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ISOLATION OF A KETO SUGAR SPECIFIC AGGLUTININ FROM RAT UTERUS

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A ketosugar-specific agglutinin has been identified and purified to apparent homogeneity by affinity chromatography from the three days post-coital rat uterus. This protein is not found in other stages of estrous cycle. The agglutinin is glycoprotein, moves as single band with molecular weight 37,000 in polyacrylamide gel electrophoresis and pI is 4.2. Among the ketosugars, fructose and ribulose effectively inhibit the agglutinin activity, beside these sugars, mannose, glucose, α methyl-mannoside and glucoside are also effective as potent inhibitors. Possible roles of this protein in pregnancy are discussed.

Agglutinins are carbohydrate-binding proteins or glycoproteins which cause agglutination of washed rabbit erythrocytes (1). Recently a sialic acid-binding agglutinin has been isolated from cycling rat uterus (2). In the present paper, identification and isolation of another agglutinin, ketosugar-binding, which was found only in the post-coital uterus, has been reported.

MATERIALS AND METHODS

Animals: Swiss albino female rats were obtained from the animal facilities of our Institute. They were maintained under standard light and temperature conditions; food and water was given ad libitum. Their vaginal smear were checked every morning. Animals in proestrus were caged with proven fertility males for overnight and the smear were checked on the next morning. The animals having sperm positive smear were taken as day 0 of pregnancy. Pregnant animals were sacrificed after 3 days of pregnancy and the uterie were collected and kept frozen at -20°C till they were processed for agglutinin isolation.

For isolation of agglutinin the uterie were weighed and homogenised in 0.02M Tris-HCl buffer pH 8.0, containing 0.15M

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NaCl and 2% NaN_3. The homogenates were centrifuged at 2000 rpm at 4°C and the crude supernatant was purified by affinity chromatography, using Concanavalin A, Sepharose-4B column prepared by the technique as described by Cutracasas and Anfinsen (3). The bound materials were eluted by 40 mM mannose in Tris buffer. The effluents and eluants were monitored at 280 nm, the eluted protein peak was dialysed and concentrated by ultrafiltration. Total protein was determined by method of Lowry et al. (4) and total carbohydrate was estimated by phenol sulphuric acid method (5).

The purified protein was analysed by 10% alkaline polyacrylamide gel electrophoresis (6) and SDS gel electrophoresis using 4% stacking and 10% sample gels containing SDS with or without β-mercaptoethanol (7). Isoelectric focussing was performed on thin layer polyacrylamide gel using 5% polyacrylamide gel containing 2% carrier pharmalytes (pH 3 to 10) by the method of Rufo et al. (8). Haemmagglutination tests were done using Takatsy microtitrator (Laxbro Mfg. Co., India) with 25 ul loops and 2% suspension of rabbit erythrocytes. Specific inhibitory effects of different mono and di-saccharides were determined by adding the sugars in serial two fold dilutions to the microtiter plates and the titer which is necessary to inhibit 50% of the haemmagglutination activity of the agglutinin was recorded. Optimum temperature required for agglutination was determined by performing the agglutination test at different temperature of 10°, 22° and 37°C. The effect of pH (5-10) and metal ions (Ca^{++}, Mg^{++}, Mn^{++}) was studied at 22°C. The purified agglutinin was assayed for estrogen and progesterone binding activities (9) and acid phosphatase (EC 3.1.3.2) and peroxidase (EC 1.11.1.7) enzyme activities (10).

RESULTS

The ketosugar specific agglutinin which was purified by Con A sepharose affinity chromatography from 3 days post-coital rat uterus was absent in the non-pregnant cycling uterus (Table 1). Optimum hemmagglutinin activity was observed at pH 7.4 and 22°C, metallic ions did not have additive effect. The agglutinin is glycoprotein in nature as shown by total carbohydrate and protein estimation.

Table 1 : Purification of ketosugar - specific uterine lectin

Source	Volume (ml)	Total protein (mg)	Total titer	Specific Activity (Total titer/mg protein)
Pregnant uterus (3 days)	2	6.64	5120	771
Affinity purified	4	0.4	5120	12800

Non-pregnant cycling uterine homogenate is -Ve for ketosugar-agglutinin activity.

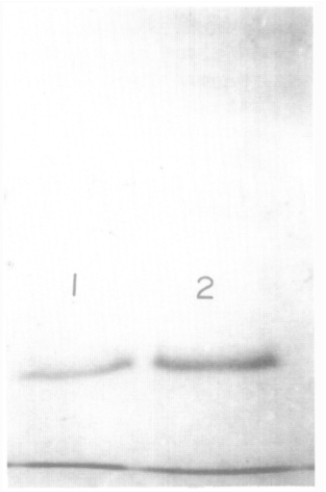

Fig. 1. Polyacrylamide gel electrophoresis under denaturing conditions of agglutinin isolated from pregnant uterus by Con A - Sepharose 4B. Lane 1-10 ug protein with SDS, Lane 2-10 ug protein with SDS and β-mercaptoethanol.

The purified protein moved as a single band in the alkaline gel, and SDS gel electrophoresis having 37,000 molecular weight, treatment with β-mercaptoethanol did not alter the band pattern (Fig. 1). The pI of the protein was found to be 4.2.

Sugar inhibition test performed with various mono and disaccharides (Table 2) showed that hexoses, D-mannose and D-fructose were the potent inhibitors of the agglutinin activity while D-glucose appeared to be less potent. Galactose, in both D or L form was found to be inactive. Of the pentoses tested, only ketopentose ribulose was found to inhibit the agglutinin activity however, another ketopentose, xylulose was without any inhibitory activity. α-methyl glucoside and mannoside were the most potent inhibitors found for this agglutinin, whereas N-acetyl glucosamine and mannosamine, N-acetyl neuraminic acid and most of the disaccharides were found to be inactive.

The purified agglutinin does not have progesterone or estrogen receptor binding activity and acid phosphatase or peroxidase enzyme activity.

Table 2. Inhibition of haemagglutination activity by various mono and di-saccharides. The lowest concentration of sugar that reduced haemmagglutination titer by one step is referred to as the concentration needed for 50% inhibition of lectin activity.

Sugar	Minimum concentration (mM)
Fructose	3.47
Mannose	3.47
Glucose	6.94
Ribulose	1.04
α-methyl mannoside	0.4
α-methyl glucoside	0.8

D and L - Galactose, ribose, fucose, arabinose, xylulose, N-acetyl glucosamine, mannosamine, sucrose, mellibiose, maltose, cellobiose, N-acetyl neuraminic acid did not inhibit binding even at 25 mM. All the sugars were purchased from Sigma Chemicals, U.S.A.

DISCUSSION

The present study shows that the agglutinin purified from the post-coital rat uterus, binds specifically to mannose and ketosugars such as fructose and ribulose which are also the active components of seminal plasma (11-12). The inhibition pattern with different sugars indicates that steric arrangement of hydroxyl groups at C3 and C4 positions are important factors to determine the potency of the sugar as an inhibitor of the agglutinin. Presence of α-methyl group at position C1 magnifies the inhibitory potency whereas presence of N-acetyl amino group at C2 position destroys the inhibitory activity. However, the presence of keto group at C2 position increases the potency as an inhibitor. In the case of xylulose the steric arrangement of hydroxyl group at C3 position may cause the loss of inhibition potency

Unlike some other uterine proteins, this protein is not associated with steroid receptor activity or peroxidase or acid phosphatase enzyme activity (9, 13-14).

The physiological role of this agglutinin is currently uncertain. Since it appears at the early part of pregnancy, it may play a role in maintenance of spermatozoa in the female tract by concentrating hexoses (fructose, mannose, glucose), the group of sugars shown to be metabolised by sperms in vitro (15). Furthermore, this agglutinin not only binds to Con A, but also has Con A-like specificity for binding to mannose and glucose. Since preimplantation embryo has Con A receptors on their surface (16-17), agglutinin may play a role in directing the embryo to its nesting site in endometrium. Although the production site of this protein is not identified yet, assumptions can be made that either uterus or oviduct-fertilized ovum complex or even the male gametes are responsible for synthesis of this protein.

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