Comment on “Revealing Nature’s Cellulase Diversity: The Digestion Mechanism of Caldicellulosiruptor bescii CelA”

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Brunecy et al. (Reports, 20 December 2013, p. 1513) compared the cellulolytic activity of bacterial multimodular cellulase CelA with fungal Cel7A (cellulohydrolase I from Trichoderma reesei). If more active Cel7A from another fungus were used as a reference enzyme under optimal conditions with β-glucosidase added, the reported difference between bacterial and fungal enzymes would be less dramatic.

Brunecy et al. (1) proposed a novel mechanism of cellulose digestion by the dominant multimodular cellulase CelA of the thermophilic bacterium Caldicellulosiruptor bescii and compared its hydrolytic performance with that of a binary mixture containing Cel7A fungal exoglucanase (cellulohydrolase I from Trichoderma reesei) and bacterial Acidothermus cellulolyticus Cel5A (E1) endoglucanase. The major finding of the Report, that CelA is able to degrade cellulose into small fragments, is very interesting and indeed reflects a novel cellulose digestion paradigm. However, a few questions may be raised with respect to the methodology of this Report, as well as the quantitative comparison of bacterial CelA with fungal Cel7A (Cel7A/E1 mixture, in which the fungal enzyme plays a major role).

Although Cel7A represents the most heavily used cellulase in industry, as a major component of T. reesei commercial cellulase preparations, it is strange that the authors did not use optimal conditions for this enzyme in a comparison experiment on Avicel hydrolysis [figure 1A in (1)]. The temperature of 50°C may be optimal for Cel7A, the pH 5.5 is optimal for CelA (2) but not for Cel7A. The latter enzyme displays maximum activity at pH 4.0 to 5.0, whereas at pH 5.5 its activity is only 60 to 80% of the maximum (3–6). The pH of the reaction system is not specified in Brunecy et al.’s main article; this information may be found only in their supplementary materials (SM). The substrate concentration, used in the experiments on hydrolysis of different substrates [figure 1 in (1)], is not specified at all elsewhere, although this parameter is very important when analyzing the final substrate conversions.

It is unclear why the authors did not try to add β-glucosidase (BGL) to the Cel7A/E1 mixture in figure 1A in (1), whereas they did add it to CelA, when the optimal conditions for this enzyme were used [upper kinetic curve in figure 1A in (1)]. Cellobiose, the product of Cel7A action, is an inhibitor of the enzyme, and adding BGL may dramatically boost the performance of Cel7A. A reference to literature data [see text S3 in the SM for (1)] that adding BGL to the Cel7A/E1 mixture would be expected to produce a 30% increase in activity is not very convincing. Experimental data obtained under conditions used in the Report would be more persuasive. One may expect that if BGL were added to the Cel7A/E1 mixture, used under optimal conditions for Cel7A (pH 5.0 instead of 5.5; see above), then the difference in performance of CelA/BGL and Cel7A/E1/BGL would be less dramatic.

Other fungal cellulase mixtures and individual cellobiohydrolases, which are more effective than those produced by T. reesei, are known (6–12). In particular, cellulases from various Penicillium species proved to be more effective in hydrolysis of different lignocellulosic residues than the cellulase system and individual Cel7A of T. reesei (9–12). At least one of the authors of (1) is also a coauthor of a patent (12) in which more than twofold higher specific performance of the P. fumiculosum Cel7A + A. cellulolyticus E1 mixture over the T. reesei Cel7A + E1 mixture has been documented. So, the P. fumiculosum Cel7A instead of T. reesei Cel7A could be used for a comparison of a fungal enzyme with bacterial CelA in figure 1A in (1). In such a case, the performance of the fungal enzyme (especially, when used together with BGL) could probably be comparable to that of the multimodular CelA.

Then we come to molar efficiency of CelA. The authors declare that the activity of CelA acting on Avicel, on a molar basis, is seven times as high as that of the Cel7A/E1 mixture [or individual Cel7A at 15 mg/g glucan loading; see text S3 in the SM for (1)]. However, one molecule of CelA contains two catalytic modules (two active sites), whereas Cel7A contains only one. So, the calculations by the authors are not correct. At least they ought to have divided the 7-fold difference by 2, obtaining a ~3.5-fold difference. It is also unclear how protein concentration in the cellulose hydrolysis experiments was determined. As described in the Materials and Methods section of the SM for (1), the Bradford method is specified for a partial extracellular protein fraction, whereas a Pierce bicinchoninic acid (BCA) protein assay was used for the final purified CelA. Nothing is said about protein dosage calculations for the Cel7A/E1 mixture. McMillan et al. (13) reported a 2.5- to 4.9-fold increase in protein concentrations measured in cellulase samples using the BCA and Bradford assays. The drawbacks of the BCA assay, giving considerable protein concentration overestimations (especially when bovine serum albumin is used as the standard), have also been reported (14). Analysis of the enzyme hydrolytic performance (per mg of protein) would be less ambiguous if protein concentrations for purified enzymes were measured using their molar extinction coefficients at 280 nm (14). The possible problems associated with the determination of protein concentration in the reaction system, together with issues discussed above, make a precise quantitative comparison of the bacterial CelA with fungal Cel7A (Cel7A/E1 mixture) rather ambiguous.

Surprisingly, electrophoretic data for the final purified CelA are not presented elsewhere, although usually this is a necessary requirement when publishing reports on enzyme properties in biochemical journals. One may only guess whether the enzyme used in kinetic and morphological studies was homogeneous or whether it also contained truncated forms of CelA. In the latter case, the mechanism of cellulose digestion could be more sophisticated than that discussed in the article.

Finally, despite the above criticism concerning a comparative analysis of the hydrolytic performance of the bacterial multifunctional cellulase versus the mixture of free microbial cellulases, data presented in the report of Brunecy et al. (1) undoubtedly provide new and important knowledge about mechanisms of cellulose biodegradation in nature. CelA may also become a prospective enzyme for biotechnological applications, especially taking into account the fact that an era of commercial cellulase biorefineries has just begun (15).

References
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