COMPARISON OF THE ENZYMATIC AND STRUCTURAL CHARACTERISTICS OF β-1,3-GLUCANASES FROM MARINE MOLLUSKS

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A comparison has been made of the enzymatic and structural characteristics of two endo-β-1,3-glutamases from the crystalline styles of bivalve mollusks. The enzymes possess similar enzymatic properties but differ in thermal stability. The molecules of the enzymes have differences in the N-terminal sequences and in the numbers of disulfide bonds, tryptophan residues, and neutral sugar residues. An homology of the primary structures is to be expected in the functionally important sections of the enzyme molecules.

We have previously shown that marine invertebrates are sources of various carbohydrases: β-1,3-glutamases, cellulases, chitinases, etc. [1-3]. We have detected the highest laminarinase activity in the crystalline styles of bivalve mollusks – unique organs representing peculiar concentrators of digestive enzymes. For the crystalline styles of the bivalve mollusks Spisula sachalinensis and Chlamys albidus we have isolated two endo-β-1,3-glucanases in the homogeneous state (L-IV and Lo, respectively), differing in some features of their action on laminarin, laminarioligosaccharides, and mixed glucans [4-6]. At the present time, there is practically no information on the link between the structure and functions of β-1,3-glucanases, and it was of interest to compare the structural characteristics of the two related enzymes isolated from different species of bivalve mollusks.

The endo-β-1,3-glucanases L-VI and Lo have close molecular weights and isoelectric points but differ in the values of their temperature optima and pH optima (Table 1). Substantial differences are also observed in the thermal stability of the enzymes under investigation (Fig. 1). A stabilizing action of NaCl is obvious, but while L-IV is approximately twice as stable in 0.5 N NaCl (for example at 40°C) as in 0.05 M buffer solution, these differences are even more considerable for Lo. An amount of Lo equal to that added in an experiment with NaCl (Fig. 1, curve 3) exhibited practically no activity in buffer.

It is possible that such differences in the thermal stabilities of the two enzymes are connected primarily with different numbers of disulfide bonds (Table 2) The disulfide bonds in Lo were determined with previous reduction by β-mercaptoethanol in 6 M urea followed by the determination of the number of SH groups formed by Ellman's method [7]. In addition, it was established that Lo, like L-IV, is a glycoprotein but with a smaller amount of neutral sugar residues (see Table 1). This may also affect the thermal stability of Lo. There is information in the literature confirming the important role of a carbohydrate component for the thermal stability of enzymes [8].

Table 2 gives the amino acid compositions of the β-1,3-glucanases. They are characterized by a high amount of acidic amino acids, which is characteristic for hydrolases, and of proline. It is interesting that a relatively high level of proline as compared with lysozyme C also distinguishes the lysozyme isolated from materials of marine origin [9].

The Tyr/Trp ratio established by the method of second-derivative UV spectroscopy [10] amounted to 1.2 for L-IV and to 2.1 for Lo. Since, according to the results of total amino acid analysis, the numbers of tyrosine residues in the glucanase molecules were the same, the difference in the Tyr/Trp ratio was due to different numbers of tryptophan residues (see Table 2). Tryptophan was determined by separate analysis after hydrolysis of the protein with methanesulfonic acid [11].

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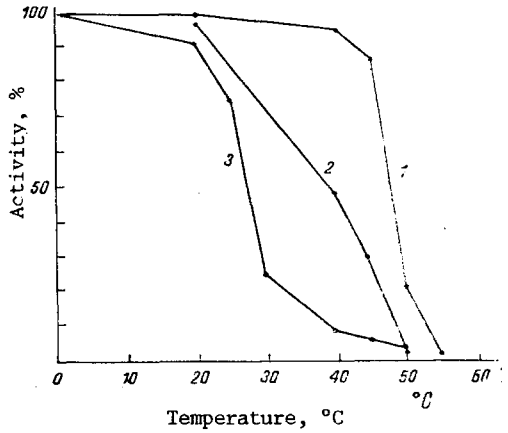

Fig. 1. Thermal stabilities of the β-1,3-glucanases: 1) L-IV in 0.05 M succinate buffer, pH 5.2, containing 0.5 M NaCl; 2) L-IV in the same buffer without NaCl; 3) Lo in a buffer containing 0.5 M NaCl.

TABLE 1. Comparison of Some Characteristics of the β-1,3-Glucanases

Characteristic	L-IV [4]	Lo [5]
Molecular weight (according to gel chromatography)	22000	25000
Isoelectric point	7.5	7.6
pH optimum	5.8	4.4
Temperature optimum, °C	45	35
Content of neutral sugars, %	6.5	3

TABLE 2. Amino Acid Compositions of the β-1,3-Glucanases*

Amino acid	Number of residues in the molecule	
	L-IV	Lo
Asp	11	17
Thr	11	11
Ser	10	12
Glu	13	11
Pro	7	8
Gly	13	18
Cys	10	4
Val	8	8
Met	3	3
Ileu	5	5
Leu	7	5
Tyr	5	5
Phe	5	6
Lys	5	4
His	10	11
Arg	7	5
Trp	4	2

*Cysteic acid was determined by Ellman's method [7] and tryptophan by separate analysis with methanesulfonic acid [11].

An immunological study of the lysozymes showed the existence of a definite correlation between the number of substitutions of the amino acids in the primary structure and the immunological distance between the proteins. The absence of a cross-reaction is observed when the difference between amino acid sequences exceeds 30-40% [12]. The degree of similarity of the amino acid sequences of L-IV and Lo was previously checked by the immunodiffusion method [13]. The β-1,3-glucanase L-IV was used as antigen. Lo did not form a precipitation band with the antiserum of L-IV, which permitted the assumption of a difference in the primary structures of the enzyme. This was confirmed by an analysis of tryptic hydrolysates of the β-1,3-glucanases by the peptide map method.

A tryptic hydrolysate of L-IV contained only acidic peptides while a similar hydrolysate of Lo contained mainly basic peptides (see the conditions of separation on electrophoresis in Fig. 2). The numbers of peptides containing tryptophan residues, the important role of which for the functioning of L-IV and Lo has been shown previously [14, 15] were different.

The N-terminal sequences of the β-1,3-glucanases determined by the automatic Edman method had no similarities:

L-IV Gly-Thr-Val-Val-Phe-Arg-Asp-Asp-Phe-...,

Lo Ala-Gly-Thr-Gly-Asp-Glu-Phe-...

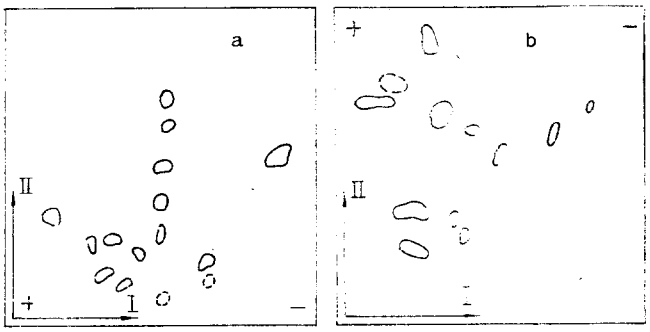

Fig. 2. Peptide maps of tryptic hydrolysates of the β-1,3-glucanases (– – – – tryptophan-containing peptides): a) L-IV, conditions of separation: I) electrophoresis at pH 5.6, 40 V/cm, 50 min; II) chromatography in the pyridine–butan-1-ol–water–acetic acid (40:60:48:12 by volume) system; b) Lo: I) electrophoresis at pH 1.9, 10 V/cm, 10 min, 50 V/cm, 90 min; II) chromatography as in case a).

On comparing the N-terminal (Arg) sequences of 29 amino acid residues of Taka-amylase A and an α-amylase from porcine pancreas, no similarity whatever was detected between them [16]. However, when the possibility appeared of comparing their complete amino aicd sequences a number of homologous sections were observed (degree of homology about 20%) [17].

The β-1,3-glucanases investigated, which had been isolated from animals close on the evolutionary level, had close specificities and similar enzymatic properties. We have recently shown with the aid of the method of chemical modification [18] that residues of tryptophan, of dicarboxylic acids, and of histidine are of fundamental importance for the activity of Lo, just as for L-IV [14, 19, 20]. These facts permit the expectation of an homology of the primary structures of the enzymes under investigation in the functionally significant parts of the molecules: in the region of the active sites of binding of the substrate. The possibility is not excluded of a spatial similarity of the β-1,3-glucanase molecules that is responsible for their close functional properties.

EXPERIMENTAL

The β-1,3-glucanases were obtained from the crystalline styles of bivalve mollusks: Lo from Chlamys albidis, and L-IV from Spisula sachalinensis, by methods described previously [4, 5]. Protein concentrations were determined spectrophotometrically on the assumption that the optical density at 280 nm of a 0.1% solution of a β-1,3-glucanase, Lo or L-IV, is 1 unit. The activities of the enzymes were determined from the increase in the amount of reducing sugars by Nelson's method [21]. The substrate – laminarin – was obtained from the brown seaweed Laminaria cichorioides [22].

Amino acid analysis was performed on a Biocal analyzer (Sweden) after hydrolysis of the protons with 6 N HCl at 105°C for 24, 48, and 72 h. Tryptophan was determined by separate analysis after hydrolysis with methanesulfonic acid [11]. The calculation was performed relative to the numbers of lysine and arginine residues found from the results of general analysis.

Neutral sugars were determined by the phenol/sulfuric acid method [22]. The amounts of proteins in samples were determined by Lowry's method [24].

The UV spectra of the β-1,3-glucanases were taken in 0.1 N KOH on a Varian-Cary-219 spectrophotometer (USA). The Tyr/Trp ratio was calculated as in [10].

Determination of the Number of S–S Bonds in the Lo Molecule. β-Mercaptoethanol was added in an amount to give a concentration of 1.5 mM to 2 ml of a solution of Lo (1.4 mg in 1 M Tris buffer, pH 8.6, with 0.02 M EDTA and 6 M urea). Then the Ellman reagent was added to a concentration of 20 mM and the optical density was measured at 412 nm. The number of SH groups was calculated with an extinction coefficient E = 13,600 [7].

N-Terminal sequences were determined by the automatic Edman method on a Rank–Hilger solid-phase sequenator (United Kingdom).

Peptide Maps. A tryptic hydrolysate of 0.005 μmole of one of the enzymes was deposited on a 20 × 20 cm plate with a layer of cellulose, and electrophoresis was carried out: for L-IV at pH 5.6 and a voltage of 40 V/cm for 50 min; for Lo, at pH 1.9 with a voltage of 10 V/cm for 10 min and 50 V/cm for 1.5 h. The dried electrophoretograms were subjected to chromatography in the pyridine–butan-1-ol–acetic acid–water (40:60:12:48, by volume) system. The peptides were revealed with a 0.3% solution of ninhydrin in acetone. Tryptophan-containing peptides were revealed with the Ehrlich reagent.

To compare the thermal stabilities of the enzymes, solutions containing equal amounts of enzymes (0.05 unit) in 0.05 M succinate buffer, pH 5.2, and in buffer with 0.5 M NaCl were used. Aliquots of 0.1 ml were kept at the required temperature for 10 min, and then 0.4 ml of a 0.04% solution of laminarin in 0.05 M succinate buffer, pH 5.2, that had previously been heated to 37°C was added. The residual activity was determined.

CONCLUSION

1. A comparison has been made of the enzymatic and structural characteristics of two endo-β-1,3-glucanases from the crystalline styles of bivalve mollusks.

2. The enzymes possess similar enzymatic properties but differ in thermal stability.

3. The enzyme molecules have differences in their N-terminal sequences and in the numbers of disulfide bonds and of tryptophan and neutral sugar residues.

4. Homology of the primary structures is to be expected in functionally significant sections of the enzyme molecules.

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