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DOMAIN STRUCTURE OF CELLOBIOHYDROLASE II AS STUDIED BY SMALL ANGLE X-RAY SCATTERING: CLOSE RESEMBLANCE TO CELLOBIOHYDROLASE I

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Evidence for a domain structure of cellobiohydrolase II (CBH II, 58 kDa) from *Trichoderma reesei* (Teeri et al., 1987; Tomme et al., 1988) is corroborated by results from SAXS experiments. They indicate a 'tadpole' structure for the intact CBH II in solution (Dmax = 21.5 ± 0.5 nm; Rg = 5.4 ± 0.1 nm) and a more isotropic, ellipsoid shape for the core protein (Dmax = 6.0 ± 0.3 nm; Rg = 2.1 ± 0.1 nm).

The latter was obtained by partial proteolysis with papain which cleaves the native CBH II to give two fragments (Tomme et al., 1988): the core (45 kDa) with the active (hydrolytic) domain and a smaller fragment (11 kDa) coinciding with the tail part of the model and containing the binding domain for unsoluble cellulose. This peptide fragment is conserved in most cellulolytic enzymes from *Trichoderma reesei* (Teeri et al., 1987). It contains a conserved region (block A) and glycosylated parts (blocks B and B' duplicated and located N-terminally in CBH II).

In spite of different domain arrangements in CBH I (blocks B-A at C-terminals) SAXS measurements (Abuja et al., 1988) indicate similar tertiary structures for both cellobiohydrolases although discrete differences in the tail parts exist.

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Cellobiohydrolase II, core CBH II, small-angle X-ray scattering, functional-structural domains, model for solution structure

Abbreviations: SAXS, small-angle X-ray scattering; cellobiohydrolase (CBH, 1.4-ß-D-glucan cellobiohydrolase, E.C. 3.2.1.91); Dmax: maximum diameter; Rg: radius of gyration

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The filamentous fungus *Trichoderma reesei* is known to produce a variety of cellulolytic enzymes, among them two types of cellobiohydrolases (CBH I and CBH II, Fägerstam and Pettersson, 1979; van Tilbeurgh et al., 1984). Their primary structures have been elucidated (Shoemaker et al., 1983; ; Teeri et al., 1983; Fägerstam et al., 1984). Both enzymes exhibit a similar action as they adsorb strongly onto insoluble substrates and lack carboxymethylcellulase activity. Differences in specifity may however be noted with respect to the degradation of small, soluble substrates (van Tilbeurgh et al., 1982) and microcrystalline cellulose (Henrissat et al., 1985).

Partial proteolysis of both CBH I and CBH II with papain (van Tilbeurgh et al.,1986, Tomme et al., 1988) cleaves these enzymes in two fragments. The larger fragment (56 kDa for CBH I, 45 kDa for CBH II), called the core protein, contains the catalytic site and the smaller consists of the terminal 35 amino acids long conserved region (block A) and the O-glycosylated regions (B in CBH I,BB' in CBH II). These homologous regions are situated at the C-terminus of CBH I (BA) but at the N-terminus of CBH II (ABB') (Teeri et al., 1987, Tomme et al., 1988). Both cores were fully active against soluble substrates but showed reduced adsorptive and hydrolytic activities against microcrystalline cellulose (Tomme et al., 1988).

SAXS studies on CBH I and its core revealed a tadpole-like structure of the intact enzyme and permitted the identification of the head part of CBH I as the core [Abuja et al., 1988]. The tail part of the tadpole is thus identical with the C-terminal peptide (BA block).

Presently we report on the results obtained with CBH II and its core.

The native CBH II was prepared from the extracellular fluid of *Trichoderma reesei* QM 9414 as reported (van Tilbeurgh et al., 1984) and its core was obtained by partial proteolysis with papain as described (Tomme et al., 1988). The purity of these preparations was checked by SDS-PAGE (CBH II, 58 kDA; core, 45 kDA) and IEF-PAG (CBH II p_i = 5.9; core p_i = 4.4). The molar absorption coefficient (280 nm) is 75000 M^{-1} cm^{-1} for CBH II and is unchanged for the core protein.

Scattering experiments were performed with a Kratky camera (improved compact type) with slit collimation system on a Philips

PW1730 X-ray generator with a copper tube which was operated at 50 kV and 30 mA. Each scattering curve was recorded up to 10 times in the range of h= 0.1 to 5.1 nm^{-1} ($h = (4 \pi \sin \Theta)/\lambda$, 2Θ : scattering angle, λ=0.154 nm , wavelength of the CuK_α - line). Measuring temperature was 4°C. A series of 5 concentrations from 8 to 37 mg/ml was measured for core-CBH II and from 8 to 31 mg/ml for intact CBH II.

Data evaluation, desmearing and indirect Fourier transformation were done as described elsewhere (Glatter, 1977; Glatter and Kratky, 1982; Pilz et al. 1977). Model calculations were performed with the MULTIBODY program (Glatter, 1980).

As for CBH I the intact enzyme is an elongated particle with a head part rich in mass and a long tail. Fig. 1 shows the model and its p(r)-function compared to the experimental p(r)-function (distance distribution function, $4\pi * p(r)$ represents the number of distances r in a homogenous particle, found by the combination of any volume element with any other within the particle; p(r) becomes zero at Dmax.) . The core protein is more isotropic and about one third of the length of the intact enzyme. The model and its p(r)-function are shown in Fig. 2. Dmax of the intact particle is 21.5 nm ± 0.5 nm, Dmax of the core is 6.0 nm ± 0.3 nm. The radii of gyration found are 5.4 nm ± 0.1 for the intact CBH II and 2.1 nm ± 0.1 for the core. For comparison we give the parameters for

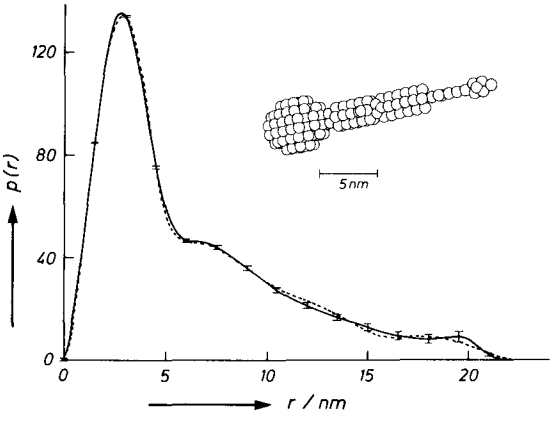

Fig. 1

Model structure and comparison of the experimental and model p(r)-functions of intact CBH II. The functions are normalized to an integral of 10^5.

experimental (————)

model (- - -)

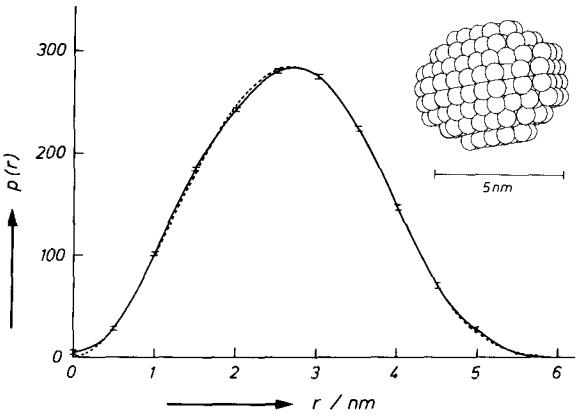

Fig. 2

Model structure and comparison of the experimental and model p(r)-functions of core CBH II. The functions are normalized to an integral of 10^5.

experimental (————)

model (- - -)

CBH I [Abuja et al., 1988]: intact - Dmax = 18.0 ± 0.5 nm, Rg = 4.3 ± 0.2 nm; core - Dmax = 6.5 ± 0.3 nm, Rg = 2.1 ± 0.2 nm.

The resemblance between the CBH II and CBH I models is striking (Fig. 3). Closer examination however reveals significant differences not only in size but also in shape. CBH II is even more elongated with a longer tail part which lacks the distinct "girdle" typical for CBH I. Studies on the primary structure of

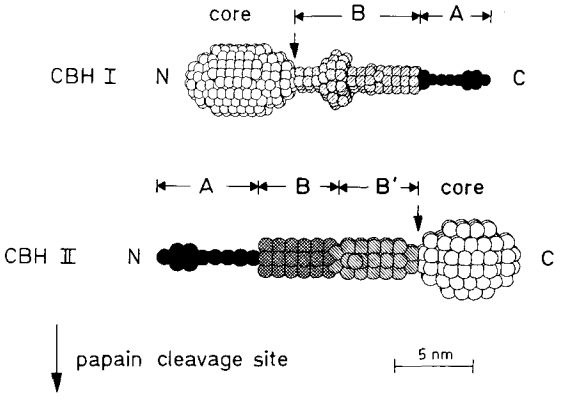

Fig. 3

Comparison between the domain arrangement of CBH I and CBH II. The core part is situated N-teminally in CBH I and C-terminally in CBH II, where block B is duplicated.

both cellobiohydrolases indicate in fact a duplication of the block B in CBH II (Tomme et al., 1988), situated in the tail part. This duplication (BB') is obviously responsible for the additional length of the tail part of CBH II.

For the core best fit was obtained with an ellipsoid with the axial ratios 1.4 : 1.0 : 1.0 which is identical with the head part of intact CBH II and has nearly the same shape and size as the core of CBH I.

Some preliminary function-structure relationships can be deduced from these and previous results. The compact heads (cores) contain the enzymes' active sites, whereas the tails (block BA and ABB') coincide with the binding domains for the insoluble substrate, cellulose.

Since the core proteins have been successfully crystallized (A. Jones, personal communication) more information about the position of the active site relative to these two structurally defined domains may be expected soon. The particular tadpole shape of the intact proteins may then functionally differentiate head (core) domains from the tail parts, the latter operating as 'anchors' to the cellulose. The very strong (nearly irreversible) adsorption of the intact enzymes seem to confirm this hypothesis.

The differences in the shapes of the tails for the two enzymes is a further intriguing observation. Although adsorptive capacities for both enzymes (onto Avicel) are comparable, specific activities are somewhat different. Also CBH I is far more sensitive to product inhibition. The synergistic action of both enzymes on the same substrate is a further peculiarity, which originates undoubtedly from the different adsorptive characteristics and which are presumably linked to the different shapes of the tails. Further investigations of the adsorptive behaviour of these enzymes and their interaction with large soluble substrates, as may be studies by SAXS, are in progress.

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