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A FUNGAL CELLULASE SHOWS SEQUENCE HOMOLOGY WITH THE ACTIVE SITE OF HEN EGG-WHITE LYSOZYME

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SUMMARY: The N-terminal amino acid sequence of an endo-β-1,4-glucanase from the cellulase complex of the white-rot fungus *Schizophyllum commune* has been determined. The sequence from Glu-33 to Tyr-51 was homologous with the active site sequences of various hen egg-white type lysozymes, including lysozyme catalytic residues (Glu-35, Asp-52) and substrate binding residue Asn-44. The homology offers evidence for a lysozyme-type mechanism in enzymic hydrolysis of cellulose.

The catalytic mechanism of hen egg-white lysozyme (HEWL), first proposed in 1967 on the basis of crystallographic studies (1), and by analogy with acid catalyzed hydrolysis of glycosides (2), involves general acid catalysis by Glu-35 and carboxonium ion stabilization by Asp-52. The essential features of the mechanism have since been confirmed, although the contribution of a third effect, namely ring distortion in the substrate during binding, is still a subject of debate (3). Other lysozymes, from bacteriophage T 4 (4), goose (5), and the mold *Chalaropsis* (6), show no sequence homology with each other or with the originally studied HEWL. However, subsequent structural studies (7, 8) have proven that phage, goose, and hen lysozyme classes are related and, indeed, that glutamic and aspartic acid residues are common active-site functionalities.

As early as 1963 it was suggested that all glycosidases may follow the same mechanistic pathway as HEWL (9). Cellulose, which like substrates of lysozyme is a β-1,4 linked polymer, is hydrolyzed to glucose by a combination of endoglucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) (10-12), but not by lysozyme (13). The available evidence to support a lysozyme-type mechanism in cellulases is very limited (for a review see 14, 15). Kinetic data has implicated a carboxylate anion (pK_a 4.0−4.5) and a protonated carboxyl group (pK_a 5.0−5.5) as the catalytic residues in a cellulase from *Aspergillus niger* (16, 17). Affinity labels were bound to aspartic acid in β-glucosidases from *Aspergillus wentii* (18) and bitter almonds (19). However, the adjacent sequences showed no identities to HEWL. The only other sequence data published for cellulases is the first 20 residues of two cellobiohydrolases (20) and an endoglucanase (21) of *Trichoderma reesei*. One of the cellobiohydrolases and the endoglucanase show some homology with each other (21), but again none of the three partial sequences has obvious homology with HEWL.

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We have isolated and characterized an endoglucanase from the Basidiomycete *Schizophyllum commune*, and determined the N-terminal amino acid sequence of the enzyme (22). An interesting homology between the endoglucanase and the active site region of HEWL will now be described.

MATERIALS AND METHODS

Strain. S. commune (ATCC 38548) was used for enzyme production as previously described (23).

Enzyme purification. The enzyme was purified to electrophoretic homogeneity from culture filtrate by a combination of fractional ethanol precipitation and ion-exchange chromatography (22).

Determination of the amino acid sequence. Automated Edman degradation of ^{14}C-carboxymethylated enzymes was performed with a Beckman model 890D protein sequencer (22).

RESULTS AND DISCUSSION

Purified endoglucanase, one of two major endoglucanases produced by *S. commune* when grown on cellulose, gave a specific activity of approx. 16 μmol bonds broken min^{-1} when assayed on carboxymethylcellulose by the procedure of Hulme (24). The enzyme displayed a typical endoglucanase-type pattern of hydrolysis when tested on cellodextrins; the hydrolysis rate increased with increasing DP and a variety of lower DP products were formed (22).

The amino acid sequence of residues 33 to 51 is shown in Figure 1 (upper sequence). Further details of the enzyme isolation, specificity and amino acid sequence are described elsewhere (22). When compared with the active site sequences of HEWL-type lysozymes (Fig. 1), sequence identity around Glu-33 and Asp-50 (endoglucanase numbering) is observed. Also of interest is the conservation of Asn-44; this residue in HEWL is implicated in substrate binding (1).

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Endo-β-1,4-glucanase from Schizophyllum commune
  33                                42              45        +          50
Glu-Ser-Cys-Ala-Glu-Phe-Gly-Asn-Gln-Asn-----Ile-Pro-Gly-Val-Lys-Asn-Thr-Asp-Tyr-

Lysozyme from baboon milk (25)
  35                                44              48                   53
Glu-Ser-Asp-Tyr-Asn-Thr-Gln-Ala-Thr-Asn-Tyr-Asn-Pro-Gly-Asp-Gln-Ser-Thr-Asp-Tyr-

Lysozyme from human milk (26)
  35                      +         44              48        +          53
Glu-Ser-Gly-Tyr-Asn-Thr-Arg-Ala-Thr-Asn-Tyr-Asn-Ala-Gly-Asp-Arg-Ser-Thr-Asp-Tyr-

Lysozyme from hen egg (27)
  35                                44  +                   49         52
Glu-Ser-Asn-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-Asn-Thr-----Asp-Gly-Ser-Thr-Asp-Tyr-

Lysozyme from duck egg (28)
  35                                44  +                   49         52
Glu-Ser-Ser-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-Asn-Thr-----Asp-Gly-Ser-Thr-Asp-Tyr-
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Figure 1. Sequence alignment of endoglucanase and four hen egg-white type lysozymes. Identities are underlined and proposed deletions are shown by dashed lines.

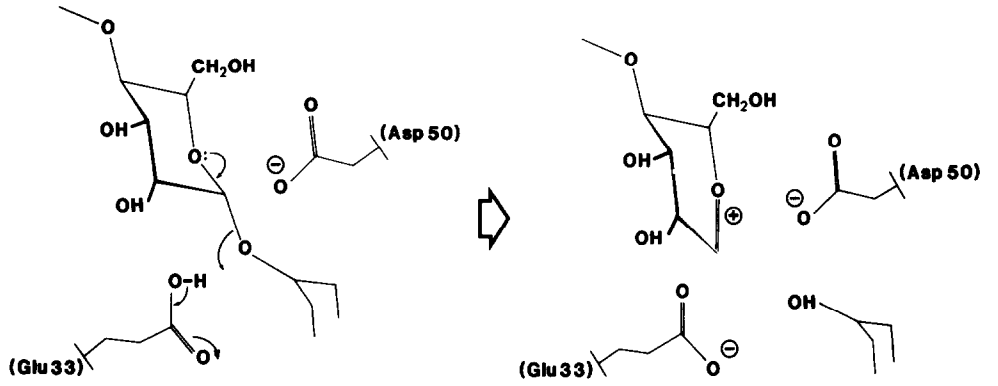

Figure 2. Proposed mechanism for endoglucanase catalyzed hydrolysis of cellulose.

The conserved sequences around Glu-33 and Asp-50 are strong evidence for the participation of these residues in catalysis of hydrolysis of cellulose, or cellodextrin chains, as shown in Fig. 2. By analogy with HEWL, Glu-33 of the endoglucanase performs general acid catalysis of cellulose hydrolysis and Asp-50 stabilizes the incipient carboxonium ion. The pH 5 optimum for activity of the endoglucanase (22) is consistent with this mechanism. Binding of the substrate chain could involve Asn-42, although in lysozyme the corresponding Asn-44 binds to an N-acetyl side chain of the substrate (1).

The sequence homology of the endoglucanase with HEWL-type lysozymes shown in Fig. 1 is remarkable since it implies a closer ancestral link between these enzymes of different specificity, than between HEWL and other types of lysozyme (goose and T4 phage (8)). In this respect the case of endoglucanase is analogous to that of lactalbumin (29).

The generality of a HEWL-type mechanism in the β-1,4 glycoside hydrolases remains to be established. As mentioned, no other sequence identity of this class of enzymes with lysozyme has been found. However, Legler (30) has previously commented on the similarity of the sequence around the active sites of β-glucosidase from *A. wentii* (Ser-Asp-Trp) and HEWL (Thr-Asp-Tyr). The published partial sequences of other β-1,4 glycoside hydrolases — a xylanase from *S. commune* (31) and the three enzymes from *T. reesei* (21) — have unfortunately been quite short, representing less than 10% of the proteins, and the active sites may therefore be found in the remaining sequences.

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