83. The Oxidation of Bacterial α -Amylase by Periodate

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(Comm. by Y. OKUDA, M.J.A., July 13, 1953)

Preceding reports have revealed that certain proteins can be oxidized without giving any coagulation under a comparatively moderate condition¹⁾. It will be of interest, accordingly, to carry out the oxidation of enzyme protein by periodate.

Jansen and others^{2,3)} recently reported that α -chymotrypsin after being oxidized by sodium metaperiodate still retained 65 per cent in its esterase activity and 35 per cent in its protease activity. Englard and others³⁾ attempted to elucidate the active centre of β -amylase by treating it with sulfhydryl-reagent such as o-iodosobenzoate. Although similar researches have hitherto been reported⁵⁾, no works have been made on the oxidation of crystalline α -amylase by the use of periodate. The present paper deals with the isolation of a crystalline form of oxidized α -amylase which still possesses its activity to some extents.

Experimental

1. *The relationship between consumed amounts of periodate and the activity of oxidized bacterial α -amylase.* A crystalline α -amylase which was used in this work, was kindly supplied by Prof. Juichiro Fukumoto of the Osaka City University. Sodium metaperiodate which was recrystallized three times from water, was dissolved in acetate buffer of pH 5.8. A 0.01N sodium metaperiodate solution kept at 4°C was mixed slowly with enzyme dissolved in the same buffer at 4°C. While the mixture was kept standing at 4°C, 1 ml each of the mixture was taken at successive intervals and determined for the quantity of reacted periodate according to the method given in the previous paper¹⁾. On the other hand, 20 ml of the enzyme solution was evaporated to dryness, digested with concentrated sulfuric acid and determined for total nitrogen by nesslerization. The amount of the enzyme was calculated based on its nitrogen content^{*)} being 14.8 per cent. During the oxidation, 1 ml each of the solution was taken and determined for the liquefying power (LP), the dextrifying power (DP) and the saccharifying power (SP). LP was determined by modified

*) Mr. T. Yamamoto's private communication.

Lintner-Sollied's method⁹⁾, DP by Somogyi's method⁶⁾⁷⁾, and SP by Balls' method⁸⁾ with some modifications. The results obtained are shown in Fig. 1.

Fig. 1. Amount of periodate reduced by α -amylase and diminution of enzymatic activities

2. *The crystallization of oxidized bacterial α -amylase.* To a suspension of 2.986 gm of crystalline bacterial α -amylase (total N: 135.4mg) which was kept at 4°C , was added 25 ml of a cold dilute sodium hydroxide solution of pH 9.8. The mixture was centrifuged, and the supernatant was filtered. The filtrate was adjusted to pH 5.8 with 0.1N acetic acid. To this enzyme solution was added with constant stirring 25 ml of 0.144N sodium metaperiodate in acetate buffer of pH 5.8. This was kept at 4°C for 24 hours and then filtered off. Then as much acetone was added to the filtrate as to make its concentration 47 per cent. Soon after the addition, the mixture became muddy, but after being kept standing overnight in an ice-chest, a crystalline precipitate was obtained. The crystal was centrifuged and washed several times with 45 per cent acetone-acetate buffer solution (pH 5.8) until the filtrate was free from iodine compound.

Recrystallization was carried out by dissolving in a small quantity of a cold sodium hydroxide solution (pH 9.5), adding acetic acid to make pH 5.8 and further adding acetone. LP of

Fig. 2. Activity-pH curves of the original and oxidized α -amylases

this oxidized enzyme, though it seems to vary more or less with different cases, was about 1/40 of the native enzyme. As shown in Fig. 2, the optimum pH for liquefying shifts to alkaline side (pH 6.2-6.5).

Fig. 3. Photomicrograph of the oxidized α -amylase ($\times 500$)

Results and Discussion

It seems that about 20 ml of 0.1N sodium metaperiodate solution reacted with 1gr of the bacterial α -amylase under the present experimental condition (Fig. 1). The results presented here show that the saccharifying power, the dextrifying power and the liquefying power decrease with oxidation by periodate, but their decreasing rates

are different from each other. In 24 hours, for example, LP decreased through oxidation to 1/40 of the original activity, but still remained somewhat even after 30 hours. From these facts it is apparent that groups susceptible to the oxidation play an important rôle in the α -amylase activities, i.e., liquefaction, saccharification and dextrification. Moreover, from the fact that, except DP, both LP and SP remained after the oxidation, it might be assumed that the greater part of active center of this enzyme, which is readily oxidizable, is common to those three activities, while there exists some sluggish part that is concerned only in the liquefaction and in the saccharification, being pretty stable to oxidation by periodate.

The authors wish to express their sincere thanks to Prof. J. Fukumoto and Instructor Y. Yamamoto of the Osaka City University for a generous supply of α -amylase.

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