Note

Modification of the Free Sulfhydryl Group of Buckwheat α -Glucosidase with 5-Alkylthiomethylhydantoin-S-oxide, a New Thiol Reagent

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It has been known that the free sulfhydryl (SH) group(s) of various kinds of amylase is unlikely to be essential for the catalytic process.^{1~3)} A role of the SH group(s) in acid or neutral α -glucosidases,^{4~8)} has been suggested from the inhibitory effect of SH reagents, but there is no conclusive evidence of whether or not this group is essential for the action of α -glucosidase.

Buckwheat α -glucosidase possesses only one SH group titrated with 5, 5'-dithiobis (2-nitrobenzoic acid) in the presence of urea, and this group is assumed to be located in or near the catalytic site.^{9,10)} This SH group was slightly susceptible to modification by *N*-acetylimidazole⁹⁾ or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide¹⁰⁾ which decreased the hydrolytic activity. In the present work we aimed to elucidate the role of the free SH group of this enzyme in the catalytic process or in the binding of substrate.

Tahara *et al.*¹¹⁾ reported that 5-alkylthiomethylhydantoin-S-oxide, an effective antimicrobial reagent, was readily decomposed to alkylthiosulfinate in neutral solution, and that this product was highly reactive with only cysteine to give S-alkylthio-L-cysteine. The reagent is also considered to react specifically with free SH group(s) of protein with the same reaction mechanism. Thus the reagent was used for the modification of the SH group of the enzyme.

The preparation and assay of the enzyme were described in the previous papers.^{10,12)} α -Glucosidase was incubated with 5-alkylthiomethylhydantoin-S-oxides, 5-methylthiomethylhydantoin-S-oxide (MHSO) or 5-propylthiomethylhydantoin-S-oxide (PHSO) for 4 hr at 37°C in 0.1 M sodium phosphate buffer (pH 7.0). The modification was terminated by separating the enzyme on a Bio-Gel P-4 column (1 $\times$ 30 cm) equilibrated with the same buffer. The incubation with MHSO or PHSO re-

sulted in a progressive decrease of the hydrolytic activity (Fig. 1). Turanose, a competitive inhibitor, provided complete protection against the reduction of the activity. However, there was no stoichiometric relation between the residual activity and the unmodified free SH group which were determined according to Ellman's method.¹³⁾ This suggests that the enzyme may not lose a part of its activity, even though modified.

Thus the modified enzyme was isolated by column chromatography on *p*-mercuric benzoyl-Sepharose 4B equilibrated with 0.1 M sodium phosphate buffer (pH 7.0). *p*-Mercuric benzoyl-Sepharose 4B was prepared according to Cuatrecasas' method,¹⁴⁾ except for the use of octa-

FIG. 1. Decrease in Hydrolytic Activity of Buckwheat α -Glucosidase with 5-Alkylthiomethylhydantoin-S-oxides.

The enzyme (10^{-5} M) was incubated in 0.1 M sodium phosphate buffer (pH 7.0) at 37°C, with 20 mM MHSO (●—●), 10 mM PHSO (○—○), or 50 mM turanose (○---○). At various intervals, an aliquot was withdrawn and diluted 500-fold with ice-cold 0.1 M sodium acetate buffer (pH 5.0) containing 0.05% Triton X-100. The residual activity was measured at pH 5.0 and 37°C using 1.56 mM maltose.

FIG. 2. pH-Activity Profiles of Native and Thiomethylated Buckwheat α -Glucosidase.

The hydrolytic activity was measured at each pH of McIlvaine buffer and 37°C using 1.94×10^{-9} M enzyme and 1.56 mM maltose. ●—●, native enzyme; ○—○, thiomethylated enzyme.

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TABLE I. RATE PARAMETERS FOR NATIVE AND THIOMETHYLATED α -GLUCOSIDASE

Substrate	K_m (mM)		V^a (μ mol glucose/mg protein/min)	
	Native	Thiomethylated	Native	Thiomethylated
Maltose	6.3	11	87.4	38.7
Maltotriose	4.0	6.4	97.7	39.1
Maltopentaose	1.8	2.6	106	46.7
Maltoheptaose	0.84	1.2	93.4	40.4
Soluble starch	7.5 ^b	9.8 ^b	74.2	33.4

^a Calculated as glucose liberated from nonreducing end.

^b mg/ml.

methylenediamine as a spacer. The modified enzyme was eluted with the same buffer containing 50 mM mercaptoethanol.

The free SH group was no longer detectable in the modified enzyme, but a partial activity remained. As shown in Fig. 2, the optimum pH slightly shifted to the alkaline side on thiomethylation of the SH group. One of the active ionizable groups which are assumed to be carboxyl groups¹⁰⁾ is not affected, but the pK value of the other shifted to the alkaline side. The rate parameters for a series of maltooligosaccharides are summarized in Table I. The K_m values were increased by modification of SH group, and the maximum velocities were reduced. These findings suggest that the free SH group of the enzyme is unlikely to be essential for its action including the catalytic process or the binding of substrate, although a possibility can not be ruled out that this group enhances the efficiency of the hydrolytic reaction.

The role of the SH group of buckwheat α -glucosidase is still obscure, but 5-alkylthiomethylhydantoin-S-oxide appears to be available as a reagent for the modification of SH groups.

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