Isolation and partial characterization of ovine lactoferrin[1]

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Summary. Ovine lactoferrin was isolated by ammonium sulfate precipitation and ion exchange chromatography. Comparative analyses were performed with the bovine protein. Differences in the amino acid compositions and the tertiary structure of the proteins were observed.

Lactoferrin is an iron-binding glycoprotein which has been isolated from milk of various species and also identified in a variety of mammalian secretions[2]. However, ovine lactoferrin has never been clearly identified in spite of several investigations[3,4]. The finding of a protein in ewe milk immunologically cross-reacting with an antibody against bovine lactoferrin encouraged us to undertake the present study.

Experimental. The protein was prepared from 6 l of ewe skim milk by successive precipitations with ammonium sulfate until a final salt concentration of 70% was obtained[5]. The sediment was throughly dialyzed against phosphate buffer, pH 7.2 0.05 M, then applied to a column (2.5 × 50 cm) filled with Sephadex DEAE A-50 equilibrated with the same buffer. The outlet of the column was connected to the inlet of a 2nd column (1.5 × 30 cm) filled with Sephadex CM C-50 equilibrated with the same buffer. Lactoferrin did not adsorb to the anion exchanger, but did to the cation exchanger, as a concentrated red band. The protein was then eluted from the 2nd column by increasing stepwise the molarity of NaCl in the buffer. Bovine lactoferrin and antibodies to ovine lactoferrin were obtained as described elsewhere[6]. Goat lactoferrin was a generous gift from Dr N. Bilic. Amino acid analyses were carried out on a Beckman Unichrom analyzer. Tryptophan determination was performed according to the method of Pajot[7]. The microcomplement fixation analyses were carried out according to Levine[8]. SDS-electrophoresis was done as described by Weber et al.[9].

Results and discussion. Ovine lactoferrin was eluted from the cation exchanger at a NaCl molarity of 0.35, thus indicating a lower pI than that of the bovine protein eluted from the cation exchanger at a NaCl molarity of 0.27[5].

The protein obtained was found to be homogenous on polyacrylamide gel electrophoresis at pH 2.5, 8.6 and 11.2 and on SDS-electrophoresis. Analyses by immuno-electrophoresis showed only one precipitin band with an antiserum against the whole ewe whey proteins.

The iron-binding capacity of the protein was studied by adding increasing amounts of iron to a solution of apolactoferrin (3.0 mg/ml) dissolved in HEPES-buffer pH 7.0 0.05 M[6]. The absorption monitored at 460 nm increased linearly until a plateau was reached corresponding to the saturation of the protein. The point of inflexion of the curve corresponded to a molar ratio of iron to protein of approximately 2-1.

The comparison of the relative electrophoretic mobilities of the ovine and bovine proteins showed a lower pI for the ovine lactoferrin than for the bovine protein, as values of 5.10 $cm^2 \cdot V^{-1} \cdot sec^{-1}$ and 2.68 $cm^2 \cdot V^{-1} \cdot sec^{-1}$ respectively were measured in a 3.5% polyacrylamide gel at pH 8.6. The reason for the lower pI of the ovine lactoferrin could be found in the composition of its amino acids, since there are 9 more acidic and 5 less basic residues in the ovine than in the bovine protein (table). The determination of the molecular weight of the molecule presented some discrepancies depending on the method used for the determination. A mol.wt of 94,000 was found by gel filtration on Sephadex G-150 (column 1.5 × 100 cm, Tris-HCl buffer pH 7.6 0.1 M containing 2.0 M NaCl). A value of 88,000 ± 1200 was obtained by the electrophoresis in the presence of SDS. Finally a value of 91,000 was found after treatment of the results of the amino acid analyses according to the calculation of Delaage[10] to find the most coherent molecular weight. Immunologically, a complete crossreaction was

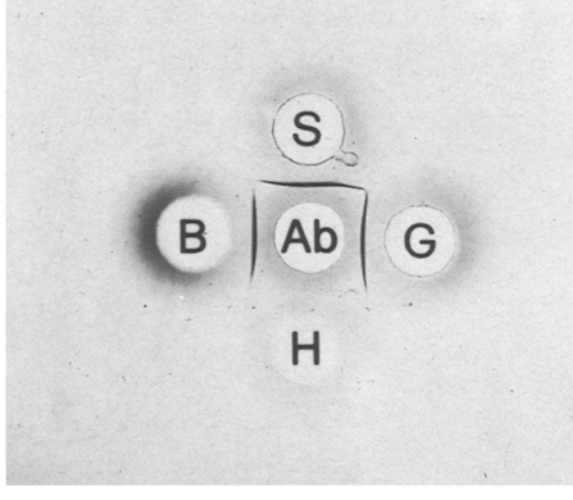

Fig. 1. Double immunodiffusion in 2 dimensions. Center well (Ab): rabbit antiserum to ovine lactoferrin. Peripheral wells (B) bovine, (S) ovine, (G) goat and (H) human lactoferrin.

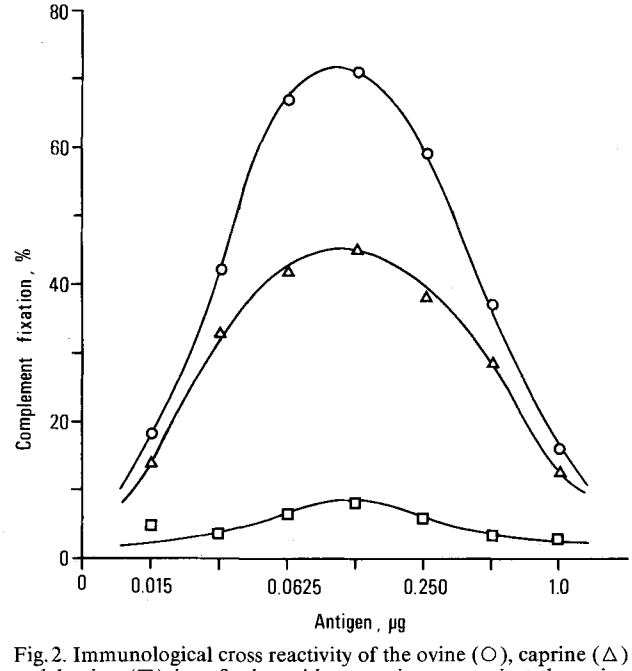

Fig. 2. Immunological cross reactivity of the ovine (○), caprine (△) and bovine (□) lactoferrins with an antiserum against the ovine protein, measured by the technique of the micro-complement fixation.

Amino acid composition of ovine and bovine lactoferrins

Amino acid residue	Ovine protein	Bovine protein
Aspartic acid	67	62
Threonine	36	37
Serine	48	44
Proline	34	35
Glutamic acid	71	67
Glycine	52	48
Alanine	67	66
Valine	44	45
Half cystine	31	35
Methionine	8	9
Isoleucine	15	17
Leucine	62	66
Tyrosine	24	21
Phenylalanine	24	29
Lysine	50	52
Histidine	9	10
Arginine	32	35
Tryptophan	15	15

The results are expressed as the number of amino acid residues per molecule, calculated on a mol. wt of 88,000 for both proteins.

observed between ovine and caprine lactoferrin using an antiserum against the ovine protein, as demonstrated by the method of double immunodiffusion in 2 dimensions (figure 1). However, only a partial fusion of the precipitin bands was observed between the ovine and bovine proteins. No cross reaction could be detected with human lactoferrin. This serological behaviour could be demonstrated much better using the method of microcomplement fixation. Figure 2 shows that the amount of complement fixed by the caprine lactoferrin reacted with the antiserum against the ovine protein was about $^2/_3$ of the amount of complement fixed by the homologous antigen, whereas there was almost no reaction with the bovine lactoferrin. These results demonstrate that ovine and bovine lactoferrin have similar characteristics. The values found for the mol.wt of the ovine protein vary between 88,000 ± 1200 and 94,000 and are in good agreement with the values published for the molecular weight of bovine lactoferrin, 86,000[11] to 93,000 ± 2000[12]. However, some differences in the amino acid compositions are responsible for a lower pI of the ovine protein, and most probably cause some subtle changes in the structural conformation of the molecules, as observed serologically.

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2 P. Masson, J.F. Heremans and C. Dive, Clin. chim. Acta *14*, 735 (1966).
3 P. Masson, in: La lactoferrine, p. 172. Arscia 1970.
4 P. Masson and J.F. Heremans, Comp. Biochem. Physiol. *39B*, 119 (1971).
5 A. Baer, M. Oroz and B. Blanc, Milchwissenschaft *31*, 649 (1976).
6 A. Baer, M. Oroz and B. Blanc, J. Dairy Res. *46*, 83 (1979).
7 P. Pajot, Eur. J. Biochem. *63*, 263 (1976).
8 L. Levine, in: Handbook of Experimental Immunology, p. 707. Ed. D.M. Weir. Blackwell Scientific Publ., Oxford 1967.
9 K. Weber and M. Osborn, The Proteins, Vol. 1, Chap. 3. Ed. H. Neurath and R. Hill. Academic Press, New York 1975.
10 M. Delaage, Biochem. biophys. Acta *168*, 573 (1968).
11 R. Parry, Jr and E. Brown, in: Advances in Experimental Medicine and Biology: Protein-Metal Interactions, p. 141. Ed. M. Friedman. Plenum Press New York 1974.
12 R. Weiner and S. Szuchet, Biochem. biophys. Acta *393*, 143 (1975).

Effect of bromocriptine on gonadal ornithine decarboxylase in vitro

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Summary. The alterations in ornithine decarboxylase activity in response to in vitro treatment with bromocriptine suggest the possible direct action of the drug on gonads.

The evidence has been reviewed previously[1,2] that bromocriptine and related drugs act, primarily, directly on the pituitary to suppress prolactin release; however, the exact mode of action has not yet been elucidated. We present here some data on the in vitro bromocriptine (CB-154) induced changes in ornithine decarboxylase (ODC) activity in the testis and ovary of the rat, since it is thought that ODC is the rate-limiting enzyme in the polyamine biosynthetic pathway[3-5] and that its activity is an indicator of hormonal stimulation.

Materials and methods. Immature (23-24-day-old) pubertal (36-37-day-old) and adult Holtzman strain, male and female rats were used. They were housed with free access to water and food, in a light controlled (14 h light 10 h dark) and temperature controlled (26 ± 1 °C) room. In order to minimize any diurnal variation in enzyme activity, experiments were designed to allow killing of the animals between 09.00 and 12.00 h. The drug, CB-154 (a gift from Sandoz Ltd, Switzerland) was dissolved in a small amount of alcohol and the solution was then diluted with distilled water to the required volume. The doses of 10, 20 and 50 ng per testis or ovary were used for incubation. The whole tissues were incubated in Krebs-Ringer buffer pH 7.2, with or without the drug, in vitro at 37 °C for 4 h in a Dubnoff metabolic shaker. After the incubation period, the tissues were homogenized in a small volume of Tris-EDTA-HCl buffer, pH 7.7, containing 1 mM dithiorthreitol. The ODC activity was determined from the 3000 × g supernatant by measuring the liberation of $^{14}CO_2$ from 0.5 µCi of D,L-[1-^{14}C] ornithine (58 mCi/mm, New England Nuclear) during incubation at 37 °C for 30 min as described previously[6,7]. Results were expressed as $^{14}CO_2$ released/mg protein. Proteins were measured according to Lowry et al.[8] using bovine serum albumin as standard.

Results and discussion. Bromocriptine, when incubated with the testis or ovary, in vitro, brought about changes in the ODC activity in prepubertal, pubertal and adult male as well as female rats. An increase in the enzyme activity was found in testis and ovary of immature animals treated with