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Note

Affinity chromatographic isolation of a major polyamine binding protein from serum and placenta, identified as serum albumin

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Polyamines are essential to many cellular processes, including DNA replication and protein synthesis [1–3]. Apart from their nucleic acid binding ability, polyamines also bind to some protein kinases and several proteins of unknown function [3–5]. In an attempt to characterize some of these proteins, the isolation of polyamine binding proteins from placenta and serum by affinity chromatography was attempted. A major polyamine binding protein was isolated from human placenta and pregnancy serum and from bovine serum, and identified as serum albumin.

EXPERIMENTAL

Spermine·4HCl and spermidine·3HCl were from Fluka (Buchs, Switzerland). NaCl, Na_2HPO_4 and NaH_2PO_4 were from Merck (Darmstadt, F.R.G.). AH-Sepharose 4B and CNBr-activated Sepharose 4B were from Pharmacia (Uppsala, Sweden).

Spermine Sepharose was made by incubating 15 g of CNBr-activated Sepharose 4B (prewashed) in 0.1 *M* $NaHCO_3$ (pH 8.3)–0.5 *M* NaCl with 10 m*M* spermine for 10 min; the pH was maintained by addition of 1 *M* NaOH. After this the coupling was allowed to proceed for an additional 12 h. The gel was finally washed with water and 50 m*M* sodium acetate (NaAc) (pH 4.5).

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Pregnancy sera and placenta were obtained from Rigshospitalet (Copenhagen, Denmark).

Placental extracts were made by homogenizing one placenta in 400 ml of ice-cold 40 m*M* phosphate buffer (pH 7.2). After centrifugation (20 000 *g*, 20 min, 5 °C), the precipitate was further extracted with 300 ml of 40 m*M* phosphate buffer (pH 7.2), 0.5% Triton-X-100 and 50 μ*M* dithiothreitol (DTT). The Triton extract was clarified by centrifugation as above. Chromatography was done in disposable 10-ml columns and on mono-S columns, using an FPLC system (Pharmacia) and the following buffers: A, 50 m*M* NaAc (pH 4.5); B, 4 m*M* spermine–50 m*M* NaAc (pH 4.5). The flow-rate was 1 ml/min. The UV detector (280 nm) was set at 0.05 a.u.f.s.

Samples for amino acid analysis were hydrolysed in 6 *M* HCl containing 0.5% phenol for 24 h at 110 °C. After drying, the samples were analysed on an ion-exchange column using a Waters HPLC system with post-column OPA quantitation of amino acids.

Protein sequence analysis was done on a Model 477A Sequenator (Applied Biosystems, Foster City, CA, U.S.A.) with on-line analysis of phenylthiohydantoin (PTH) amino acids.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli [6] using a 12% separating gel.

Nitrocellulose membrane (0.45 μm) were from Millipore (Milford, MA, U.S.A.). Bovine serum albumin was from Sigma (St. Louis, MO, U.S.A.).

[^{14}C]Spermine (50 μCi/ml) was from New England Nuclear (Boston, MA, U.S.A.). Binding of spermine to serum albumin and purified proteins was assayed by incubating nitrocellulose filters (0.5 or 1 cm diameter), which had been saturated with protein in 1 ml of 0.2 *M* phosphate buffer (pH 7.2) with increasing amounts of labelled spermine. After 1 h incubation at 37 °C, the filters were washed and placed in scintillation vials, and 5 ml of scintillation liquid were added (Maxifluor, J.T. Baker, Deventer, The Netherlands).

RESULTS

Affinity chromatography on spermine-Sepharose of a human placenta extract (Fig. 1) led to the isolation of a single protein of relative molecular weight (M_r) 67 000 (Fig. 2). Chromatography of the same extract on aminohexyl-Sepharose (Fig. 1) did not give rise to this protein, but did lead to the isolation of a small amount of a protein with M_r 80 000 (Fig. 2). The specificity of the former protein for spermine itself and not merely for primary amino groups thus seems to be quite high. N-Terminal sequence analysis of this protein showed two sequences, the major corresponding to residues 1–20 of human serum albumin and the minor to residues 84–99 of human serum albumin.

Ion-exchange affinity chromatography (Fig. 3) of human and bovine sera on a mono-S column saturated with spermine and eluted with a gradient of

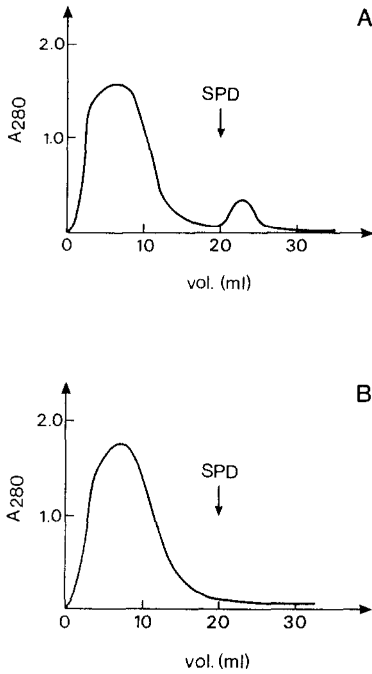

Fig. 1. Affinity chromatography of placental proteins on spermine-Sepharose (A) and aminohexyl-Sepharose (B). The sample load was 5 ml and the column washed with phosphate buffer. Bound proteins were eluted with 10 m*M* spermidine (SPD) in phosphate buffer.

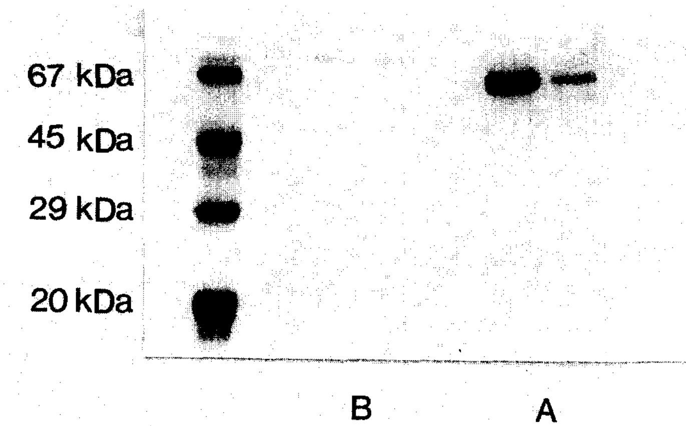

Fig. 2. SDS-PAGE analysis of pooled fractions from Fig. 1. (A) Fractions 21-25 ml and 26-30 ml from the spermine-Sepharose column. (B) Fractions 21-25 ml and 26-30 ml from the aminohexyl-Sepharose column. Molecular mass standards (Sigma) were bovine serum albumin (67 000), ovalbumin (45 000), carbonic anhydrase (29 000) and soybean trypsin inhibitor (20 000).

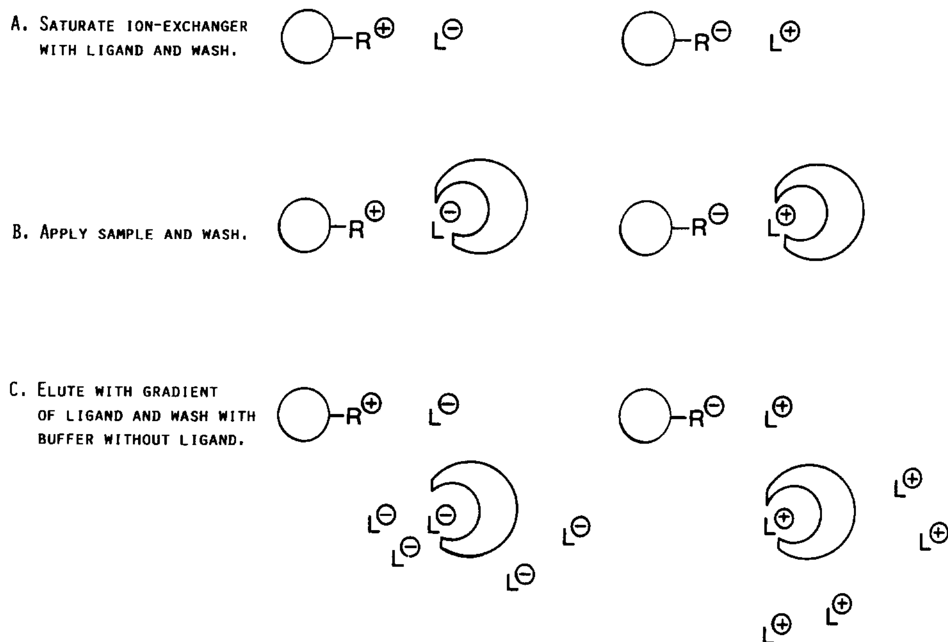

Fig. 3. Principle of ion-exchange affinity chromatography.

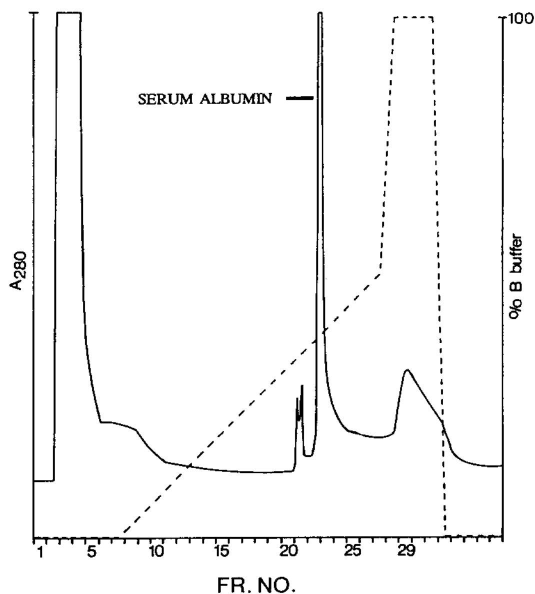

Fig. 4. Chromatography of 1 ml of foetal bovine serum on a mono-S column saturated with spermine and eluted with a linear gradient of spermine. The column was first washed with buffer B (4 m*M* spermine in 50 m*M* NaAc) and then with buffer A (50 m*M* NaAc). After injection of the sample, the column was eluted with buffer B as shown (----).

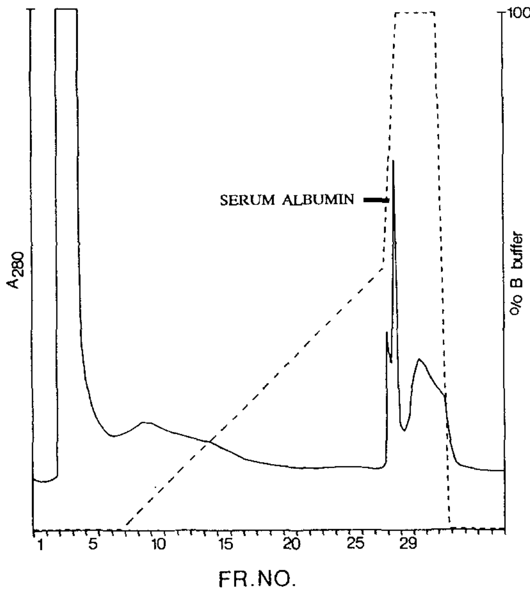

Fig. 5. Chromatography of 1 ml of human pregnancy serum on a mono-S column saturated with spermine and eluted with a gradient of spermine. Experimental details as in Fig. 4.

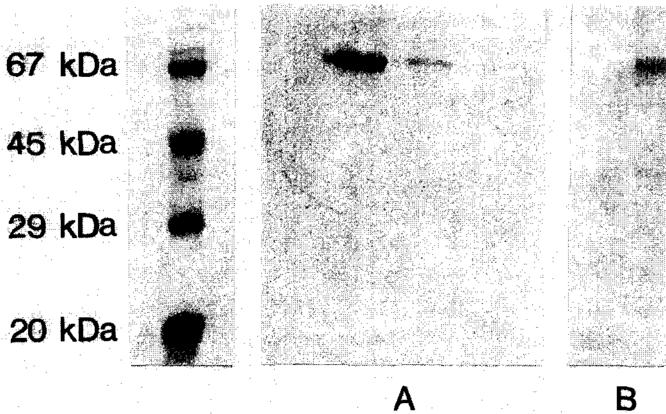

Fig. 6. SDS-PAGE analysis of fractions from ion-exchange affinity chromatography. (A) Fractions 21–24 from Fig. 4. (B) Fractions 27 and 28 from Fig. 5. Molecular mass standards as in Fig. 2.

spermine (Figs. 4 and 5) also led to the isolation of proteins with M_r 67 000 (Fig. 6). Amino acid analysis and N-terminal sequence analysis of these proteins were identical with those of serum albumin.

Preliminary binding experiments with bovine serum albumin immobilized

on nitrocellulose indicated the existence of several low affinity binding sites (data not shown).

DISCUSSION

The results presented show that polyamines bind to serum albumin. Since serum albumin did not bind to aminohexyl-Sepharose it seems likely that the secondary amino groups of the polyamines are of importance for binding. Serum albumin is known to bind several low-molecular-mass components, including Cu^{2+}, fatty acids, bilirubin, thyroxine, indole, tryptophan, imidazole and tuftsin [7–10]. For all of these compounds there are several binding sites distributed on the three homologous subdomains. Since the secondary amino group of the polyamines appears to be important for binding it is likely that they bind to the same sites as bilirubin, indole and imidazole. The ability of polyamines to bind to serum albumin emphasizes the role of this protein as a transport protein, since polyamines are present in serum and are excreted in urine [2].

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