USE OF AN AFFINITY IMMUNOSORBENT FOR THE ISOLATION OF LACTOFERRIN FROM BREAST MILK SERUM

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An affinity or biospecific method of isolating an iron-binding glycoprotein — lactoferrin — from breast milk is described which permits in a single chromatographic operation the isolation of lactoferrin homogeneous according to the results of electrophoresis in polyacrylamide gel. The affinity method of isolating lactoferrin is based on the use of a specific immunosorbent consisting of antibodies to lactoferrin covalently conjugated with cyanogen-bromide-activated Sepharose 4B.

Traditional methods of isolating pure proteins, consisting of different variants of salt precipitation, ion-exchange and gel-permeation chromatography, preparative electrophoresis, etc., are, as a rule, lengthy and require special apparatus for the fractionation of proteins together with various ion-exchange resins and inert gels — Sephadexes. In the case of the ion-binding protein lactoferrin the use of ion-exchange chromatography on carboxymethyl-Sephadex as described previously [1] permits a protein to be obtained which is present predominantly in the apo form [2], and this, of course, lowers the yield of lactoferrin. The use of sorbents of the heparin-Sepharose type, possessing an increased affinity for lactoferrin [3], however, likewise does not, in a number of cases, permit the lactoferrin to be freed from protein impurities in the course of a single chromatographic operation.

In the present paper we describe the isolation of lactoferrin by the use of a specific affinity immunosorbent consisting of monospecific rabbit antibodies to lactoferrin covalently conjugated with Sepharose 4B activated by cyanogen bromide.

Lactoferrin was isolated from breast milk serum by a known method [1], all the procedures being performed in the microvariant. Thus, about 10 mg of lactoferrin was obtained, and its purity was checked in two variants of electrophoresis in polyacrylamide gel (PAAG) — in an acid medium and in the presence of the denaturing agent sodium dodecyl sulfate (NaDDS) — and also by N-terminal amino acid analysis. The protein homogeneous with respect to these parameters was used to obtain monovalent antisera. The latter were obtained by immunizing rabbits of the Chinchilla breed by a scheme that we have described previously [4]. To isolate from the antisera monospecific antibodies to lactoferrin we used a prepared affinity sorbent — lactoferrin-Sepharose. The antibodies isolated on this sorbent were checked for specificity in the Ouchterlony double radial immunodiffusion reaction, where a single homogeneous precipitation zone indicating the monospecificity of the antibodies to lactoferrin was obtained.

The monospecific antibodies to lactoferrin were covalently conjugated with cyanogen-bromide-activated Sepharose 4B. The immunosorbent obtained was used for isolating lactoferrin from milk serum.

The positive aspects of the affinity method for isolating lactoferrin include the possibility of obtaining purified lactoferrin in only one chromatographic operation lasting a short time, but the yield of lactoferrin may vary according to the capacity of the affinity immunosorbent. A given immunosorbent after suitable regeneration can be used repeatedly if it is stored appropriately (at +4°C with the addition of preservatives).

EXPERIMENTAL

The isolation of lactoferrin was carried out in the microvariant according to [1] with modifications consisting in the preparative defatting of the milk by centrifugation at 3000 rpm for 20 min, and the performance of chromatography in a column with elution of the iron-saturated lactoferrin by a buffer with a higher ionic strength (0.5 M NaCl).

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Electrophoresis in PAAG in an acid medium was carried out by a standard method [5].

Electrophoresis in PAAG in the presence of NaDDS was carried out as described by Weber and Osborn [6].

The N-terminal acid in the protein preparation obtained was analyzed by the method of Belen'kii et al. [7].

Activation of the Support. Sepharose 4B was taken as the support for the mobilization of the antibodies to lactoferrin. Sepharose 4B (10 ml) was washed on a No. 3 glass filter with 150 ml of distilled water. It was then suspended in 30 ml of distilled water and the pH was brought to 11 with the aid of 2 M NaOH. With stirring, 25 ml of an aqueous solution of cyanogen bromide (25 mg/ml) was added [8]. The reaction was performed, with the pH being kept constant, for several minutes at room temperature. The activated Sepharose was then washed with 500 ml of 0.1 M $NaHCO_3$, pH 8.3, on a glass filter.

The immobilization of the antibodies to lactoferrin on the activated Sepharose was carried out in accordance with the "Pharmacia" recommendations for a commercial preparation of BrCN-Sepharose [8]. The unoccupied active sites of the aminosorbent were blocked by the passage through it of a 0.2 M solution of glycine at pH 8.0. The lactoferrin was eluted from the immunosorbent with 0.02 M phosphate-citrate buffer, pH 2.0, containing 0.25 M NaCl. The lactoferrin eluted from the immunosorbent was dialyzed against distilled water and freeze-dried.

SUMMARY

An affinity immunosorbent permitting the isolation of homogeneous lactoferrin from breast milk serum has been obtained by the covalent conjugation of monospecific antibodies to lactoferrin with an inert Sepharose support.

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