoles of $Na_2[^{75}Se]O_3$ and applied to a Sephadex G-100 column (2.5 x 100 cm) equilibrated and developed with the standard buffer at a flow rate of 25 ml/h. For molecular weight determinations 5 mg each of thyroglobulin, bovine serum albumin, β-lactoglobulin, myoglobin and cytochrome c were applied to the column and developed under identical conditions.

RESULTS

Figure 1 presents the sucrose density gradient profile of rat colon and intestine cytosol incubated with 300 pmoles of $Na_2[^{75}Se]O_3$. Both the extracts produced a prominent radioactive peak corresponding to an $S_{20,w}$ value of 2. Treatment of the extracts with pronase, subsequent dialysis and sucrose density gradient sedimentation completely eliminated the 2S peak showing the protein nature of the binding component. About 60 pmoles of selenium was bound to one mg of extractable total protein from the

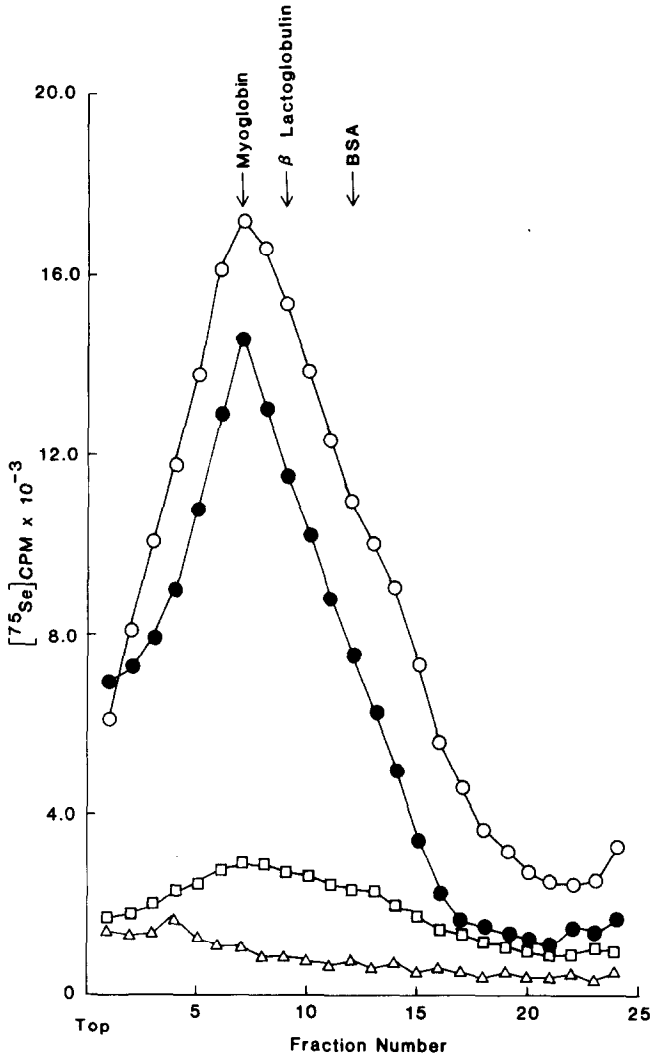

Figure 1. Sucrose density gradient sedimentation patterns of rat tissue cytosols incubated with 300 pmoles of $Na_2[^{75}Se]O_3$. ● , colon; O, intestine; □, same as O, plus 200-fold molar excess of unlabeled ligand; △, effect of pronase (100 μg) on the intestine extract-[^{75}Se]complex.

intestine. The specificity of the selenium binding activity was indicated by the virtual elimination of the 2S peak when challenged with 200-fold molar excess of unlabeled selenium (Fig. 1). When increasing concentrations of selenium were present in the incubation mixture, a second radioactive peak at fractions corresponding to a sedimentation coefficient of 5-6 S was observed (not shown). This peak may be due to serum contamination since sucrose density gradient sedimentation pattern of rat serum also produced a similar 5-6S peak.

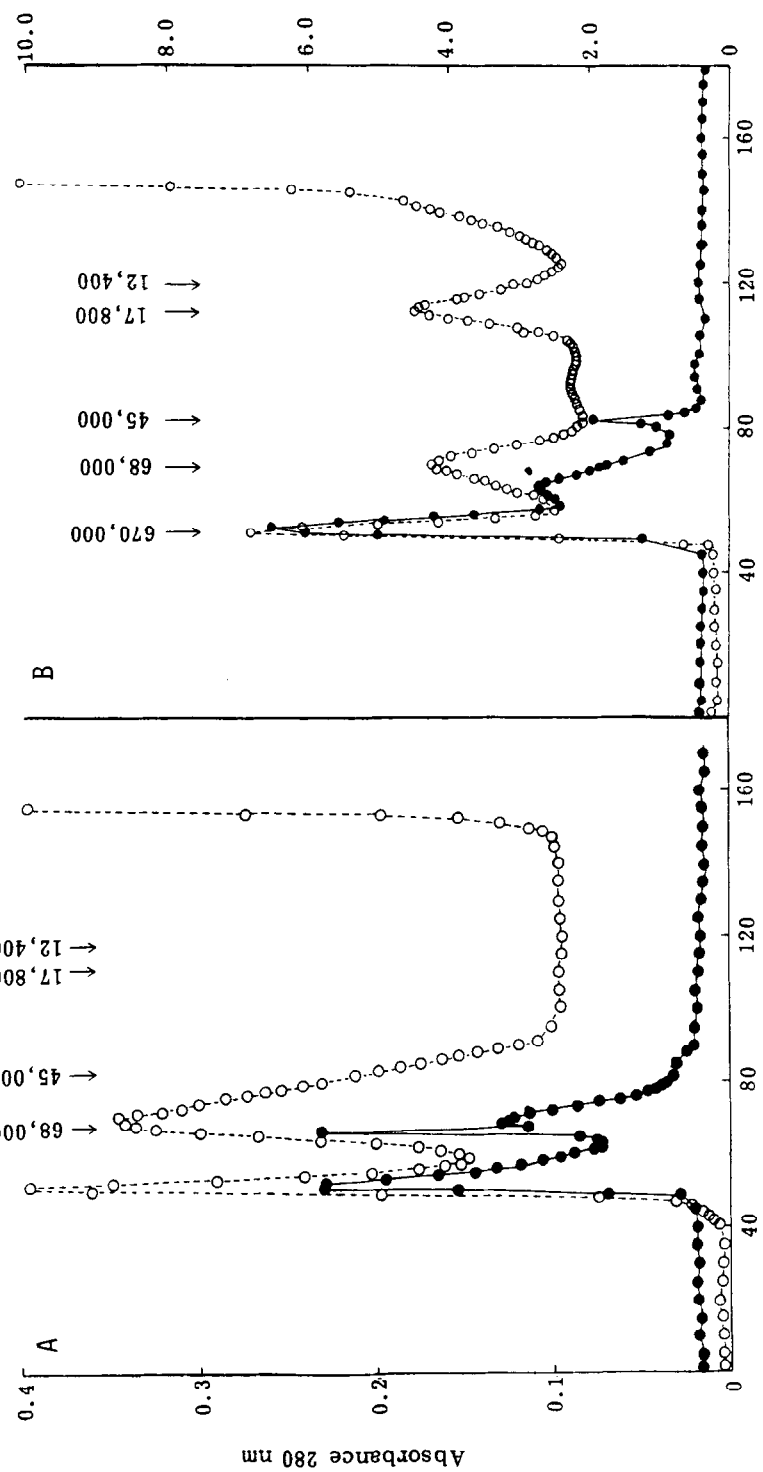

Figure 2. Sephadex G-100 gel filtration pattern of (A), rat serum-[^{75}Se]complex; (B), rat intestine extract-[^{75}Se]complex. ●, absorbance 280 nm; O, radioactivity.

The binding of selenium to the 2S protein was strong and could withstand dialysis up to 20 h. The binding protein-Se complex was also resistant to dialysis against 50 mM carbonate-bicarbonate buffer, pH 11.0, since all the radioactivity was retained in the dialysis tube after 16 h dialysis. The binding activity was stable for several months when stored at -60°; however at -20° there was a 30% loss of binding activity after one month of storage.

The binding activities of the serum and of the cytosol from intestine were further studied by incubating them with selenium, followed by Sephadex G-100 chromatographic analysis. Fig. 2A presents the Sephadex G-100 gel chromatographic profile of serum protein-selenium complex. A protein-selenium complex of high molecular weight (>90,000) emerged from the column with the void volume. The second radioactive peak that was eluted, corresponded to a molecular weight of 68,000. Fig. 2B presents the Sephadex G-100 chromatographic profile of intestinal extract-[^{75}Se] complex run under identical chromatographic conditions as serum. In addition to the two peaks seen in the serum profile, a third radioactive peak corresponding to a molecular weight of 17,000 was evidenced. The amount of Se bound in the 17,000 dalton region of the profile approximated to 0.3 mole per mole of the protein(s) in that region. This binding protein showed an $S_{20,w}$ value of 2.0 upon sucrose density gradient sedimentation.

The effect of thiol inhibitors on the selenium binding activity of the 2S protein was also studied. Both iodoacetamide (2 mM) and CMPS (1 mM) completely inhibited the binding (not shown) indicating that thiol groups may exist in the protein ligand interactions.

DISCUSSION

Chromatography of the intestine extract-[^{75}Se] complex on Sephadex G-100 column produced three distinct radioactive peaks. The protein peak corresponding to a molecular weight of 17,000 and an $S_{20,w}$ value of 2.0 could not be detected in the corresponding serum profile. Although the molar ratio of selenium bound to one mg protein of the crude extract was quite low, the 2S protein resolved from the Sephadex G-100 column bound about 0.3 mole of selenium per mole of 17,000 dalton protein. A quantitative estimation of the molar ratio, however, must wait until a homogeneously

purified binding protien is available. The high molar binding of selenium to the 2S protein as well as the elimination of the 2S radioactive peak by using unlabeled selenium in the competitive experiments (Fig. 1) allows the conclusion that the selenium binding to the 17,000 dalton protein is highly specific. It has been shown that binding of selenium to amino acids and polypeptides is facilitated by sulfur containing amino acids, the S-S groups binding more predominantly (11). The S-S groups form selenotrisulfides through loose ionic bonds which are susceptible to high molarities of mercaptoethanol or alkaline pH (12). However, selenium binding to the 2S protein described here probably involved SH groups since the binding could be eliminated by exposure to thiol inhibitors such as iodoacetamide and CMPS. A tight binding was inferred from the fact that the protein retained the bound ligand on dialysis for 16 h against a pH 11.0 buffer. The 2S protein detected in the colon cytosol exhibited similar properties as the intestinal protein. The relationship of these 2S binding proteins to the testis and sperm tail proteins described by Prohaska (13) and by Calvin (14) after intratesticular administration of [^{75}Se] to rats is not presently known. The 2S protein may mediate some of the cellular actions of selenium and thus may be similar in function to the hormone and vitamin receptors.

The two other peaks with selenium binding activity in the Sephadex G-100 chromatographic profile of the intestinal extract corresponded to molecular weights of >90,000 and 68,000 respectively. A comparison of the chromatographic profiles of serum and intestinal extract revealed striking similarities in the binding activity of the high molecular weight region, thereby suggesting that the heavier binding proteins in the intestinal extract may have originated from serum as a contaminant. Loose binding of selenium to alubmin *in vivo* has been described (15); such binding occurred only at high ligand concentrations. In the *in vitro* experiments described here the radioactive peak corresponding to 68,000 molecular weight was not due to albumin since passage of the extract through Affi-Gel Blue column, which effectively adsorbs albumin (16), did not reduce the size of the radioactive peak on sucrose density gradient profiles (not shown). The two plasma proteins reported here might function in the transport of selenium to various tissues.

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REFERENCES

1. Underwood, E. J. (1977) Trace Elements in Human and Animal Nutrition. Academic Press, New York.
2. Wu, S. H., Oldfield, J. E., Whanger, P. D., and Weswig, P. H. (1973) Biol. Reprod. 8, 625-629.
3. Muth, O. H., Oldfield, J. E., Remmert, L. F., and Shubert, J. R. (1958) Science 128, 1090.
4. Jacobs, M. M., Janson, B., and Griffin A. C. (1977) Cancer Lett. 2, 133-138.
5. Daoud, A. H., and Griffin, A. C. (1980) Cancer Lett. 9, 299-304.
6. Wedderburn, J. (1972) New Zealand Veterinary J. 20, 56-57.
7. Shamburger, R. J. (1970) J. Natl. Cancer Inst. 44, 931-936.
8. Ip, C. (1981) Cancer Res. 41, 2683-2686.
9. Stadtman, T. C. (1980) Ann. Rev. Biochem. 49, 93-100.
10. Baumgartner, W. A. (1979) In: Kharasch, N. Ed. Trace Metals in Health and Disease pp. 287-305, Raven Press, New York.
11. Schwarz, K., and Sweeney, E. (1964) Fed. Proceed. 23, 421.
12. Cumins, L. M., and Martin, J. L. (1967) Biochemistry 6, 3162-3168.
13. Prohaska, J. R., Mowafy, M., Ganther, H. E. (1977) Chem.-Biol. Interact. 18, 253-265.
14. Calvin, H. I. (1978) J. Exp. Zool. 204, 445-452.
15. Sandholm, M. (1973) Acta Pharmacol. et Toxicol. 33, 1-5.
16. Sani, B. P., Condon, S. M., Brockman, R. W., Weiland, L. H., and Schutt, A. J. (1980) Cancer 45, 1199-1206.