87. Studies on Secretor Specific H Antigen of Water-Soluble Blood Group Substances. I

Findings of Anti-H(Se) Precipitin in Eel Sera

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An antibody present in eel sera which agglutinates group O red cells was first reported by Miyazaki.¹⁾ Sugishita²⁾ classified eel sera into two types; type I reacting to the same extent with groups O, A, B and AB red cells and type II reacting much stronger with group O red cells than with groups A, B and AB red cells. Additional evidence indicated that agglutinins in eel sera display a spectrum of specificities against antigenic determinants of H and I antigens.³⁾ Precipitation reactions were observed when H substances, some plant polysaccharides and invertebrate extracts, were diffused in agar gel against eel serum.^{4),5)} The present report concerns the anti-H(Se) precipitin in eel sera which reacts specifically with water-soluble blood group substances from human ABH secretors.

Materials and methods. 1) *Antisera.* Eel anti-H sera were obtained from eels (*Anguilla japonica*), chicken anti-H serum was prepared by intravenous injection of group O red cells and anti-Le^b serum was obtained by immunization of rabbit with group O, Le (a-b+) secretor saliva.

2) *Blood group substances.* Human and hog stomach H substances were prepared from mucosa by pepsin digestion and phenol extraction. H substances from submaxillary glands, intestinal mucosa, meconium and semen were prepared from saline extract by ethanol precipitation. Salivas were used after heating for 20 min in a boiling water-bath.

3) *Inhibition of antibodies by sugars.* For agglutination inhibition experiments, sugar solution (0.5 M in monosaccharide and 0.8% in oligosaccharide) was serially diluted and equal volumes of diluted eel serum were added. The reaction mixtures were kept overnight at 4°C. Precipitation inhibition experiments were carried out by absorption test.

4) α -(1→2)-*Fucosidases.* Preparations from *Bacillus ful-*

minans^{6),7)} and *Clostridium tertium* H, Le^{a8)} were used for H- and Le^b-decomposing enzymes.

Results. 1) *Antibodies in eel sera.* Agglutinin types according to Sugishita²⁾ and precipitins against human H substances were shown in Table I. Of the 87 eel sera tested, frequencies of agglutinin type I

Table I. Reaction types of eel sera by anti-H agglutinin, and anti-H and anti-H(Se) precipitins

Reaction type	Number of eel	Frequency %
Hemagglutinin		
Type I*	15	17.2
Type II*	68	78.2
No agglutinin	4	4.6
Precipitin		
1. Anti-H	36	41.4
2. Anti-H(Se)	4	4.6
3. Anti-H+H(Se)	43	49.4
4. No precipitin	4	4.6

* According to Sugishita.²⁾

and type II were 17.2% and 78.2% respectively. On immunodiffusion in agar gel, 49.4% of eel sera gave two main precipitation lines against H substance from the human stomach (Fig. 1, wells 1 and 4). The first precipitation line which migrates faster into H substance side fused with precipitation line of hog stomach H substance (Fig. 1, wells 1 and 6). This precipitin was removed by absorption with human group O red cells or with stomach H substance together with

Fig. 1. Agar gel diffusion patterns between eel serum and blood group substances. Center well: Eel serum. Peripheral wells: (1) Human stomach H substance, (2) Human submaxillary H substance, (3) Human O, Le (a-b+) secretor saliva, (4) Human stomach H substance, (5) Human stomach Le^a substance, and (6) Hog stomach H substance.

anti-H agglutinin in the serum.

A second slower migrating precipitation line formed in front of the center well containing eel serum joined with lines of H substances from human submaxillary glands and human group O, Le (a-b+) secretor saliva (Fig. 1, wells 1 to 4). Hog gastric H substance gave the first precipitation line only and no precipitation occurred between the eel serum and human Le^a substance (Fig. 1, well 5). Immuno-diffusion patterns against different types of saliva samples were shown in Fig. 2. The line formed from the interaction of the eel serum

Fig. 2. Agar gel diffusion patterns between eel serum and human salivas. Center well: Eel serum. Peripheral wells: (1) O, Le (a-b+) secretor saliva, (2) O, Le (a+b-) non-secretor saliva, (3) O, Le (a-b-) secretor saliva, (4) O, Le (a-b-) non-secretor saliva, (5) Oh (Bombay) Le (a+b-) saliva and (6) O, Le (a-b-) secretor saliva.

with O, Le (a-b+) secretor saliva linked up with the line formed by the interaction with O, Le (a-b-) secretor saliva. No precipitation occurred between the eel serum and salivas of group O, L (a+b-) non-secretor, O, Le (a-b-) non-secretor and Oh, Le (a+b-).

The precipitation reaction of the eel serum against Le (a-b-) secretor salivas indicated that the reaction does not participate in Le^b substance. The second precipitin was not absorbed with group O red cells. The human secretor specific antigen found in water-soluble H substances by the precipitation reaction with eel sera was designated 'H(Se)' to distinguish from H antigen common to erythrocytes and water-soluble H substances. Two precipitation lines were demonstrated between the human ovarian cyst H substance and eel anti-H+H(Se) serum, while only H(Se) precipitation was observed for preparation from the oesophagus, jejunum, ileum, meconium and

semen against anti-H+H(Se) serum. H substance from hog submaxillary glands reacted neither with anti-H nor with anti-H(Se) precipitins by the methods of agar gel diffusion and ring test.

2) *Inhibition of eel anti-H and anti-H(Se) antibodies by sugars.* The reactions of group O red cells by type I and type II eel anti-H agglutinins and the reaction of human stomach H substance by eel anti-H precipitin were completely inhibited by L-fucose. 2'fucosyl-lactose from human milk weakly inhibited agglutination and precipitation of eel anti-H antibodies. The reaction of H substance by anti-H(Se) precipitin was scarcely inhibited by L-fucose or any of oligosaccharides containing L-fucose from human milk.

3) *Action of fucosidases on H and H(Se) substances.* When α -(1 $\rightarrow$ 2)-fucosidase from *Bac. fulminans* or *Cl. tertium* H. Le^a was acted on the human stomach H substance, the H inhibiting capacity against eel anti-H agglutinin was destroyed. The precipitation line in agar gel of the enzyme-treated H substance with eel anti-H and anti-H(Se) precipitins disappeared. The enzymic destructions of H and H(Se) specificities were inhibited in the presence of L-fucose.

4) *Inactivation of H and H(Se) activity by alkaline and acid degradation.* Blood group H activity to inhibit the agglutination of group O red cells by anti-H chicken serum and the precipitation of H substance in agar gel with anti-H eel serum was lost by heating 2% solution of the human stomach H substance with 50% of aqueous methanol containing 2.5% triethylamine in a boiling water-bath for 6 hr. The H(Se) activity was still retained in this treatment. Acid hydrolysis of H substance in 0.1 N HCl in a boiling water-bath for 10 min or at 50°C for 3 hr reduced the precipitation line in agar gel with anti-H(Se) eel serum.

Discussion. The presence of ABH secretor specific precipitin designated anti-H(Se) was demonstrated by precipitation lines formed between eel sera and water-soluble H substances in agar gel immunodiffusion. Anti-H(Se) precipitin reacts with H substances derived from human secretors of group Le(a-b+) and Le(a-b-), but not with substances derived from non-secretors of group Le(a+b-) and Le(a-b-). Anti-H(Se) precipitin is not absorbed with human group O erythrocytes. Hog stomach H substance does not react with anti-H(Se) precipitin. When anti-H+H(Se) eel serum was used, the anti-H(Se) precipitation line in agar gel was definitely distinguished from the anti-H precipitation line by the reaction with the human stomach or ovarian cyst H substances. The eel serum gave a clear anti-H(Se) precipitation line with secretor saliva and 1% solutions of the preparation of H substances from the small intestine, meconium and semen.

Summary. The presence of two kind of precipitins against blood group H substances from the human stomach and ovarian cyst was demonstrated in eel (*Anguilla japonica*) sera by immunodiffusion techniques in agar gel. One of them which forms the precipitation line to antigen side is the known anti-H precipitin containing anti-H agglutinin activity. The precipitating activity of the eel anti-H serum with H substance was inhibited by L-fucose and 2'fucosyllactose. The anti-H precipitin also reacts well with H substance from the hog stomach, but does not show clear precipitation lines with human secretor salivas and 1% solution of preparations of H substances from the small intestine, meconium and semen. Another precipitin which forms a precipitation line to the antiserum side in agar gel reacts well with H substances from human secretions and tissues. Because this precipitin reacted with water-soluble substances from human secretor, the antigen was named H(Se) and antibody anti-H(Se). The anti-H(Se) precipitin is not absorbed with group O red cells and the H and H(Se) activities of H substance against eel precipitins are decomposed by α -(1 $\rightarrow$ 2)-fucosidase.

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