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Immunochemical Studies on Blood Group A Substance from Human Hair

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Summary. Blood group A-active substance was extracted from urea-treated human hair with methanol-ethyl ether 1:1, v/v) or chloroform-methanol (1:1, v/v). The serological activity of blood group A substance in the hair was destroyed by A-decomposing enzyme from *Clostridium tertium* with concomitant development of blood group H activity. It is concluded therefore that the extract from the hair of group A contained blood group A-active glycolipid with *N*-acetylgalactosamine as the non-reducing sugar.

Zusammenfassung. Das A-blutgruppenaktive Glycolipoid wurde mit Methanol-Äther (1:1, v/v) oder Chloroform-Methanol (1:1, v/v) aus Harnstoff-behandelten Haaren extrahiert. Durch Einwirkung von A-zerstörendem Enzym aus *Clostridium tertium* auf das A-Glycolipoid aus Haaren, kommt nunmehr die H-Eigenschaft nach Inaktivierung von A zum Vorschein. Darum kann es sein, daß *N*-Acetyl-Galactosamin für die A-Aktivität vom Haarglycolipoid benötigt wird.

Key words: Blood groups, A substance from hair – Hair, A-active substance

Previous investigations of human hairs have dealt almost exclusively with their blood grouping [1]. Little attention has been paid to the immunochemical properties of blood group-specific substances obtained from human hair. Sakai [2] has shown that blood group-active substances are present in both water-soluble and ethanol-soluble fractions of human hair.

The present paper concerns certain of the properties of glycolipid obtained from human hair.

Materials and Methods

1. Hair. Hair was collected from the head of an adult woman of group A.

2. Preparation of substances. Water-soluble material was extracted according to the procedure of Steinert and Rogers [3]. The hair was cleaned by immersion in petroleum ether, ethanol and water and then air-dried. It was cut into small pieces (1–2 mm) and crushed with a hammer on an iron bed. The hair was homogenized efficiently in a buffer of 8 M urea, 0,1 M Tris-HCl (pH 9.0), 1 mM EDTA and 0.1 M 2-mercaptoethanol (25 ml/g of hair). The solution was stirred mechanically for 5 hrs, and then treated with iodoacetic acid (200 mg/ml), to a final concentration of 50 mM. This mixture was then stirred for a further 5 hrs. Excess iodoacetic acid was destroyed by the addition

of 2-mercaptoethanol. The solution was filtered through gauze, centrifuged at 38000 g for 10 min to remove debris and then dialysed against 10 mM Tris-HCl (pH 7.6) – 1 mM EDTA buffer. The non-dialysable residue was lyophilized (soluble fraction).

The non-filtrable portion, which could not be dissolved in urea solution, was dialysed against distilled water and also recovered by lyophilization (insoluble fraction).

3. Purification of the soluble fraction. The soluble fraction was purified by the phenol method of Morgan and King [4]. That is to say, 1 g of the lyophilized material was shaken for 1 day and the viscous supernatant was centrifuged. To the phenol solution, which was stirred mechanically, a mixture of equal volumes of ethanol and 90 per cent phenol was slowly added in a dropwise fashion until the final alcohol concentration reached 10 per cent by volume, and after standing overnight at room temperature, no precipitate separated out on centrifugation. The mixture was dialysed against water for 3 days, and then lyophilized after centrifugation.

4. Isolation of glycolipid from the insoluble fraction. Glycolipid was extracted from the insoluble fraction with methanol-ethyl ether (1:1, v/v) and then with chloroform-methanol (1:1, v/v) [5]. Each extract was concentrated to a small volume by rotatory evaporation under reduced pressure and then 20 volumes of cold acetone were added. The white precipitate which separated out on centrifugation was washed with acetone and dried *in vacuo* over P_2O_5.

5. Blood group substance-decomposing enzymes. A substance-decomposing enzyme from *Clostridium tertium* [6] was prepared by the method of Yamamoto and Iseki [7], B substance-decomposing enzyme from *Clostridium sporogenes* Maebashi by the method of Iseki et al. [8], and H substance-decomposing enzyme from *Bacillus fulminans* [9] by the method of Iseki et al. [10].

6. Conditions of incubation of the enzymes. A mixture of equal volumes of a 1 per cent solution of the extract and a 1 per cent solution of the enzyme in phosphate buffer at pH 6.8–7.0 was incubated at 37° C for 2 days.

7. Antisera. Human iso-agglutinins were used for A and B haemagglutination inhibition tests. H-specific haemagglutination inhibition tests were performed with immune chicken anti-H serum and natural eel anti-H serum.

Results

1. Yields of different fractions. Ten g of human hair gave 4 g of water-soluble fraction and 6 g of insoluble fraction after treatment with urea solution. Six g of insoluble fraction gave 5 mg of acetone precipitate after extraction with the methanol-ethyl ether mixture, followed by 4 mg of acetone precipitate after extraction with the chloroform-methanol mixture. One g of the soluble fraction gave 100 mg of purified water-soluble fraction after treatment with phenol.

2. Blood group activity of the soluble fraction. No detectable blood group activity was found in the solube fraction by haemagglutination inhibition tests with either human, eel or chicken anti-reagents (Table 1). Steinert and Rogers [3] have reported that this fraction contains sulphur keratins and other proteins.

The soluble fraction was further purified by the phenol method of Morgan and King [4] and the product obtained also showed no A, B or H activity by haemagglutination inhibition tests (Table 1).

Table 1. Haemagglutination inhibition tests of the urea-soluble fraction

Soluble fraction	Antisera			
	Anti-H (chicken)	Anti-H (eel)	Anti-A (human)	Anti-B (human)
Urea-soluble fr.	>10000*	>10000	>10000	>10000
Phenol-soluble fr.	>10000	>10000	>10000	>10000

* Minimum concentration of each fraction giving complete inhibition (μg/0.1 ml)

3. Blood group activity of the insoluble fraction. Although no blood group activity was present in the soluble fraction as described above, the insoluble residue after urea extraction showed blood group A activity to the same degree as the original hair, as determined by the elution technique. The residue was stirred in methanol-ethyl ether (1:1, v/v) and then in chloroform-methanol (1:1, v/v). The acetone precipitate was assayed for blood group A, B and H activities by the above haemagglutination inhibition tests and it was found that it strongly inhibited human anti-A and immune chicken anti-H agglutinins and wealkly inhibited human anti-B agglutinin (Table 2).

In contrast, no blood group-active substance was obtained from urea-untreated hair by the extraction method with methanol-ethyl ether (1:1, v/v) or chloroform-methanol (1:1, v/v).

4. Action of blood group substance-decomposing enzymes. The acetone precipitates of the extracts obtained from the insoluble fraction with organic solvents, exhibited strong inhibition in an A red cells-Anti-A system and weak inhibition in a B red cells-anti-B system.

To examine the serological activity and chemical structure of these substances, the chloroform-methanol extract was treated with blood group substance-decomposing enzymes, *viz.* A-enzyme from *Clostridium tertium* [6], B-enzyme from *Clostridium sporogenes* Maebashi [8], and H-enzyme from *Bacillus fulminans* [9]. A-enzyme acted on the extract to give loss of A activity and development of H activity. Such a serologica change is to be expected since removal of the terminal *N*-acetyl and galacto-

Table 2. Haemagglutination inhibition tests of the urea-insoluble fraction

Insoluble fraction	Antisera			
	Anti-H (chicken)	Anti-H (eel)	Anti-A (human)	Anti-B (human)
Methanol-ethyl ether extract	125*	>1000	125	500
Chloroform-methanol extract	31.3	>1000	125	500

* Minimum concentration of each fraction giving complete inhibition (μg/0.1 ml)

Table 3. Serological changes in the chloroform-methanol extract with blood group-decomposing enzymes

Enzymatic treatment	Antisera			
	Anti-H (chicken)	Anti-H (eel)	Anti-A (human)	Anti-B (human)
Before	62.5*	>500	125	500
After				
A-decomposing enzyme	15.6	>500	>500	500
B-decomposing enzyme	62.5	>500	125	500
H-decomposing enzyme	31.3	>500	125	500

* Minimum concentration of each fraction giving complete inhibition (μg/0.1 ml)

saminyl residues from the extract would leave the structure characterized as the H determinant. On the other hand, treatment of the extract with B-enzyme caused no change in its inhibiting capacties. This result is considered to show that the weak B inhibiting power of the extract was not specific to blood group-active antigen (Tab. 3).

Discussion

The data given in this communication clearly demonstrate the existence of blood group-active glycolipid in human group A hair treated with urea.

Treatment of the hair with urea gave both soluble and insoluble fractions. These fractions were assayed for blood group A activity by haemagglutination inhibition tests or the elution technique and it was found that only the insoluble fraction showed A activity. Glycolipid was extracted from this fraction with methanol-ethyl ether and then with chloroform-methanol. Blood group A activity could be demonstrated in the extract by inhibition studies. Sakai [2] reported that „carbohydrate" and „lipoid" fractions obtained by ethanol extraction of human A, B or H hair contained blood group activity.

Important information on the structures that are associated with serological activity has been obtained using microbial blood group-specific glycosidases [11]. Yamamoto and Iseki [7] showed that A-decomposing enzyme from *Cl. tertium* [6] can be separated into two principal fractions: one fraction, comprised solely of deacetylase, destroys A activity without enhancement of H activity [12], whereas the other fraction, comprised principally of galactosaminidase containing a deacetylase, destroys A activity with the development of H activity. It can be said therefore that the serological activity of the A substance in human hair was destroyed by the A-enzyme from *Cl. tertium* with concomitant development of blood group H activity. Based on the reults of this investigation, it thus seems reasonable to conclude that the group A determinant of the hair contained terminal α-*N*-acetylgalactosaminyl residue.

Slight inhibitory capactiy was found in the glycolipid in a B red cells-anti-B system. However, B-decomposing enzyme from *Cl.sporogenes* Maebashi [8], which destroys the B activity of group B substance with concomitant development of H activity, failed to induce any serological change in the power of the glycolipid to inhibit haemagglutination of B red cells by an anti-B serum and of O red cells by an anti-H serum. This result suggests that the inhibitory capacity of the glycolipid against anti-B agglutinin was not group B specific.

References

1. Yada, S., Okane, M., Sano, Y.: Blood grouping of a single human hair by means of elution technique. Acta Crim. Jap. **32**, 7–8 (1966)
2. Sakai, T.: Studies on blood group substances of human hairs (Japanese). Jap. J. Leg. Med. **5**, 19–41 (1951)
3. Steinert, P.M., Rogers, G.: Characterization of the proteins of guinea-pig hair and hair-follicle tissue. Biochem. J. **135**, 759–771 (1973)
4. Morgan, W.T.J., King, H.K.: Studies in immunochemistry. 7. The isolation from hog gastric mucin of the polysaccharide-amino acid complex possessing blood group A specificity. Biochem. J. 37, 640–651 (1943)

5. Takizawa, H.: Blood group substances of human erythrocyte. 1. Sugar structures of blood group-active oligosaccharides from stroma by means of ozonolysis (Japanese). Kitakanto Med. J. **20**, 171–194 (1970)
6. Iseki, S., Okada, S.: On a specific enzyme which decomposes group A substances. Proc. Jap. Acad. **27**, 455–458 (1951)
7. Yamamoto, H., Iseki, S.: Development of the H specificity in A substance by A-decomposing enzyme from *Clostridium tertium* A. Proc. Jap. Acad. **44**, 263–268 (1968)
8. Iseki, S., Furukawa, K., Yamamoto, S.: B substance-decomposing enzyme produced by an anaerobic bacterium. I. Serological action on the B-decomposing enzyme. Proc. Jap. Acad. **35**, 507–512 (1959)
9. Iseki, S., Tsunoda, S.: On a bacterial enzyme which specifically decomposes O substance. Proc. Jap. Acad. **28**, 370–373 (1952)
10. Iseki, S., Yamamoto, S., Furukawa, K.: A new O (H) substance-decomposing enzyme produced by an aerobic bacterium. II. Chemical action of the O (H)-decomposing enzyme. Proc. Jap. Acad. **38**, 57–62 (1962)
11. Iseki, S.: Glycosidases and serological changes in blood group substances. In: Blood and tissue antigesn (Aminoff, D. ed.). pp. 379–394, New York: Academic Press Inc. 1970
12. Marcus, D.M., Kabat, E.A., Schiffman, G.: Immunochemical studies on blood groups. XXXI. Destruction of blood group A activity by an enzyme from *Clostridium tertium* which deacetylates *N*-acetylgalactosamine in intact blood group substances. Biochemistry 3, 437–443 (1964)

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