47. On Bacterial Enzyme Specifically Decomposing Group B Substance

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In 1951, Iseki and Okada¹⁾ reported on an enzyme derived from *Clostridium tertium* and specifically decomposing group A substance. Later, Iseki and Tsunoda²⁾ reported on an enzyme derived from *Bacillus fulminans* and specifically decomposing O(H) substance. Further, Iseki and Masaki³⁾⁴⁾ clarified that these enzymes annihilated the group specificities by separating L-fucose from the group specific substances, and that the enzyme derived from *Clostridium tertium*, acting upon A substance, transformed it into O(H) substance. The present paper reports on a bacterium which has recently been isolated by the present authors from the soil, and which releases an enzyme specifically decomposing B substance.

Materials and Methods

1) Enzyme liquid. The bacterium was cultured in broth at 37°C for 48 hours, the culture liquid adjusted to pH 7.8, centrifuged with a high speed centrifuge, and the supernatant was used as the enzyme liquid.

It was added to the same volume of the group substance solution, the whole adjusted to pH 7.8-8.2, allowed to stand at 37°C for 48 hours, and heated in a water bath of 100°C for 5 minutes to destroy enzymic action. Then it was centrifuged, and the supernatant was subjected to an agglutinin absorption test and a group specific precipitation test to examine for the decomposition of the blood group substance.

The examination for the same object was also made by culturing bacterium in a synthetic minimal medium in which glucose was replaced by group B substance. The composition of the minimal medium was as follows: K₂HPO₄, 7 g; KH₂PO₄, 3 g; sodium citrate, 0.5 g; MgSO₄·7H₂O, 0.1 g; (NH₄)₂SO₄, 1 g; glucose, 2 g; distilled water, 1000 cc.

2) Blood group substances. Human secretor salivas of O, A, and B groups were used.

3) Antisera. As anti-O antibody, anti-O human blood cells immune chicken serum was used, as anti-A and anti-B precipitins,

anti-A and anti-B human blood cells immune rabbit sera, respectively, and as anti-A and anti-B agglutinins, groups B and A human sera, respectively.

Results

1. Properties and identification of the bacterium. This bacterium is an aerobic bacillus, Gram-positive and motile, and has a central spore. The colony is circular, grayish white, and opaque, and has a rough surface. It grows by anaerobic culture on blood agar plate, producing hemolysis. Biochemically it was found to be catalase-positive, to reduce nitrates but not to form indole. It liquefies gelatine and coagulated serum, peptonizes milk and hydrolyzes starch. It ferments glucose, fructose, maltose, dextrin, and saccharose in a day, glycerol in 3 days but not lactose, galactose, arabinose, rhamnose, xylose, raffinose, mannitol, inulin, and inositol. These properties are identical with those of *Bacillus cereus* described in Bergey's Manual of Determinative Bacteriology.⁵⁾

2. Group specific substance decomposing action. When an enzyme derived from this bacterium was allowed to act upon each group specific substance, group B saliva lost anti-B agglutinin absorbing ability and precipitability against anti-B precipitin, but group O and A substances did not lose the absorbing ability and precipitability against anti-O and anti-A antibodies, respectively, as shown in Table I.

Table I. Enzymic action against group specific substances

Reaction		Agglutinin absorption					Precipitation				
Antigen dilution		1/80	1/160	1/320	1/640	1/1280	1/20	1/40	1/80	1/160	1/320
Antiserum		Anti-O chicken serum					Anti-O chicken serum				
Group O saliva	Before action	-	-	-	-	+	+	+	+	+	-
	After action	-	-	-	-	+	+	+	+	+	-
Antiserum		Anti-A human serum					Anti-A rabbit serum				
Group A saliva	Before action	-	-	-	-	+	+	+	+	-	-
	After action	-	-	-	-	+	+	+	+	-	-
Antiserum		Anti-B human serum					Anti-B rabbit serum				
Group B saliva	Before action	-	-	-	-	+	+	+	+	+	-
	After action	+	+	+	+	+	-	-	-	-	-

These results indicate that an enzyme released by *Bacillus cereus* specifically decomposes group B substance.

3. Properties of the B-decomposing enzyme. To investigate the action power of this enzyme, it was allowed to act at various pH upon the group B substance in human secretor saliva. The result is represented in the figure.

The optimum pH was 7.8–8.2, and no reaction occurred below pH 5.0 and above pH 9.4.

The optimum temperature at the optimum pH was 30–37°C, but the reaction was effected sufficiently well at 20°C. When the enzyme liquid was heated at 50°C for 5 minutes, the enzymic action was considerably weakened. It was almost lost by heating at 60°C for 5 minutes, and completely lost at 70°C for 5 minutes.

Relation between reaction power and reaction pH of enzyme

4. Transformation from B to O(H) substance by enzyme. When *Bacillus cereus* was cultured in the above described synthetic medium containing B substance, the absorbing ability and precipitability against anti-B agglutinin and anti-B precipitin were lost, and those against anti-O agglutinin and anti-O precipitin were increased, as shown in Table II. However, group A specificity was not revealed in this case.

Table II. Action of B-decomposing enzyme against group B substance

Reaction		Agglutinin absorption					Precipitation				
Antigen dilution		1/80	1/160	1/320	1/640	1/1280	1/80	1/160	1/320	1/640	1/1280
Antiserum		Anti-O chicken serum					Anti-O chicken serum				
Group B saliva	Before action	—	—	+	+	+	+	+	—	—	—
	After action	—	—	—	—	+	+	+	+	+	—
Antiserum		Anti-B human serum					Anti-B rabbit serum				
Group B saliva	Before action	—	—	—	—	+	+	+	—	—	—
	After action	+	+	+	+	+	—	—	—	—	—

In the following experiment, *Bacillus cereus* was cultured in the synthetic medium containing group B saliva which was preliminarily acted upon by the O-decomposing enzyme from *Bacillus fulminans*. As shown in Table III, the agglutinin absorption test and group specific precipitation test revealed that the O(H) specificity of B saliva which was lost by the O-decomposing enzyme, was reproduced by the action of B-decomposing enzyme.

Table III. O- and B-decomposing enzymic actions against group B substance

Reaction	Agglutinin absorption					Precipitation				
Antigen dilution	1/80	1/160	1/320	1/640	1/1280	1/40	1/80	1/160	1/320	1/640
Antiserum	Anti-O chicken serum					Anti-O chicken serum				
Before action	-	-	+	+	+	+	+	+	-	-
Group B saliva After action by O-enzyme	+	+	+	+	+	-	-	-	-	-
After action by B-enzyme	-	-	-	+	+	+	+	+	+	-
Antiserum	Anti-B human serum					Anti-B rabbit serum				
Before action	-	-	-	-	+	+	+	+	-	-
Group B saliva After action by O-enzyme	-	-	-	-	+	+	+	+	-	-
After action by B-enzyme	+	+	+	+	+	-	-	-	-	-

The results indicate that the B-decomposing enzyme from *Bacillus cereus* transforms group B substance into O(H) substance by annihilating the group B specificity.

Summary

1. The authors discovered *Bacillus cereus* in the soil which produces an enzyme decomposing neither O(H) nor A substance but specifically B substance.

2. This enzyme can be almost destroyed by heating at 60°C for 5 minutes, and completely destroyed at 70°C for 5 minutes. The optimum pH for the enzymic action ranges 7.8-8.2, and the optimum temperature at these pH, 30-37°C.

3. The enzyme, acting upon B substance, transforms it into O(H) substance by annihilating its B specificity.

References

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