PROTEASE ACTIVITY IN RABBIT UTERINE SECRETION 24 HOURS BEFORE IMPLANTATION

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Summary. Protease activity in rabbit uterine secretion, 24 hr before implantation, was demonstrated by film gelatine lysis. The enzyme activity could be localized in the β-glycoprotein fraction. The authors assume a relation between uterine protease and the implantation of the blastocyst.

During the preimplantation period, the protein pattern of rabbit uterine secretion is characterized by several pregnancy-specific proteins. One of these, the β-glycoprotein (synonymous with glycoprotein II) is only secreted after the 6th day (i.e. 144 hr) post coitum (Kirchner, 1969a, b, 1971). At the same time, it was possible to demonstrate an increasing proteolytic activity in the uterine secretion. The method of collection of the uterine secretion and the purification of several uterine protein fractions by gel chromatography has been described previously (Kirchner, 1969a). The mean purification steps were ion-exchange chromatography on DEAE Sephadex A 50, NaCl gradient 0 to 0·4 m in 0·1 n-tris-HCl buffer, pH 8·0. The polyacrylamide (PAA) disc electrophoresis was carried out with buffer, pH 9·0; 2 mA/column; 3 cm. The cellulose acetate electrophoresis was carried out in the Phoroslide—Millipore system using a veronal buffer pH 8·5, I = 0·1, 100 V, 20 min. The proteolytic activity was detected by film gelatine lysis (Adox Kb 14). The film was incubated with single droplets of test solution for 24 hr at 37°C, and then examined. Semi-quantitative estimation of protease activity was obtained by comparison with trypsin solutions of various known concentrations.

It was possible to obtain 20 mg dry material of uterine secretion from one doe 162 hr post coitum and 0·2 mg of this material dissolved in 10 µl distilled water gave a significant clearing of the film layer (Pl. 1, Fig. 1, dd). Using similar protein amounts of the uterine secretion 120 hr post coitum, we found no clearing of the film layer. The secretion of 144 hr post coitum showed only slight enzyme activity (Pl. 1, fig. 1, bb, cc). An increase of β-glycoprotein secretion appeared to parallel the increase in protease activity (Pl. 1, Fig. 1, b to d).

We incubated complete gel columns after PAA disc electrophoresis of uterine secretion (162 hr post coitum) and were able to localize the protease activity in the β-glycoprotein region (Pl. 1, Fig. 1, d, d'). After isolation of the uterine proteins by gel and ion-exchange chromatography, we found proteolytic activity only in the β-glycoprotein fraction.
By the same technique as described above, the uterine protease was found to be stable to freeze drying, gel filtration, ion-exchange chromatography and electrophoresis. The proteolytic activity was greatly decreased at pH 4. In comparative investigations, 2.5 ng trypsin showed the same clearing after 4 and 7 hr as 200 μg pure β-glycoprotein material. The molecular weight, affinity to basic ion-exchangers, electrophoretic mobility of the uterine protease and its concentration in uterine secretion are all comparable to β-glycoprotein. Iodoacetic acid, e-aminocaproic acid and soy bean inhibitor showed no inhibition, whereas EDTA was perhaps able to inhibit the uterine protease (Pl. 1, Fig. 2, d to h). If the inhibition by EDTA is due to the fact that the enzyme needs metal ions for optimal proteolytic activity, there is no similarity to trypsin and plasmin and the comparison with trypsin is only of theoretical interest.

Dissolution of the zona pellucida and implantation are closely connected with one another in rabbits. Both processes may involve the action of enzymes which are produced and controlled by the maternal organism. The neuraminidase activity in uterine secretion (Schwick, 1965) seems to be of no importance for the dissolution of the zona pellucida (Denker, 1970): it is possible to dissolve the zona by trypsin-like enzymes (Bowmann & McLaren, 1970), as described in the rabbit sperm acrosome (Stambaugh & Buckley, 1969; Zanefeld, Srivastava & Williams, 1969). While Denker (1971) assumes a lysis by a protease localized in the trophoblast, we suggest that there is also a possible lysis by the uterine secretion. It is known that, if the secretion of β-glycoprotein is delayed (e.g. by oestradiol treatment), the blastocysts are coated with a stable zona pellucida (Beier, Kühnel & Petry, 1971). We assume, therefore, that the β-glycoprotein and the uterine protease are really identical.

REFERENCES


Fig. 1. (a–d) Disc electrophoresis of uterine fluids 120 hr (b), 144 hr (c), and 162 hr (d) post coitum in comparison to oestrus (a). Film layers after incubation of droplets of the single uterine fluids (bb–dd). Film layer after incubation of a disc column of uterine fluid 162 hr post coitum (d'). β-Glyco = β-glycoprotein, Tf = transferrin, Ugl = uteroglobin, PA = postalbumin, Alb = albumin, Pr = prealbumin. Arrow: boundary between large pore gel and small pore gel.

Fig. 2. (a–c) Disc (a) and cellulose acetate electrophoresis (c) of β-glycoprotein, purified by Sephadex G 150, and DEAE Sephadex A50. Points = subfractions. Film layers after incubation of a disc column (a') and a cellulose acetate strip (c') of β-glycoprotein. Inhibition tests: film layers after incubation of 0.2 mg β-glycoprotein dissolved in 10 µl distilled water (d), mixed 1:1 with 10% ε-aminocaproic acid (EACA) (e), 10⁻² M-iodoacetic acid (JAA) (f), 10⁻² M ethylenediamine tetraacetate (EDTA) (g), and 0.2 mg/ml soy bean inhibitor (Sbl) (h).

(Facing p. 260)