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The Cellulase Proteins of *Trichoderma reesei*: Structure, Multiplicity, Mode of Action and Regulation of Formation

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The filamentous fungus *Trichoderma reesei* is the predominant industrial producer of cellulolytic enzymes by secreting an enzyme system capable of degrading crystalline cellulose, which consists of several cellobiohydrolases, endoglucanases and β-glucosidases. All of these enzymes occur in multiple forms. A critical appraisal of the methods used to assess cellulase multiplicity is presented. By the aid of gene technology, advanced protein analytics and immunology, "true" isoenzymes and proteolytic fragments of all of these enzymes could be identified, and their structure and properties are described. Also, the recent elucidation of the three-dimensional domain structure of cellulases, their active center, and the role of both in the hydrolysis of cellulose are dealt with. Particular emphasis is presented on the differences in the enzymatic reaction mechanisms of cellobiohydrolase I and II, and their synergism.

Recent developments in the understanding of the triggering of cellulase formation by cellulose and its inhibition by readily metabolizable carbon sources are also presented.

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1 Introduction

Cellulose is by far the most abundant renewable carbohydrate source with an estimated synthesis rate of 4×10^7 tons per year. Many attempts have been made to utilize this enormous amount through enzymatic hydrolysis into glucose. Cellulolytic enzyme mixtures may be obtained from several microorganisms, but due to the physical nature of the cellulose molecule and the fact that its natural occurrence is always accompanied by associated materials (hemicelluloses, lignin), their efficiency deserves improvement in most cases, and different enzymes may be preferred for different purposes. However, the inherent complexity of cellulolytic enzyme mixtures in terms of number of enzyme components and their specificity has caused a still prevailing lack of sufficient understanding. This situation is still valid even for the most extensively studied cellulolytic microorganism, the fungus *Trichoderma reesei* and its mutant strains. As I will explain later, this is in part due to the difficulties to purify some of these enzymes to homogeneity. Considerable progress in our understanding of *T. reesei* cellulases has however recently been provided by studies involving genetic engineering techniques, monoclonal antibodies and physicochemical protein analytics (for Refs. see below). It is therefore the purpose of this paper to critically assess the current state of knowledge about the nature of the *T. reesei* enzymes involved in cellulose breakdown, and to define important areas still unsufficiently understood. It is noted that related aspects as conditions for production and application of the enzymes, albeit equally important, will not be covered here. These topics have already been subject of several detailed reviews recently [1–5].

2 General Molecular Properties of *T. reesei* Cellulolytic Enzymes

Cellulose is a linear β-1,4-glucosidically linked homopolymer of around 8000–12000 glucose units, which forms a crystalline unit held together by hydrogen bonding [6]). According to this structure, Mandels and Reese [7] postulated the involvement of two different types of enzymes in the degradation of natural cellulose: a "C_1"-enzyme, which renders the cellulose crystal accessible for hydrolytic attack, and a "C_x"-enzyme, which subsequently degrades cellulose by both endo- as well as exo-type attack. As will be reviewed in chapter 6, evidence for the existence of the C_1-enzyme is still obscure, but at least two exocellobiohydrolases (EC 3.2.1.91, CBH), several endo-β-1,4-glucanases (EC 3.2.1.4, EG), and β-glucosidases (EC 3.2.1.21) have been identified and characterized.

Enzymes acting on molecules like cellulose, which are insoluble and of similar, or even greater size than themselves, obviously require delicate tertiary structures: comparison of the sequence of approximately 50 fungal and bacterial cellulase genes and of some other polysaccharide hydrolase (for review see [8]) has offered evidence that such proteins are composed of separate domains, which allow a spacial separation of the sites involved in substrate recognition and enzymatic activity.

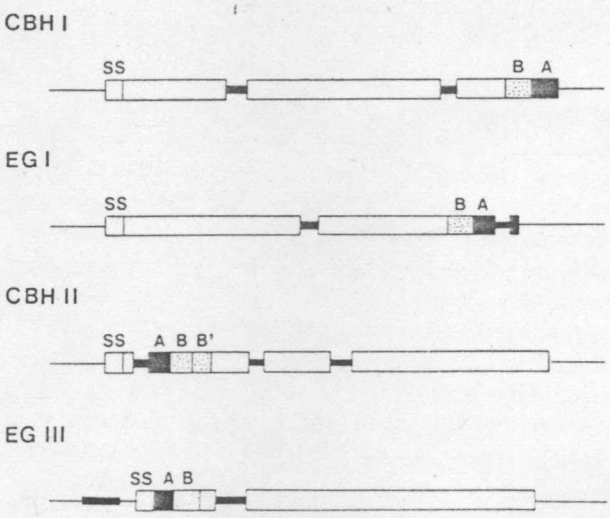

Fig. 1. Domain structure of the four major cellulase genes from *T. reesei*. Taken from Ref. [101], by permission

First evidence for a domain organization of the *T. reesei* cellulases came from the finding that partial proteolysis by papain can cleave CBH I and CBH II into an enzymatic active "core", and a cellulose adsorbing "tail" domain [9, 10]. These studies were complemented by data on the gene sequences of four main cellulase components from *T. reesei* (CBH I, CBH II, EG I, EG III), which revealed a strikingly conserved terminal domain in all four species [11], which is joined to the rest of the protein by a similarly conserved "hinge" domain (Fig. 1).

Coinciding evidence for such a domain organization of CBH I and II came from the pioneering work of I. Pilz and coworkers using small-angle X-ray scattering [12, 14]. These studies revealed a rather unusual tadpole like shape of both CBH I and II, with isotropic heads and protruding tails (Fig. 2). Corresponding data on the size estimates are given in Table 2. Due to the duplication of the B region in CBH II, this molecule is somewhat longer despite its lower molecular mass. Crystallization of either of these enzymes or any other cellulase has not yet been possible. However, after proteolytic removal of the AB-region, CBH II was recently crystallized [15]. This indicates that the A-, B-, or AB-region provides some flexibility to the CBH molecules. In Pettersons' group, AB-domains of CBH I from *T. reesei* and *P. chrysosporium* were isolated by proteolytic cleavage, and compared to a synthetic peptide synthesized according to the residues 462–497 of *T. reesei* CBH I. They were able to show [16] that the synthetic sequence (which lacks the carbohydrate rich B region) binds equally well to cellulose, but requires intact disulfide bonds. These studies support the idea that the AB-domain forms a functional domain wherein the A part interacts with cellulose, whereas the B-region provides a flexible arm connecting the catalytic and the adsorptive regions of the enzyme. The structure of this synthetic peptide in solution has more recently

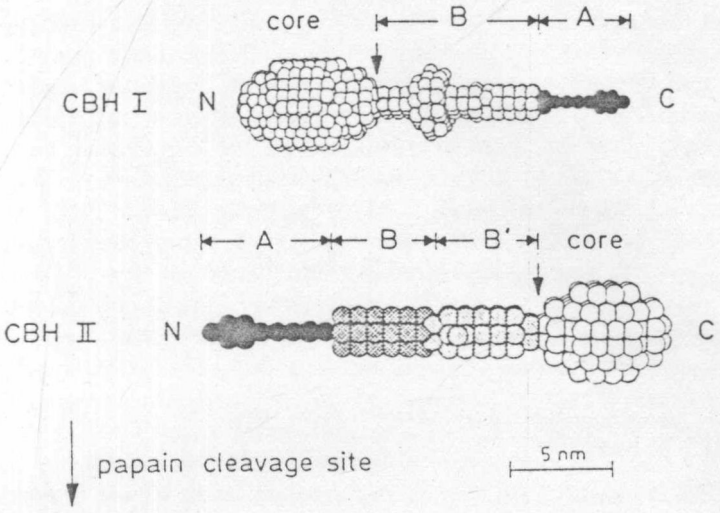

Fig. 2. Model structure of CBH I and CBH II from *T. reesei* taken from Ref. [14], by permission

been investigated by NMR and hybrid distance geometry-dynamical simulated annealing [17]. It was found to be made up of an irregular triple-stranded antiparallel β-sheet (β1, β2, β3), in which β3 is hydrogen bonded to the two other sheets. Its three-dimensional structure exhibits a wedgelike shape with an amphiphilic character, one face being predominantly hydrophilic, and the other hydrophobic. Both faces would be ideally suited to interact with cellulose, which has a flat, layered structure with hydrogen bondings in the plane of the layer and Van der Waals interactions holding the layer together [18].

The B-region carries heavy O-glycosylation, but the reason for this is still speculative. It has been assumed that it may serve to protect the enzymes against proteolysis [16]; however, proteolytic cleavage in fact starts by attacking within the B-region [10].

Detailed knowledge on the three-dimensional structure of cellulases has been strongly hampered by the failure to crystallize these proteins, however, recently crystallization of the catalytic core proteins of CBH II (and CBH I) has been successful [15]. CBH II consists of a seven strand singly wound parallel α/β-barrel (Fig. 3). Extended loops from the barrel produce a large channel for cellulose binding. The active site is located at the C-terminus of the β-sheet and can be clearly identified using data collected with an inhibitor diffused into the crystal. It is present in the tunnel, through which the cellulose threads, and two aspartic acid residues most probably form the catalytic residues (see also chapter 6). In this structure, the ABB' block (which is about 140 A long [14], see Table 1) is only 45 A apart from the entrance of the tunnel. It is therefore tempting to speculate that the anchoring non-catalytic domain holds the cellulose crystal in the appropriate position for the active center to attack.

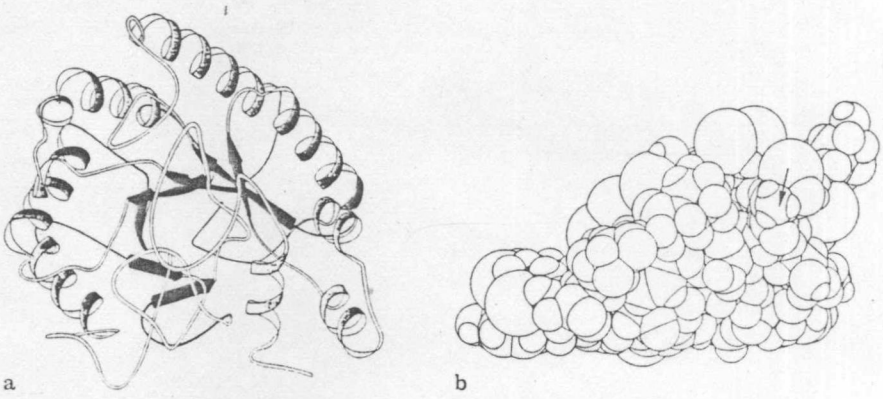

Fig. 3a, b. Schematic representation of the crystal structure of *T. reesei* CBH II core protein. Taken from Ref. [199], by permission

Although corresponding data for CBH I is still lacking, the structure outlined above may be typical for cellobiohydrolases, i.e. exoglucanases. Comparable investigations for endoglucanases are also still lacking, but a comparison of respective amino acid sequences (derived from gene sequences) show that e.g. EG I shows four clear deletions relative to CBH I; these deletions result in a failure to form the tunnel and probably result in a groove for multiple subsites [17]. Kraulis et al. [17] supposed that the various cellulases secreted by *T. reesei* may differ in the number of surface loops forming the active site. Moreover, the tunnel-like structure may be typical for exoglucanases, since it allows an attack only from the end of the cellulose chain.

Being secreted proteins, most of the cellulases from *T. reesei* have proven to be glycoproteins. Their carbohydrate structure may be deduced from detailed analysis with CBH I and in part EG I: it is composed both of N- as well as O-linked chains; most of the O-glycosylation occurs in the B ("hinge") region of the protein, whereas N-glycosylation is restricted to the core domain, most

Table 1. Concentrations of individual cellulases in the culture fluid upon cultivation of *T. reesei* QM 9414 under different conditions*

Conditions	Cellulase concentration (mg l^{-1})			
	CBH I	CBH II	EG I	β-Gluc
Glucose	<5	12	<5	7
Cellulose	960	270	250	12
Lactose	231	69	87	8
Sophorose	140	25	15	<5

* Data are taken from Ref. [66], by permission; β-glucosidase data (β-gluc) are from unpublished results in the authors laboratory

probably at β-turns. Such a location would be consistent with its protective role against proteolysis [19]. The N-linked carbohydrate resembles the highly conserved $(Man)_9(ManNAc)_2$ structure, found in other organisms, but some trimmed $(Man)_5(GlcNAc)_2$ antennae were also found [20]. In CBH I, three-N-glycosylation sites are occupied, but the proportion of Man_9 and Man_5 structures is roughly 1:4, hence suggesting microheterogeneity [20]. Carbohydrate microheterogeneity of CBH I has also been reported by Gum and Brown [21], yet these authors were unable to detect the N-glycosylation of CBH I. On the other hand, PSMS analysis of CNBr-peptides from CBH I confirmed the presence of 3-N-glycosylation sites, but failed to reveal any heterogeneity [21, 22].

The situation with O-glycosylation is even less satisfying: since this type of glycosylation lacks a defined target sequence, no predictions from the aa-sequence are possible. Analyses by different authors [20, 23, 24] revealed the presence of mono-, di-, tri- and tetrasaccharide chains. Attachment of these oligosaccharides to protein has also been observed in microsomes of *T. reesei* [25]. The relative proportions of these oligosaccharides in CBH I, however, differed strongly in different reports. Heterogeneity of O-Glycosylation of the CBH I-AB-region was analyzed by NMR [26], thereby showing that one enzyme population carries 24, and another one only 19 mannose units. The function of O-Glycosylation was discussed with respect to protection against proteolyses or adsorption to cellulose, but current knowledge for this is rather contradictory. On the other hand, there is some evidence, that O-glycosylation may be required for efficient secretion of cellulases [27, 28].

3 Enzyme Composition of *T. reesei* Cellulase Preparations — A Survey of Methods and Tools

While considerable progress has so far been obtained in the understanding of the structure and function of a few individual cellulases, the number and types of cellulases present in *T. reesei* are still a matter of dispute. This is in part caused by the fact that the method of their assessment has so far often been the stumblestone in the identification of the number of enzymes, which are involved in cellulose breakdown by *T. reesei*.

Electrophoretic techniques, especially IEF, have been frequently applied to examine the cellulase spectrum secreted by *T. reesei* [29, 32]. Identification of cellulases within the separated proteins can be conveniently achieved by overlaying the gels with cellulose followed by Congo Red [33] or dyed cellulose [34], and differentiation of different cellulases may be acchieved by staining with specific chromogenic artificial substrates [35]. Results obtained by IEF lead to the believe that the number of cellulase isoenzymes is very high [36, 37]. IEF is usually a powerful tool to separate isoenzymes with very similar physicochemical properties, and some of the multiple cellulases detected thereby may in fact be the result of carbohydrate heterogeneity [20, 21, 26], partial proteolysis [38, 39], transcript

heterogeneity [40] or other processes. IEF, however, is also subject to some pitfalls: firstly, ampholines may complex with some proteins and thereby produce multiple bands [41]; some cellulases may also form very stable complexes with each other: Sprey has shown that single bands of cellulases in IEF reflect the purity of (multi)enzyme complexes rather than that of the pure enzymes [42–45]. The forces involved in the association of cellulase proteins are unclear: some cellulases contain strongly hydrophobic domains, which may lead to their interaction with each other in aqueous environment [46, 47]. Sprey favored the role of an acidic, cell-wall derived polysaccharide, which may bind different cellulases [43], and hence lead to complex formation. Evidence for this polysaccharide has so far not been presented, but Sprey cites unpublished data that it contains „acidic carbohydrate chains". We have recently purified and characterized an acidic heteroglycan from *T. reesei* [48]. However, its role as an anchor for cellulase complex formation has yet to be assessed. In practice, all these factors may contribute to one or other extent to the multitude of bands seen in IEF, and therefore, render this technique of doubtful value in the analysis of cellulases.

Chromatographic techniques, particularly in combination with HPLC or FPLC, have also frequently been used to analyze the composition of cellulase enzymes [49–55]. Basically, these methods suffer from the same disadvantages as described above for IEF techniques. However, higher amounts of protein can be applied, and critical peaks can therefore be re-investigated by other techniques, or checked for cross-contamination by other enzymes [55].

Immunological techniques, using polyclonal antibodies, were introduced into cellulase analytics very early [56–58], but their specificity and hence the result obtained was always a matter of dispute [59, 60]. The specificity of polyclonal antibodies, raised against CBH I, CBH II and EG I, among which each polyclonal antiserum showed cross-reactivity with other cellulases, has recently been analyzed in detail [61]: cDNAs lacking regions coding for certain functional domains were produced by preparing series of 3'-end deletions, and the corresponding truncated proteins were obtained by expressing the cDNAs in yeast. The corresponding Western blots showed that all antibodies were almost entirely directed against the conserved terminal regions of the cellulase enzymes (see later for their description). This clearly emphasises caution when quantitative immunological assays are to be used to analyze the contents of individual cellulases.

Monoclonal antibodies clearly do not show this disadvantage, since – despite of high homology between some cellulases – some epitopes exist which are not shared by different cellulase enzymes. The isolation and properties of monoclonal antibodies against CBH I, CBH II, EG I, EG III and β-glucosidase have now been described [46, 47, 62, 63] and their application in cellulase quantitation by dot-blot-scanning [64, 65] and ELISA [66, 67] has been reported. The availability of monoclonal antibodies against different epitopes of CBH I and II [62] also offers the quantitation of intact and truncated forms of these enzymes [66]. Interestingly, some cellulase preparations contain unknown components interfering with ELISA, which have to be removed by precipitation of the cellulases with ethanol [66] or heat-treatment [67]. The cellulase composition of different *T. reesei* culture fluids, analyzed by ELISA, is given in Table 2.

Table 2. Molecular dimensions of CBH I, CBH II and their cores

Domain		Intact [nm]	Core [nm]	Tail [nm]
Head	CBH I			
	l	6.7	6.7	12.9
	d	4.4	4.5	3.2
	CBH II			
	l	5.4	6.0	15.2
	d	5.0	5.0	3.5

Data taken from Ref. [14], by permission

4 Properties of Individual Cellulases

4.1 Cellobiohydrolases

Cellobiohydrolases, as explained earlier, are defined as enzymes which split off cellobiose units from the non-reducing end of the chain. Two such enzymes, each occurring in several isoenzymic forms, have so far been identified in *T. reesei* – CBH I and CBH II – which are dealt with below:

4.1.1 Cellobiohydrolase I

CBH I comprises the major part of the cellulolytic enzyme mixture secreted by *T. reesei* (cf. Table 2). Its gene has been cloned and sequenced [69, 70]. It codes for a polypeptide with a corresponding M_r of 58 kDa. As emphasized earlier, it is both N- and O-glycosylated. Data from gene sequencing was completely consistent with the primary structure of CBH I protein by automatic liquid phase Edman degradation [71], when two different mutants were compared (L27 and QM 9414). However, a comparison of the gene sequence of *T. reesei* L27 and *T. viride* indicates only 95% homology, hence indicating several differences in the aa sequence of CBH I purified from different *Trichoderma* species.

Purification of CBH I has been reported by several authors [72–80]. The corresponding data is compiled in Table 3, which indicates considerable differences in the M_r of the purified proteins. However, the relative proportions of amino acids present in these proteins are well comparable. It therefore appears that the molecular weight determination of cellulases is subject to severe pitfalls. In any case, from this data there is little evidence for more than one single CBH I enzyme secreted by *T. reesei*.

Purified CBH I exhibits pronounced heterogeneity, but the reason for this has so far not been assessed clearly: early evidence of carbohydrate heterogeneity [21] has more recently been specified as O- and N-linked carbohydrate microheterogeneity [20, 26]. However, differential transcription termination has also been

Table 3. Amino acid composition and some properties of CBH I isolated from *T. reesei*

Ref.	[79]	[59]	[21]	[78]	[73]	[77]
Cys	4.8	3.8	4.1	4.7	4.3	4.1
Asx	11.3	11.9	12.5	12.4	12.0	11.2
Thr	11.5	11.3	10.7	12.7	10.7	11.3
Ser	11.3	10.8	10.4	10.8	11.2	11.3
Glx	8.2	9.0	8.2	10.0	8.8	8.2
Pro	5.6	5.6	6.4	5.0	5.7	6.0
Gly	12.5	13.3	13.2	15.1	12.3	12.7
Ala	5.8	6.3	6.8	7.1	6.0	6.2
Val	4.6	5.1	4.9	5.3	4.8	4.5
Met	1.2	1.4	1.6	1.5	1.1	1.4
Ile	2.4	2.4	2.3	2.1	2.2	1.9
Leu	5.6	5.9	5.5	6.1	5.8	5.2
Tyr	4.8	5.0	5.0	5.0	5.2	5.1
Phe	3.0	3.2	3.0	3.4	2.5	3.1
His	1.0	1.1	1.0	1.0	1.0	0.9
Lys	2.6	2.8	2.5	2.9	2.7	2.6
Trp	1.8	ND	ND	2.4	1.6	1.5
Arg	1.8	1.9	1.7	2.1	1.6	2.2
[kDa]	64.0	66.0	48.3	50.3	42.0	61.0*
CH [%]	6.0	ND	10.4	7.2	9.2	7.0

All amino acid values are given as % of total residues; ND, not determined; [kDa] indicates the M_r; CH [%] the carbohydrate content; * value for protein lacking carbohydrate given

observed [40]. Furthermore, tight binding of oligosaccharides from the medium to CBH I during purification has been reported [81]. All these observations may be the result of a very relaxed mechanism for glycoprotein formation and processing in *T. reesei*. However, multiplicity of CBH I does not appear randomly during cultivation, suggesting that it is an inherent property of this enzyme or its formation. It remains to be assessed whether enzyme microheterogeneity in fact fulfills a yet unknown physiological function.

4.1.2 Cellobiohydrolase II

The purification and characterization of the second cellobiohydrolase secreted by *T. reesei* was first described as an enzyme immunologically distinct from CBH I, which produced cellobiose from cellulose [57]. However, for a long time its purification presented a major methodological problem because its physicochemical properties (IEP, M_r) are very similar to EG I. This was finally overcome by using immunoadsorption [82] or affinity chromatography on thiocellobiose coupled to Affigel [83]. The *cbh 2* gene has been cloned and sequenced [84, 85]. It codes for a 471 aa protein, whose aa composition is identical to that of purified CBH II [79]. It contains a duplicated B-motive, and therefore, is glycosylated stronger than CBH I. Probably owing to the paucity of reports on purification of CBH II, no multiple forms have yet been described.

4.2 Endoglucanases

Endoglucanases hydrolyze β-1,4-glycosidic linkages randomly. They do not attack cellobiose but hydrolyze cellodextrins, phosphoric-acid swollen cellulose and substituted celluloses such as carboxymethyl (CM)- and hydroxyethyl (HE)-cellulose. *Trichoderma* seems to secrete a number of respective enzymes into the medium, of which two (EG I, EG III) have been the subject of a more detailed investigation. It should be noted that some confusion has arisen in the past from the fact that EG III was termed EG II by several authors from the US. However, this has been overcome now.

4.2.1 Endoglucanase I

EG I is the major endoglucanase secreted by *T. reesei*, whose purification has been reported by several authors [46, 68, 74, 78, 79, 86–93]. The precise structure of EG I has been revealed by the isolation of its gene [94, 95]. It codes for a 437 aa long polypeptide with an M_r of 46 kDa. The corresponding aa composition coincides very well with that of EG I proteins purified by Shoemaker et al. [68] and Bhikhabhai et al. [79]. It also coincides with aa compositions of endoglucanases purified by other workers (see Table 4), if their molecular weight determinations are considered erroneous (cf. 4.1.1) and adjusted to 55 kDa. The native M_r of EG I, when determined by LDSM is 52110 Da, indicating that a M_r of 6.1 Da is due to carbohydrate residues. The nature of this carbohydrate residues is however unclear: according to the aa sequence, 6 putative N-glycosylation sites would be present (at aa 78, 164, 204, 208, 281, and 394). However, PDMS of EG I CNBr-fragments showed that those aa at 78 and 164 are obviously not occupied. Nevertheless, the size of the glycosylated fragments was not compatible with the usual $(Man)_9(GlcNAc)_2$ or $(Man)_5(GlcNAc)_2$ structure [20]. Salovuori [96] reported that 70% of EG I carbohydrate was O-glycosidically linked. This would indicate that less of the resulting N-glycosylation sites are occupied in EG I, and that the differences in M_r found in the corresponding fragments must be due to other reasons (i.e., adsorption of cellooligosaccharides, non-enzymatic glucosylation etc.). Postsecretional modification appears to contribute only little to the strikingly high number of multiple forms of endoglucanases found by some workers [30–32, 37, 51, 52], since the enzyme is comparably resistant to proteolysis [46] and enzymatic deglycosylation [28]. The purification of a 50 kDa endoglucanase I, which can be identified according to its aa composition, and lack of detectable glycosylation [89] as EG I is so far the only result pointing to a possible postsecretional modification. Stahlberg et al. [97] cited unpublished results to have purified a truncated, AB-region less EG I from *T. reesei* culture filtrates. The occurrence of such a fragment has indeed been observed in some commercial cellulase preparations, albeit in very low amounts [98]. Since EG I exhibits a strong tendency for di- and trimerization, these oligomers are very stable and SDS-resistant, and can only be separated by treatment with non-polar solvents, indicating the involvement of hydrophobic forces in the aggregation process [99]. The EG I displays di- and trimer bands at IEP 4.7 and 5.1 (unpublished data), hence being responsible for at least two of the multiple EG I bands seen in IEF or chromatofocusing [100].

Table 4. Properties of various endoglucanases from *T. reesei*

Ref.	EG I						EG III				"small EG"		"EG III"
	[79]	[59]	[78]	[89]	[91]	[91]	[78]	[101]	[101]	[91]	[105]	[103]	[79]
Ala	5.5	5.9	6.0	5.8	5.7	7.3	7.2	7.0	7.8	8.0	8.6	8.6	11.6
Arg	1.8	1.8	2.0	1.7	1.8	1.4	2.6	2.5	2.8	2.8	1.2	2.0	1.9
Asx	13.9	12.7	13.6	13.8	12.8	12.7	13.7	12.3	13.1	12.3	10.5	11.1	7.0
Cys	5.0	4.7	4.1	ND	5.1	3.3	ND	3.0	ND	3.0	2.9	2.0	3.6
Glx	6.6	7.0	7.0	6.8	6.8	6.6	7.5	8.0	7.5	8.5	11.5	7.6	7.0
Gly	11.1	11.0	11.7	12.4	11.1	10.7	11.6	10.6	12.0	11.8	16.3	14.7	10.0
His	1.4	2.0	1.5	1.0	ND	1.2	1.1	1.2	1.4	1.5	6.4	1.5	0.7
Ile	2.7	2.9	2.8	3.1	2.8	3.8	4.5	5.3	5.0	4.8	3.0	3.3	3.6
Leu	5.5	5.4	6.0	6.2	5.4	5.7	6.0	5.8	6.1	5.2	4.9	4.0	3.9
Lys	2.3	2.1	2.3	2.4	2.2	1.6	1.5	1.5	2.1	1.7	4.6	2.5	2.0
Met	2.0	2.2	2.0	2.4	2.2	1.2	0.8	1.0	0.9	0.7	1.3	1.0	0.4
Phe	2.0	2.2	2.2	2.4	2.0	2.1	3.8	3.2	3.3	3.0	2.5	2.5	1.8
Pro	5.5	5.6	5.7	5.5	5.3	7.6	6.0	4.7	5.2	5.8	ND	4.6	6.7
Ser	13.4	13.5	12.9	11.7	13.4	11.8	8.7	10.5	11.7	9.6	8.5	11.7	11.7
Thr	10.5	10.3	10.6	10.3	10.3	11.1	10.9	11.0	11.4	10.0	5.8	9.1	15.0
Tyr	5.2	5.3	5.4	6.2	5.1	4.0	3.3	3.5	3.6	3.3	ND	5.5	9.1
Trp	1.6	1.6	ND	2.4	1.4	2.6	3.0	2.8	ND	2.5	2.1	2.5	1.6
Val	4.3	4.5	4.9	4.2	4.3	5.2	6.0	5.5	5.9	5.3	6.6	2.8	7.1
[kDA]	46	55	54	44.7	51	52	34.5	42.2	48.0		25	20.5	48
CH [%]	ND	11	ND	12	0	ND	15	18	15		0	0	12

Abbreviations as in Table 3