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Different activities of a hydrolase under pressure

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Abstract

The general effect of High Hydrostatic Pressure on biopolymers and, especially, on proteins, is the well-known destabilization of their solution structures, leading, depending of pressure, temperature and treatment time, to dissociation of structural elements or partial or total denaturation (with the implicit reduction or loss of enzymatic activity). These effects can be reversible after return to atmospheric pressure or longer lasting, depending on factors kinetically controlling the process. However, in a number of enzymatic systems, the effect can be an enhancement of the activity or, at least, an overall performance improvement. This behavior deserves our attention, both as a technological improvement and as a chance for advancing in our understanding of pressure effects on biopolymers and of their structure-function-stability relationships, themselves.

Cellulases, hydrolases able to cut (1→4)-β-D-glucosidic bonds present in many polysaccharides, such as cellulose, are known to be sensitive to pressure, in given conditions improving its catalytic performance. It has been reported in the fungal origin (Trichoderma reesei) endoglucanase II, reacting with several plant substrates. Here, its effect on artichoke cellulose-rich is reported.

T. reesei hydrolytic activity is actually carried out by an enzymatic complex, containing different activities, cleaving polysaccharides in different positions. It is commercially produced under the name Celluclast™. Additionally, different domains carry out different functions, some of them catalytic, but others related to interactions with substrates. To obtain information on the mechanism in which activity is modified by pressure, the catalytic domain of T. reesei endoglucanase II was heterologously expressed in E. coli and purified. Its cellulose-cleaving activity was extensively characterized at atmospheric conditions, showing a behavior and stability similar to that within the complex, in spite of not being associated to the rest of proteins. Its activity was found to be enhanced by pressure.

These pressure-promotion effects on cellulose-cleaving activity were observed when the reaction took place under pressure, by measuring reactants and products before and after given periods of pressure treatment.

Celluclast™ commercial cocktail also shows a somehow unexpected hydrolytic activity towards chitosan. This linear, chitin-derived polymer by partial deacetylation, is also bound by (1→4)-β-D-glucosidic bonds, after all, not so different from cellulose bonds, but steric differences, as well as the net charge of chitosan in solution are differential factors to be noted.

In a different type of experiment, Celluclast™ previously treated in solution under pressure showed a significant increase of activity towards chitosan. The possible action mechanisms under these similar but not equal responses to pressure are discussed, proposing lines of research to ascertain the ways to increase this enzyme performance, as well as to understand pressure-related proteins structure-function relations.