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Immunological Characterization of Blood Group A* Reactive Protectins from Helix Pomatia

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Summary. The immunoelectrophoretic analysis of the albumin gland extract of Helix pomatia reveals at least 10 precipitation arcs. Absorption experiments have shown 2 of them represent that antibody-like protectins reacting with blood group A rezeptors. One of these protectins reacts with the Forssman antigen as well the other one does not.

Zusammenfassung. Die immunelektrophoretische Analyse des Eiweißdrüsenextraktes von Helix pomatia liefert mindestens 10 Präcipitationsbanden. Absorptionsversuche haben ergeben, daß 2 davon antikörperähnliche Protectine sind, die mit dem Receptor der Blutgruppe A reagieren. Eines davon reagiert auch mit dem Forssman-Antigen, das andere nicht.

Key words: Albumin gland, Helix pomatia — Blood group A antigen — Forssman antigen.

The protein spectrum of serologically active snail albumin gland extracts has been studied and described by several authors [1—3].

This paper is concerned with the immunoelectrophoretic characterization of protein constituents occurring in the albumin gland of Helix pomatia, that are responsible for the prezipitation of human seminal plasma constituents and the agglutination of human sperm cells from donors with different blood groups as described in preceeding papers [4].

These earlier investigations provided some new informations concerning the nature of the blood group A reactive receptor occurring in the male accessory fluids of reproduction [4]. Most recently this receptor has been isolated and purified to a certain degree; it was found to be a glycoprotein containing 7.38% of N-acetyl-neuraminic acid reacting strongly with extracts from the albumin glands of Helix pomatia [5].

The experiments presented here (in Table 1) have shown that our extracts from the albumin glands of Helix pomatia contain two agglutinins reacting with blood group substances A: a major component designated as P_1 and a minor anti-A-(like) protectin designated as P_2.

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Table 1. Characterization of the protectins No. 1 and No. 2 from Helix pomatia by absorption with blood group reactive cells and preparations of various types and origins

No.	Anti-A_{HP} absorbed with	P_1	P_2
1	not absorbed control	+	+
2	human rbc, A-type	∅	∅
3	human rbc, A-type treated with RDE	∅	∅
4	human rbc, B-type	+	+
5	human rbc, 0-type	+	+
6	human rbc, 0-type treated with RDE	∅	∅
7	sheep rbc	∅	∅
8	pig rbc, (hel group)	+	+
9	Forssman antigen (Glycolipid)	∅	+
10	Ovine submaxillary mucine (Gottschalk)	∅	+
11	horse serum (Forssman active)	+	+
12	horse rbc, treated with RDE	∅	+
13	human spermatozoa, A-type	(+)[a]	+
14	human spermatozoa, B-type	+	+
15	human spermatozoa, 0-type treated with RDE	∅	(+)[a]
16	human seminal plasma, pooled	∅	∅
17	blood group A reactive glycoprotein prepared from human seminal plasma	∅	∅
18	blood group A reactive glycoprotein prepared from peptone	∅	∅

[a] Precipitations are markedly reduced as a result of incomplete absorption.

Materials

1. Saline extracts from albumin glands of Helix pomatia were prepared as previously described [6] = *anti-A_{HP}*.

2. Rabbit antiserum No. 129/71 expecially directed against the proteins extracted from albumin glands of Helix pomatia (RAHP) was kindly supplied by Prof. Dr. I. Ishiyama, Tokyo, Japan.

3. Red blood cells were obtained by venipuncture (with sodium citrate), separated by centrifugation for 5 min at 2500 rpm and washed 3 times with saline.

4. Semen from donors with blood group A, B, and 0, were obtained from patients attending the Department of Dermatology for infertility examination. Spermatozoa were separated by centrifugation for ca. 20 min at 3000 rpm. Spermatozoa from 10 patients with blood group A, B, and 0, respectively, were pooled and washed 3 times with saline.

5. Lyophilized seminal plasma prepared from pooled seminal plasma irrespective of donor's blood groups = HSP.

6. Neuraminidase (receptor destroying enzyme) from Vibrio cholerae (500 U/ml) was purchased from Behring-Werke, Marburg (Lahn), GFR = *RDE*.

Methods

1. Immunoelectrophoretic analysis on microscope slides according the method of Scheidegger [7].

2. Absorption of anti-A_{HP} with red blood cells was performed by mixing one volume of anti-A_{HP} with two volumes of packed erythrocytes; after 10 min of incubation at room temperature the mixtures were centrifuged. The sediments were discarded. The supernatants were checked for agglutinin activities and the absorption procedures were continued until no further agglutinations could be demonstrated. Absorption with spermatozoa was done

by the same procedure using 1—3 Mrd washed sperm cells per 0.5 ml anti-A_{HP}. Absorptions with blood group A substances and the Forssman antigen, respectively, were done by mixing 1.0 ml of anti-A_{HP} with 10 mg of the purified lyophilized substances. The mixtures were incubated for 15 min at $+37°C$ and for 16 hrs at $+4°C$, then centrifuged for 15 min at 3000 rpm. The sediments were discarded, the supernatants were checked for complete absorption by precipitation reactions.

3. Treatment with RDE was performed as previously described [8].

Results

The immunoelectrophoretic analysis of anti-A_{HP} with rabbit antiserum No. 129/71 yielded a characteristic protein pattern consisting of at least 10 different precipitation arcs (Fig. 1 a). If anti-A_{HP} was absorbed with blood group A reactive cells and preparations of various origin one or two of these precipitation arcs disappeared (Fig 1 b).

These findings indicate that our extract from the albumin glands of Helix pomatia contains at least 10 different proteins 2 of which are capable of reacting with blood group A reactive materials.

Consequently, the 2 proteins were designated as protectin No. 1 (P_1) and protectin No. 2 (P_2), respectively. P_1 has the electrophoretic mobility of a γ-globulin, P_2 migrates in the region of β-globulins.

The results of our absorption experiments are summarized in Table 1.

When anti-A_{HP} was completely absorbed with the Forssman glycolipid and ovine submaxillary mucine the supernatant was found to contain no further agglutinating activities towards human-A and other animal (enzyme-treated) red cells.

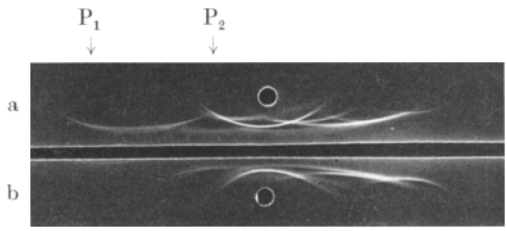

Fig. 1. Immunoelectrophoretic analysis of anti-A_{HP} (upper well) with a specific rabbit antiserum (trough), showing a total of 10 precipitation lines. After absorption with blood group A reactive material of porcine origin (lower well) 2 of these precipitation arcs (P_1 and P_2) have disappeared

Discussion

In contrast to the findings of other authors [1] we were able to show that extracts from the albumin glands of Helix pomatia contain not only one but two proteins reacting with blood group substance A of different origin.

These two proteins (P_1 and P_2) were found to differ with respect to their antigenicities, their electrophoretic mobilities, and even specifities.

Earlier investigations had already shown that the blood group A reactivity of anti-A_{HP} could easily be absorbed with the Forssman glycolipid, ovine sub-

maxillary mucine, and RDE-treated horse erythrocytes [9]. This, however, has now been found to be true only for P_1 whereas P_2 could not be absorbed by these antigens.

Since the supernatants of such absorbed extracts did not contain any further agglutinating or precipitating activities towards A-type cells and blood group substances, it may be concluded that P_2 behaves like an "incomplete" protectin, a fact which should be taken into account when hemagglutination inhibition tests with anti-A_{HP} are interpreted.

With human A-type rbc and sperm cells, however, as well as with RDE-treated O-type rbc and sperm cells, sheep rbc, human seminal plasma, and blood group substance A prepared from peptone and human seminal plasma, both protectins (P_1 and P_2) could be completely absorbed.

These findings clearly indicate that P_1 and P_2 are both reacting with blood group A reactive materials of the glycoprotein type and the glycolipid type; nevertheless, their serological specificities are not completely identical.

We wish to emphasize that the presence of an incomplete agglutinin with a similar anti-A-like specificity may interfere with the saline agglutinating protectin hemagglutination and hemagglutination inhibition studies with crude extracts from Helix pomatia.

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