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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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

ODC activity in the rat testes and ovaries after in vitro treatment with CB-154

		Control	CB-154 10 ng	20 ng	50 ng
Males	24–26 days	1.11 ± 0.08	0.73* ± 0.09	0.58* ± 0.02	3.99** ± 0.67
	35–37 days	4.80 ± 0.28	61.16** ± 3.71	7.35* ± 1.52	3.84 ± 0.46
	90–92 days	4.43 ± 0.07	0.95** ± 0.14	0.94** ± 0.16	4.32 ± 0.31
Females	24–26 days	3.68 ± 0.15	5.22* ± 0.40	3.89 ± 0.27	22.7** ± 4.71
	35–37 days	66.98 ± 2.16	3.89** ± 0.39	26.57** ± 2.04	4.59** ± 0.57
	90–92 days, diestrous	3.29 ± 0.25	4.18 ± 0.35	4.71* ± 0.27	28.01** ± 1.56
	90–92 days, proestrous	9.84 ± 0.44	10.26 ± 0.44	15.80 ± 0.32	18.43** ± 1.36
	90–92 days, estrous	8.83 ± 0.44	4.16* ± 0.32	3.14** ± 0.08	3.46** ± 0.23

Values are expressed as mean ± SD, pmoles $^{14}CO_2$ released/mg protein (n = 5). * $p < 0.02$; ** $p < 0.001$.

50 ng of bromocriptine. In case of 37-day-old male rats, a drastic increase in the enzyme activity was observed at the 10-ng dose level. Unlike the male rats, female rat ovaries showed low enzyme activity at all the 3 dose levels. CB-154 also decreased ODC activity in the testis of adult male rats, at the dose level of 10 ng and 20 ng. In addition, ovaries of adult animals responded to CB-154, and the ODC activity was higher in proestrous and diestrous females, whereas ovaries from estrous females showed a low enzyme activity after the in vitro drug incubation. These alterations in the ODC activity are not dose-related in all cases. Seppala et al.[9] have suggested 4 possible actions of CB-154 in normoprolactinaemic patients: direct action on gonads, stimulation of secretion of gonadotropins, stimulation of secretion of LHRH and prolactin suppression. The recent report by Sheth and Shah[10] indicates a possible action of the drug at the hypothalamic level. The present findings also suggest a direct action of bromocriptine on the gonads.

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Purification of an L-fucose binding lectin from *Ulex europeus* by affinity column chromatography

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Summary. An L-fucose binding lectin from *Ulex europeus* was purified by affinity column chromatography using an L-fucose-starch complex. The lectin thus purified had a mol.wt of 60,000, and consisted of 2 glycoprotein subunits with mol.wt 29,000 and 31,000, respectively.

It has been known for some time that extracts of many plant seeds contain hemagglutinins which are called lectins[1]. Lectins have recently been used for the determination of human and animal blood group factors[2], examination of the surface structure of various animal cells[3,4] and clarification of the mechanism of blast formation of lymphocytes[5]. In order to carry out further studies precisely, purification and characterization of the lectins is required. In this report, we describe attempts to purify and characterize the L-fucose binding lectin of *Ulex europeus* seeds, the crude extract of which has been routinely used for the diagnosis of secretors[6] and for the determination of subgroups of A and AB blood groups[7]. In addition, differences found between our purified lectin and that so far reported[8] are discussed.

Materials and methods. It was already known that Ulex seeds contain 2 kinds of lectins with distinct anti-H(O) specifities. The 2 lectins are designated type I and type II; they are able to bind specifically to L-fucose and di-N-acetylchitobiose, respectively[8]. In our case, extraction and salt precipitation with $(NH_4)_2SO_4$ were performed on type I lectin by the method of Matsumoto and Osawa[8]. In brief, 100 g of powdered seeds (P-L, Biochemicals Inc.) were extracted with 500 ml of phosphate buffered saline (PBS, pH 7.4) at 4 °C for 1 day. Then the extract was centrifuged to obtain a crude supernatant and saturated. $(NH_4)_2SO_4$ was added slowly to the supernatant with stirring to give 0.4 saturation. The precipitate thus formed was taken and dissolved in 100 ml of PBS and dialysed against the same buffer. The dialysed solution was centrifuged in order to apply the supernatant to the affinity column (2×4 cm) consisting of an L-fucose-starch complex prepared by the method used by Matsumoto and Osawa[9]. The column was eluted with the same buffer and the absorbance at 280 nm was determined. After disappearence of the protein from the effluents, the lectin which was absorbed to the column was eluted out with 0.05 M glycine buffer (pH 3.0). The eluates were then dialysed against PBS, concentrated using an Amicon diaflow membrane apparatus with a UM10 membrane, and submitted to tests for purity and hemagglutinating activity. The latter test was carried out on human erythrocytes according to the standard method[8]. Disc electrophoresis was performed in 10% polyacrylamid in gels at pH 9.5 by Davis' method[10] and SDS-polyacrylamide gel electrophoresis (SDS-Page) was done in the presence or absence of 2-mercaptoethanol, a reducing agent, by the method of Weber and Osborn[11].