ISOLATION OF LACTOFERRIN FROM BREAST MILK AND ITS PHYSICOCHEMICAL PARAMETERS

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Lactoferrin or lactotransferrin – a nonheme iron-containing protein of various exocrine secretions of the organism and also of neutrophilic blood granulocytes – fulfills the function of transporting iron and other metal ions [1, 2] and is at the same time one of the factors of the bactericidal system of the phagocytes [3].

In the present paper we describe the isolation of lactoferrin from breast milk by a modified method giving a higher yield of this protein as compared with the methods proposed by other workers [4]. The modification consists in the preliminary defatting of the milk by centrifugation, performing the chromatographic operation in a column, and eluting the lactoferrin from a cation-exchange resin with 0.5 M NaCl in neutral 0.01 M sodium phosphate buffer. Figure 1 gives a typical elution profile of the proteins of the milk serum from CM-Sephadex. The fraction of the fifth chromatographic peak, eluted at a 0.5 M concentration of salt also had a characteristic pink coloration. To identify the protein present in the fractions of the fifth chromatographic peak as lactoferrin, it was subjected to physicochemical investigation and the results obtained were compared with those known from the literature for lactoferrin.

The purity of the protein obtained was estimated with the aid of two variants of electrophoresis in polyacrylamide gel (PAAG). On electrophoresis in an acid medium, a single protein zone was revealed. On electrophoresis in PAAG with NaSDS, the protein of the fifth chromatographic peak likewise migrated in the gel as a single component which corresponded in its electrophoretic mobility to a polypeptide with a molecular weight of about 79,000 daltons. The lactoferrins of cows' milk [2] and of mouse milk [5] have similar molecular weights. When the gel was stained by Rafikov's method [6], these protein zones were colored red-brown, which is characteristic for iron-binding proteins. The capacity of the protein that we isolated from the breast milk serum for binding iron in stoichiometric ratios in the presence

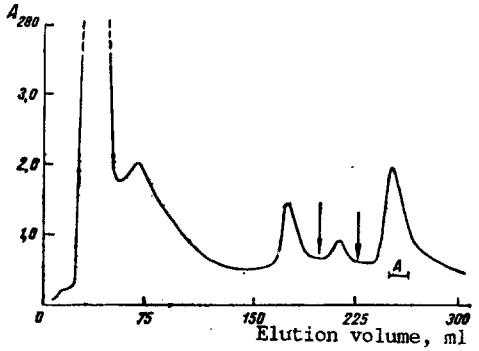

Fig. 1. Elution profile of the proteins of breast milk serum on a column of CM-Sephadex. The first arrow shows the beginning of elution with 0.01 M sodium phosphate buffer, pH 7.0, containing 0.33 M NaCl as proposed in [4], and the second arrow the same buffer but containing 0.5 M NaCl with which the lactoferrin (fraction A) is eluted completely.

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of obligate bicarbonate anions with the formation of pink-colored complexes possessing a characteristic absorption spectrum in the visible region with a maximum at 470 nm also permitted it to be identified as lactoferrin. Since transferrin, which is present in milk in definite amounts, possesses similar properties we made a qualitative analysis of the N-terminal amino acid. In the protein analyzed this was glycine, which is characteristic for lactoferrin, and not valine, which is characteristic for transferrin.

Using this method, it is possible to obtain about 150 mg of lactoferrin from 100 ml of milk.

LITERATURE CITED

1. E. Ainscough, A. M. Brodie, and J. E. Plowman, Am. J. Clin. Nutr., 33, 1314 (1980).
2. C. S. Wang, W. Y. Chan, and H. U. Loer, Comparative Biochem. Physiol., B 78, No. 3, p. 575 (1984).
3. A. I. Kudryavitskii, Lab. Delo, 1, 45 (1980).
4. P. Querinjean, P. L. Masson, and J. F. Heremans, Eur. J. Biochem., 20, 420 (1971).
5. J. M. Kinkade, W. W. Miller, and F. M. Segars, Biochim. Biophys. Acta, 446, 407 (1976).
6. Kh. S. Rafikov, Lab. Delo, 12, 7 (1977).

PREPARATION AND PROPERTIES OF A CYSTEINE ANALOG OF HUMAN INSULIN

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In connection with a study of the structural and functional organization of insulin, we have obtained a previously unknown analog of human insulin differing from the natural hormone by the replacement of the L-threonine residue in the B^{30} position by a protected L-cysteine residue.

The tert-butyl ester of S-tert-butyl-L-cysteine-B^{30}-insulin (I, R = de-Thr-B^{30}-(human insulin)) was obtaned by an enzymatic-chemical method using a scheme involving the tryptic transamidation of porcine insulin (II, R = de-Ala-B^{30}-(porcine insulin)).

$$\underset{\text{I}}{R-Cys\,(Bu^t)-OBu^t} \qquad \underset{\text{II}}{R-Ala-OH} \qquad \underset{\text{III}}{H-Cys\,(Bu^t)-OBu^t}$$

The trypsin-catalyzed transamidation of porcine insulin (II) takes place on the interaction of the latter with the tert-butyl ester of S-tert-butyl-L-cysteine (III) in an aqueous organic medium (water–dimethylformamide) at 25°C and pH 6.3. Under these conditions, transamidation takes place only at the Lys^{B29} residue, and the undesirable side reaction of the Arg^{B22} residue does not take place.

The compound (I) formed as the result of transamidation was purified by ion-exchange chromatography on DEAE-Sephadex A-25 followed by gel filtration on Sephadex G-25F. The course and degree of purification were monitored by thin-layer chromatography on silica gel, by electrophoresis in cellulose, and by disc electrophoresis in polyacrylamide gel.

After the lyophilization of the eluate, compound (I) was obtained in analytically pure form.

tert-Butyl Ester of S-tert-Butyl-L-cysteine-B^{30}-(human insulin) (I). R_f 0.48 (C_5H_5N–C_4H_9OH–H_2O, 10:15:3:12), 0.78 (iso-C_3H_7OH – 25% NH_4OH, 7:4, 0.8 (C_5H_5N–CH_3COCH_3–H_2O, 1:1:2), 0.96 (iso-C_3H_7OH – 25% NH_4OH–H_2O, 7:4:6) (TLC on Silufol UV-254 plates; Pauly revealing reagent [1]). Electrophoretic mobility: 1.5 (electrophoresis on Whatman No. 1 paper, 450 V, 7 mV, pH 1.9; reference standard: the bis-S-sulfonate of the B-chain of human

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