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].

Purification of *Ulex europeus* type I lectin

Fraction	Total protein (mg)	M.H.D.[a] (μg/ml)	Specific activity	Total activity	Recovery (%)
Original	63.7	38	1	63.7	100
Affinity column					
Fraction 1[b]	63.5	995	0.038	-	-
Fraction 2[c]	0.62	1.3	29	18.0	28.3

[a]Minimal hemagglutinating dose against human 0 type erythrocytes; [b]column not adsorbed fraction; [c]column adsorbed fraction. -; not done.

Proteins were stained with Coomassie brilliant blue and glycoproteins by PAS staining[10].

Results. As described above, the crude Ulex lectin (type I) was applied to the affinity column and eluted with glycine buffer. The results pertaining to purification of the lectin are summarized in the table. The purified lectin had a hemagglutinating activity about 30-fold higher than the original preparation. The recovery rate by this method was 28%. Then disc gel electrophoresis was carried out to examine the homogeneity of the lectin as indicated in figure 1. Here, as shown in *A*, a major band was observed in the polyacrylamide gel electrophoresis at pH 9.5. In *B* and *C*, 2 glycoprotein bands were seen in the SDS-polyacrylamide gel electrophoresis irrespective of the presence or absence of the reducing agent. 2 subunits were found to have the mol.wts of 29,000 and 31,000 respectively on the basis of several markers with varying molecular weights. When the purified lectin was chromatographed on Sephadex G-150, a single symmetrical peak corresponding to the mol.wt of 60,000 was observed (figure 2). These results indicate that the purified Ulex lectin (type I) had a mol.wt of 60,000, consisting of 2 noncovalently associated glycoproteins.

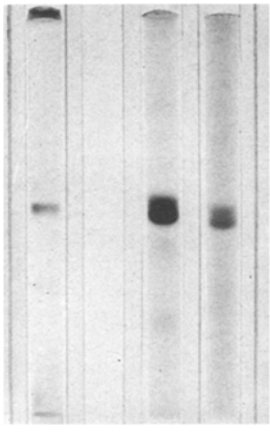

Fig. 1. Polyacrylamide gel electrophoresis (Page) patterns of purified lectin. *A* Page at pH 9.5, Coomassie brilliant blue stain; *B* SDS-Page in the presence of 2-mercaptoethanol, Coomassie brilliant blue stain, *C* same as *B* PAS stain instead of Coomassie brilliant blue stain.

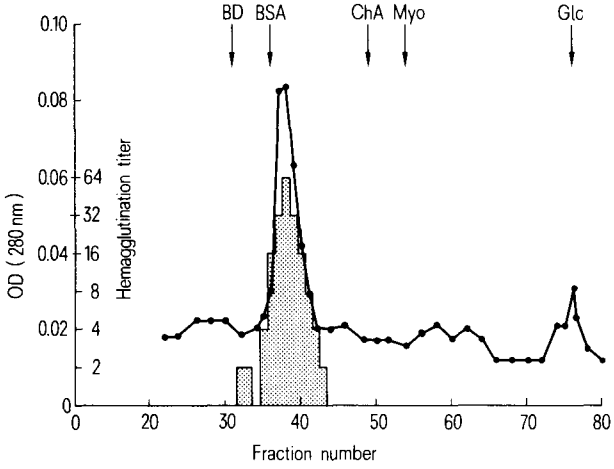

Fig. 2. Gel filtration of purified lectin on Sephadex G-150. 0.5 mg of purified lectin was applied onto the Sephadex G-150 column (1.2×65 cm). Elution was carried out with PBS and fractions of 1 ml were collected at a flow rate of 3 ml/h. ●——●, OD_{280}; hemagglutinating activity is denoted by shaded portions. The elution fractions of blue dextran, bovine serum albumin, chymotrypsinogen A, myoglobin and glucose were indicated.

Discussion. Affinity column chromatography using a sugar-starch complex, such as L-fucose- and tri-N-acetylchitotriose-starch, has successfully been applied to the purification of various lectins from eel serum, *Ulex europeus* (type II) and *Cytisus sessilifolius* by Matsumoto and Osawa[9]. However, when they applied the Ulex lectin (tape I) to the L-fucose-starch complex column, the lectin was not adsorbed to the column, though the elution was apparently retarded. In our experiments, we succeeded in purifying Ulex lectin (type I) on the same adsorbent column. Namely, almost all the hemagglutinating activity applied to the column was adsorbed and recovered in an active form. The apparently low recovery, however, would be due to the inactivation or loss of lectin caused by concentration through diaflow membrane (Amicon). The activity towards human O type erythrocytes was inhibited by L-fucose, but not by other sugars, such as D-galactose, D-glucose, D-mannose, L-Rhamnose, N-acetylglucosamine and di-N-acetylchitobiose, indicating that the lectin was specific for L-fucose (data not shown). The results of our experiments revealed that the purified lectin had a mol.wt of 60,000, consisting of 2 associated glycoproteins with mol.wts of 29,000 (α) and 31,000 (β). However, because the 2 lines detected by SDS-Page are not of equal intensity, there is another possibility, that the lectin is composed of a mixture of associated subunits, $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$. The type I lectin purified by other investigators using ion-exchange and gel filtration column chromatography had a mol.wt of 170,000[8] As a small amount of hemagglutinating activity was found in the void volume of the Sephadex G-150 column in our experiments, this might correspond to the lectin of mol.wt 170,000. On the basis of these results, we suggest that the lectin of high mol.wt might be a complex of 3 lectin molecules.

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