Short Communications

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Diversity in Immunological Nature of Microbial α -Amylase

Knowledges of microbial α -amylase, a typical exo-enzyme, are now abundantly accumulating, but only a little is known of its immunological characters.¹⁻⁵⁾ During the course of research on α -amylase produced by *Streptococcus bovis*, we obtained some experimental data on the immunological characters of α -amylase proteins suggesting a conformational alteration of those enzyme proteins corresponding to the kind of microorganisms producing them.

Crystallized preparation of bacterial α -amylase (B α A) was obtained by Hagiwara's method⁶⁾ from the culture broth of *Bacillus subtilis* st. H, which was stocked at our laboratory as a representative of bacterial α -amylase producers. Taka-amylase A (TAA) was crystallized from water extracts of Taka-diastase,⁷⁾ a commercial product of fungal α -amylase by Sankyo Pharm. Co., Ltd. These two enzyme proteins were recognized to be pure by ultracentrifugation test and used for the immunological research as antigens.

Each rabbit was injected subcutaneously with 2mg. of the enzyme protein emulsionized in 1ml. of Freund-incomplete type adjuvant mixture. After 20 days from the first injection, 5mg. of antigen in 1ml. of saline was injected again into vein to boost the formation of the antibody. 7 more days later, the animal was decapitated, and blood was col-

lected to prepare the antiserum by the usual way. Tests of capillary precipitation, gel-diffusion, quantitative precipitation, and neutralization of enzyme activity were performed on each of the above prepared antisera. As shown in Fig. 1, both enzyme proteins were quite distinctive from each other on the immunological bases.

FIG. 1. Gel-diffusion Test of TAA and B α A.

A: TAA; B: B α A

a₁~a₄: antiserum against TAA.

b₁~b₄: antiserum against B α A.

a₀, b₀: normal serum.

24 strains belonging to the genus *Bacillus* were cultured for 48 hrs. in liquid medium containing 1% starch, and the amylolytic activity of these strains was estimated from the amount of starch remaining in the medium. 11 strains including *B. subtilis* st. N and st. K were found to have an intensive amylolytic activity. Capillary precipitation test with anti-B α A was carried out on the neutralized culture broth of each strain (Table I), and it was found that the α -amylases of the above two strains were precipitated with

1) M. Nomura and T. Wada, *J. Biochem.*, **45**, 629 (1958).

2) T. Wada and M. Nomura, *ibid.*, **45**, 639 (1958).

3) K. Onoue, Y. Okada, S. Nakashima, K. Shimada and Y. Yamamura, *ibid.*, **53**, 472 (1963).

4) Y. Okada, Y. Matsuoka, T. Yagura, T. Ikenaka and Y. Yamanura, *ibid.*, **55**, 446 (1964).

5) A. Yoshida, T. Tobita and J. Koyama, *Biochem. Biophys. Acta*, **44**, 388 (1960).

6) Hagiwara, *Proceed. Jap. Acad.*, **27**, 364 (1951).

7) Akabori, Ikenaka, Hagiwara, *J. Biochem.*, **41**, 577 (1954).

TABLE I. CAPILLARY PRECIPITATION TEST ON α -AMYLASES OF VARIOUS BACILLI WITH ANTI-B α A

Strain		Amylolytic Activity	Precipitation with Anti-B α A
<i>B. cereus</i>	IAM 1072	++	—
<i>B. circulans</i>	IAM 1112	+++	—
<i>B. macerans</i>	IAM 1227	+++	—
<i>B. subtilis</i> st. H	IAM 1521	+++	+
<i>B. subtilis</i> st. N	IAM 1522	+++	+
<i>B. subtilis</i> st. K	IAM 1523	+++	+
<i>B. subtilis</i> var. <i>globigi</i>	IAM 1633	+++	—
<i>B. thiaminolyticus</i>	IAM 1034	++	—
<i>B. megaterium</i>	IFO 3003	++	—
<i>B. megaterium</i>	NI 8100	++	—
<i>B. megaterium</i>	RIMD 899	++	—

B. brevis, *B. firmus*, *B. roseus*, *B. sphaericus*, *B. aneurinolyticus* and *B. pulvificiens* showed rather weak amylolytic activity.

anti-B α A. The minimum detection range of α -amylase by capillary precipitation test was 10 μ g/ml. Specificity of B α A, thus, seems to be commonly observable only in amylases produced by the strains belonging to *B. subtilis*.

Then, various types of *B. subtilis*, which had been classified by Dr. Azuma on the basis of the taxonomic concept proposed by Smith and were kindly supplied by him, were tested. Of these strains, 62 strains were intensively amylolytic but α -amylases produced by only two strains, so far tested, were found to be precipitated with the anti-B α A (Table II). It is conceivable that the specificity of B α A ranges in a considerably limited group of *B. subtilis* or closely related strains of this

TABLE II. CAPILLARY PRECIPITATION TEST ON α -AMYLASES OF 95 STRAINS OF *B. subtilis* CLASSIFIED BY DR. AZUMA*

Amylolytic Activity	Strong 62 strains	Rather weak 33 strains
Precipitation with Anti-B α A	Positive 2 strains	Negative 93 strains

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species.

The specificity of TAA was further examined using 9 strains of various fungi. Of these strains, only one strain was positive; the α -amylase of *Aspergillus oryzae* var. *globosus* was precipitated with the anti-TAA (Table III). In this case, it is noticeable that α -amylases of *A. oryzae* IAM 2748 and *A. oryzae* var. *microsporus* IAM 2651 never reacted with the anti-TAA. The α -amylases of the other species of *Aspergillus* or of *Rhizopus* species were inert in the reaction with anti-TAA.

TABLE III. CAPILLARY PRECIPITATION TEST ON α -AMYLASES OF VARIOUS α -AMYLASE-PRODUCING FUNGI

Strain		Precipitation with Anti-TAA
<i>Asp. oryzae</i>	IAM 2748	—
<i>Asp. oryzae</i> var. <i>microsporus</i>	IAM 2651	—
<i>Asp. oryzae</i> var. <i>globosus</i>	IAM 2625	+
<i>Asp. niger</i>	IAM 2533	—
<i>Asp. awamori</i> Nakazawa	IAM 2299	—
<i>Asp. awamori</i> Sakaguchi et al.	IAM 2334	—
<i>Asp. usamii</i>	IAM 2185	—
<i>Rhi. delemar</i>	IAM 6015	—
<i>Rhi. oryzae</i>	IAM 6001	—

Then the specificity of fungal amylase might be well discernible to some extent, since Taka-diastase, though a commercial preparation, mainly contains the fungal α -amylase.

From the results above obtained, it might be mentioned that α -amylase proteins produced by various kinds of microorganisms are immunologically different from each other and their specificity may be lined on the same level as that of the microbial cell body.

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