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INFLUENCE OF THE CHEMICAL MODIFICATION OF THE FUNCTIONAL GROUPS OF THE β-1,3-GLUCANASE L IV ON ITS CAPACITY FOR HYDROLYSIS AND TRANSGLYCOSYLATION REACTIONS

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UDC 577.154.26

The influence of the chemical modification of the functional groups of the β-1,3-glucanase L IV on its capacity for performing hydrolysis and transglycosylation reactions has been investigated. On the modification of the lysine, tryptophan, histidine, and dicarboxylic acid residues and on the oxidation of the carbohydrate component in the L IV molecule the ratio of the hydrolase and transglycosylating activities does not change. It is likely that the hydrolysis and transglycosylation reactions take place at the same active site with the participation of the same catalytic groups.

It is known that endoglycanases and glycosidases catalyze hydrolysis and transglycosylation reactions simultaneously. A capacity for transglycosylation has been detected in endo-β-1,3-glucanases from marine molluscs [1, 2]. Investigations of the laws of the combined occurrence of hydrolysis and transglycosylation reactions under the action of β-1,3-glucanases L IV from *Spisula sachalinensis* and L_{Cr} from *Chlamys albidus* have shown that the pH optima of the two reactions coincide [2]. The hypothesis has been expressed that identical catalytic groupings of the active sites of the enzyme studied take part in the processes of hydrolysis and of transglycosylation. To check this hypothesis we have used the method of chemically modifying functional groups, which has been used previously with success for studying the influence of modification mainly on the hydrolase activity of the β-1,3-glucanase L IV [3-7]. In an investigation of the chemical modification of lysine residues [8], the kinetics of the action on laminarin of β-1,3-glucanase L IV modified with pentane-2,4-dione was analyzed in detail in comparison with the native enzyme. It was established that modification of the lysine residues led to no change in the hydrolase and transglycosylating activities.

All the chemical modifications were carried out by the procedures described previously for β-1,3-glucanase L IV, the degree of modification and the change in activity being monitored. Transglycosylating activity was determined from the accumulation of p-nitrophenol [1] and hydrolase activity from the increase in reducing power by Nelson's method [9]. The results are given below: (Top, following page.)

The oxidation by N-bromosuccinimide of more than one tryptophan residue in the β-1,3-glucanase L IV molecule and the esterification of the carboxy groups led to the complete loss of both the hydrolase and the transglycosylating capacity of the enzyme.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 716-719, November-December, 1986. Original article submitted June 5, 1986.

 0009-3130/86/2206-0668$12.50

Reagent	Residue modified	Activity, %	
		Hydrolysis	Transglycosylation
Pentane-2,4-dione	Two lysine residues [8]	100	100
N-Bromosuccinimide	One tryptophan residue	0	0
Propylene oxide	Dicarboxylic amino acids	0	0
Diethyl pyrocarbonate	Two histidine residues	65	65
Sodium periodate	Carbohydrate component	100	100

Sodium periodate oxidation was carried out with monitoring of the shape of the UV spectrum of the protein. We have shown previously [10] that a considerable excess of periodate leads to a destruction of the tryptophan residues. On the addition of an amount of periodate causing practically no change in the shape of the UV spectrum of the enzyme, but, according to the literature, causing the oxidation of the terminal residues of the carbohydrate components [11], the magnitudes of the hydrolase and transglycosylating activities did not change.

We made a more detailed study of the properties of the β-1,3-glucanase L IV modified at two histidine residues with retention of 65% of its hydrolase activity. The kinetics of the action of the β-1,3-glucanase L IV modified at the histidine residues in comparison with the native enzyme was investigated. The two samples had identical activities with respect to laminarin. To determine the transglycosylation products in samples, the reaction mixture was passed through a column of Sephadex G-15, with the separation of the p-nitrophenyl glucoside. The hydrolase activity was determined by Nelson's method [9] in parallel samples containing no p-nitrophenyl glucoside. At each point we calculated the ratio of the products of transglycosylation to the amount of reduced ends. The magnitude of this ratio scarcely changed and was the same for the modified and native enzymes.

In addition, we investigated the action of β-1,3-glucanase L-IV modified at two histidine residues on laminarin labeled at the reducing end with ^3H by the procedure described in [12]. The separation of the labeled oligomers was carried out by repeated paper chromatography with the subsequent measurement of the radioactivities of sections corresponding to individual sugars. The patterns of distribution of the labeled products were identical for the modified and native enzymes. It may be considered that the modification of the two histidine residues most accessible to the reagent had no appreciable effect on the properties of the active site of the enzyme.

Thus, on the modification of lysine, tryptophan, histidine, and dicarboxylic acid residues and on the periodate oxidation of the carbohydrate component in the β-1,3-glucanase L IV molecule the ratio of the hydrolase and transglycosylating activities did not change. Modification of lysine [8] and histidine residues, when the activity of the enzyme was retained completely or partially, did not change the nature of the distribution of the reaction products, either. The results given in this paper confirm the hypothesis that the hydrolysis and transglycosylation reactions take place at a single active site with the participation of the same functional groups.

EXPERIMENTAL

The β-1,3-glucanase L IV was obtained from the crystalline style of the bivalve mollusc *Spisula sachalinensis* [13]. The concentration of the enzyme was determined spectrophotometrically. The optical density at 280 nm of a 0.1% solution of the β-1,3-glucanase L IV was 1 OU. The laminarin was obtained from the brown alga *Laminaria cichorioides* by a published method [14].

Koch-Light diethyl pyrocarbonate and Chemapol p-nitrophenyl glucoside were used. The other reagents were products of the Soyuzglavreaktiv All-Union Amalgamation. The N-bromosuccinimide was purified by recrystallization from benzene.

Determination of Transglycosylating Activity. The reaction mixture contained 2 mg of laminarin, 5 mg of p-nitrophenyl glucoside, and about 0.01-0.02 units of the enzyme in 1 ml of 0.05 M succinate buffer. Samples were taken after predetermined intervals of time and the reaction in them was stopped by boiling. They were analyzed for their p-nitrophenol contents [1].

To determine the amounts of transglycosylation products in the samples, the reaction mixtures were deposited on a column of Sephadex G-15 and were eluted with water, the outflow of the products being recorded on a Uvicord spectrophotometer. The fractions containing the reac-

tion products, except for p-nitrophenyl glucoside, were evaporated to 2.5 ml and the transglycosylation products were determined in them from their absorption at 300 nm.

Hydrolase activity was determined from the rise in reducing capacity by Nelson's method [9] using laminarin as substrate.

The oxidation of the β-1,3-glucanase L IV by N-bromosuccinimide was carried out at pH 4.0 with a 3- to 4-molar excess of the reagent, the number of modified tryptophan residues being monitored spectrophotometrically [3].

Esterification with Propylene Oxide. Propylene oxide in 0.05-ml portions was added to 1 ml of a solution containing 0.8 mg of the enzyme in 0.05 M acetate buffer, pH 4.0. After each addition, aliquots were taken for activity determinations.

The modification of the enzyme by diethyl pyrocarbonate was carried out by the method described in [5]. During the modification process, differential spectra were recorded in order to calculate the number of histidine residues modified. After the modification of two histidine residues per molecule, the sample was deposited on a column of Sephadex G-25 equilibrated with 0.05 M succinate buffer, pH 5.2, to eliminate the excess of reagent.

Periodate Oxidation. The periodate oxidation of 0.8 g of enzyme in 0.05 M succinate buffer was carried out with 0.01 M sodium metaperiodate at 25°C in the dark for 2 h [10]. The process was monitored from the absorption at 280 nm.

The action of the β-1,3-glucanase L IV modified at two histidine residues on laminarin labeled at the reducing end was checked by a known method [12].

The authors express their gratitude to P. V. Bezukladnikov for performing the experiment with the labeled substrate.

SUMMARY

1. On the modification of lysine, tryptophan, histidine, and dicarboxylic acid residues and on the oxidation of the terminal residues of the carbohydrate component in the molecule of β-1,3-glucanase L IV the ratio of the hydrolase and transglycosylating activities does not change. The results obtained confirm the hypothesis that the hydrolysis and transglycosylation reactions take place in a single active site with the participation of the same functional groups.

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