

Note to the Editor

Does the increased hydrophobicity of the interior and hydrophilicity of the exterior of an enzyme structure reflect its increased thermostability?

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The values of hydrophobicity of internal and external elements of the secondary structure of three *Bacillus* α -amylase ($\beta\alpha$)₈ barrel domains have been calculated in order to investigate whether there is some correlation between the values and the enzyme stability. All the values have been referred to the number of amino acids in the given β -sheet or α -helix to eliminate the differences caused by non-equal length of the sheet or helix. Hydrophobicity units obtained have been averaged according to the number of internal (all β -strands and helix α 7) and external (helices α 1– α 6 and α 8) elements of secondary structure of the α -amylase ($\beta\alpha$)₈ barrel. The averaged hydrophobicity units have been found to correlate with the thermal stability of the three *Bacillus* α -amylases in terms of the increased hydrophobicity of the interior as well as the increased hydrophilicity of the exterior of the ($\beta\alpha$)₈ barrel domain for the α -amylase with increased thermostability.

Keywords: α -Amylase; amino acid sequence; ($\beta\alpha$)₈ barrel; hydrophobicity; thermostability

α -Amylases (EC 3.2.1.1) are the enzymes hydrolysing the internal α -1,4-glucosidic linkages in starch. Most α -amylases derived from animals, plants and microorganisms keep their unique enzymatic properties only in normal physiological conditions¹. Nevertheless, there are some microbial α -amylases of extremophiles which exhibit exceptional stability². Since the temperature is the best optimized physical variable in chemical reactions³, the efforts aimed at elucidation of structure–stability relationships in enzymes or their stabilization have been focused mostly on the thermostability⁴. Among the different types of physical forces that participate in stabilization of native protein structure, the hydrophobic effect may play a crucial role⁵. The contribution to protein stability of a buried methyl/methylene group has been demonstrated recently⁶ to be on average 6.3 kJ mol⁻¹, being the difference in Gibbs free energy between the native and the unfolded state of a protein, only about –40 kJ mol⁻¹ in general⁷. Therefore, the existence of some relation between hydrophobicity of internal, buried parts of a protein structure (e.g. the elements of secondary structure) and its thermostability might not be very surprising, α -amylase being a suitable subject for such an orientated study because its basic

structural features are known to adopt the eight-folded ($\beta\alpha$)₈ superstructure⁸, i.e. a barrel of eight parallel β -strands surrounded by eight α -helices. Moreover, there is a wide spectrum of microbial α -amylases that differ from each other in their thermostabilities. The aim of this work was to compare three *Bacillus* α -amylase amino acid sequences in an effort to find a key to the enzyme thermostability depending on the distribution of hydrophobicity along their ($\beta\alpha$)₈ barrel domains.

The amino acid sequences of the α -amylases from *Bacillus subtilis*⁹, *Bacillus amyloliquefaciens*¹⁰ and *Bacillus stearothermophilus*¹¹, used in this study, were taken from the literature. *B. subtilis* α -amylase is a thermolabile enzyme, whereas the other two are thermostable enzymes, the enzyme from *B. stearothermophilus* being more thermostable than that from *B. amyloliquefaciens*². The predicted locations of the secondary structure elements in the three *Bacillus* α -amylase ($\beta\alpha$)₈ barrels were taken from MacGregor⁸ (*B. amyloliquefaciens*) and Raimbaud *et al.*¹² (*B. subtilis*, *B. stearothermophilus*). The hydrophobicities (HBs) of individual α -helices and β -sheets were calculated according to the hydropathy scale of Kyte and Doolittle¹³. Their values for β -sheets are summarized in Table 1a. It is clear that, except for the strands β 2 and β 5, there is an ambiguous correlation between the increased HB of β -sheets and the α -amylase thermostability. For the strand β 3, there is the opposite trend from what has been expected. In order to get better correlation, the HB values were referred to the number of amino acids forming the individual stretches of secondary structure (hydrophobicity units, HUs), but the results (Table 1b) remained similar. By analogy, correlation between the increased hydrophilicity of α -helices and the α -amylase thermostability was not observed (data not shown).

The value for the helix α 7, which, due to its high hydrophobicity⁸, is assumed to be located in the interior of the α -amylase ($\beta\alpha$)₈ barrel, is of importance. Its HBs do not correlate with the thermostabilities of the dependent α -amylases. Moreover, the HUs for this helix show an opposite trend from what might be assumed. This fact could indicate that, although it is located in the interior, the helix α 7 probably has no special function in supporting the tertiary structure of the α -amylase ($\beta\alpha$)₈ barrels.

The fact that the results presented in Table 1 do not obey the hypothesis on correlation between the increased hydrophobicity of the internal parts of a protein structure and the increased thermal stability of the protein may reflect the existence of a moderate difference in thermostability of *B. subtilis* and *B. amyloliquefaciens* α -amylases². Furthermore, the isokinetic effect has been reported for the kinetics of the heat inactivation of chemically modified *B. subtilis* α -amylase^{14,15}, i.e. there is the so-called temperature of compensation, T_c , at which no effect on the thermostability of the modified α -amylase is seen and the opposite effects are observed both sides of T_c . The identified values of T_c (around 65°C)^{14,15} are very close to the temperature optimum for *B. amyloliquefaciens* α -amylase². And finally, the values of

Table 1 Hydrophobicities (a) and hydrophobicity units (b) of β -sheets from three *Bacillus* α -amylase ($\beta\alpha$)₈ barrels

α -Amylase	β 1	β 2	β 3	β 4	β 5	β 6	β 7	β 8
(a) <i>B. subtilis</i>	5.8	-2.7	7.3	-2.8	-7.6	-2.5	3.2	0.9
<i>B. amyloliquefaciens</i>	-4.5	5.7	3.9	-1.1	3.3	6.9	12.3	-1.1
<i>B. stearothermophilus</i>	-2.4	6.3	3.8	-1.8	8.1	4.4	8.8	4.3
(b) <i>B. subtilis</i>	0.97	-0.30	1.22	-0.56	-1.09	-0.42	0.46	0.13
<i>B. amyloliquefaciens</i>	-0.75	0.81	0.56	-0.22	0.55	1.38	2.46	-0.16
<i>B. stearothermophilus</i>	-0.34	1.05	0.54	-0.36	1.35	0.63	1.47	0.72

Table 2 Averaged hydrophobicity units of internal and external parts of three *Bacillus* α -amylase ($\beta\alpha$)₈ barrel domains

α -Amylase	HU _{int}	HU _{ext}
<i>B. subtilis</i>	0.17	-0.58
<i>B. amyloliquefaciens</i>	0.63	-0.58
<i>B. stearothermophilus</i>	0.66	-0.64

hydrophobicity of the individual amino acid stretches always depend on the hydrophobicity scale used for calculation¹⁶.

To find a relation, if any, between the distribution of hydrophobicity in the structures of the α -amylases and their thermostabilities, all β -strands and the helix α 7 should be taken into account together because they have been proposed to form the internal parts of the ($\beta\alpha$)₈ barrel domain⁸. The hydrophobicity of the interior of the barrels ought to grow as the thermostability of the α -amylases rises. On the other hand, the opposite trend might be observed for the rest of the α -helices, i.e. the external β -barrel-surrounding elements of secondary structure. Therefore, the individual HUs calculated for the internal parts (all the β -sheets and the helix α 7) as well as the external parts (the rest of α -helices) of the three *Bacillus* α -amylase ($\beta\alpha$)₈ barrel domains were averaged (HU_{int} and HU_{ext}) according to the number of internal (9) or external (7) elements in the ($\beta\alpha$)₈ barrel domain. These values (Table 2) were found to correlate with the thermostabilities of the individual α -amylases, such that increased hydrophobicity of the interior and simultaneously increased hydrophilicity of the exterior of the *Bacillus* α -amylase ($\beta\alpha$)₈ barrels were observed for more thermostable α -amylase.

These results are not conclusive criteria for the evaluation of protein thermostability but, on the other

hand, they may indicate the differences in the thermostability of enzymes derived from closely related origins, such as the three *Bacillus* α -amylases in the present study. Moreover, they show that one of the ways leading to the stabilization of a protein might be increasing the hydrophobicity of the interior or decreasing that of the exterior of the protein structure.

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