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Distribution of Charged and Hydrophobic Amino Acids on the Surfaces of Two Types of Beta-Fructosidase from Thermotoga Maritima †

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Abstract: *Thermotoga maritima* beta-fructosidases are enzymes which release beta-D-fructose from sucrose, raffinose and fructan polymers such as inulin. Surfaces of beta-fructosidases 1UYP and 1W2T from *Thermotoga maritima* was studied in this work. It was showed that amino acids are distributed not equally on the surfaces of the enzymes. Several clusters of charged and hydrophobic residues were detected at pH 7.0. Such clusters were detected by calculation of distances between them. It was determined that on surfaces of beta-fructosidases PDB ID: 1UYP and PDB ID: 1W2T respectively 96 and 95% of charged amino acids and also 50 and 42% of hydrophobic amino acids form clusters. 6 clusters of charged amino acids on surface of beta-fructosidase 1UYP and 5 clusters on surface of beta-fructosidase 1W2T was detected. Composition of such clusters is presented. Both types of beta-fructosidase have 3 clusters of hydrophobic amino acids on their surface. These facts should be considered when choosing immobilization conditions. It was showed that charged matrix is more promising to immobilization of beta-fructosidases 1UYP and 1W2T from *Thermotoga maritima* due to possibility of binding without significant loss of activity due to overlapping active center. Hydrophobic carriers are less promising due to probable active site overlap. Such binding may have a loss of enzyme activity as a result.

Keywords: beta-fructosidases; immobilization; molecular surface; charged amino acids; hydrophobic amino acids

1. Introduction

Thermotoga maritima beta-fructosidases are enzymes which releases beta-D-fructose from sucrose, raffinose and fructan polymers such as inulin. These biocatalysts are capable of hydrolyzing inulin, which can be obtained from cheap raw materials. Inulin (2,1-β-D fructan) is a linear homopolymer consisting of fructose units linked by β-2,1-bonds with a terminal glucose residue [1]. This polysaccharide is found in the roots and tubers of plants such as Jerusalem artichoke, chicory, leeks, onions, garlic, artichoke, wheat, banana and dahlia [2].

Enzymes immobilization is a promising method of creating of industrial catalysts and medical agents. Due to high specificity and activity enzymes are very affective catalysts. They are less toxic then many different substances that can be used as catalytic agents. However, using of soluble forms of enzymes has several limitations. For example, it's difficult to extract enzymes molecules from complete product. Also, soluble forms of enzymes can be degraded quickly in human organism by its protective systems so it's unable to create stability drugs based on soluble forms of enzyme. Such limitations can be resolved by immobilization [3,4].

There are several methods of enzymes immobilization. More stability immobilized biocatalysts can be developed by covalent bonds. Nevertheless, this method of immobilization limits conformational mobility and decreases the enzymatic activity significantly. The development of a heterogeneous catalyst based on an enzyme immobilized by adsorption on a carrier is a promising method. In this case, weak interactions limit conformational mobility to a lesser extent than covalent binding, which allows preserving the native structure of the enzyme and the highest activity as a result [5–7].

2. Methods

Beta-fructosidases from *Thermotoga maritima* PDB ID: 1UYP and PDB ID: 1W2T was chosen as objects of this work. Molecular structures were visualized using the Maestro 10.3 software (Schrödinger, New York, NY, USA).

The degree of remoteness of amino acid residues from each other was a criterion for the grouping of amino acid residues into a local cluster. The residues were assigned to a certain cluster if the distance between the nearest atoms of these amino acids did not exceed 10 Å. The distance was calculated based on the formula:

$$r = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}, \tag{1}$$

3. Results and Discussion

Hydrophobic and charged residues is distributed not equally on the surfaces of molecules of beta-fructosidase presented in this work. There are several clusters of amino acids were detected on the enzymes.

3.1. The Number and Composition of Amino Acids Clusters on Surfaces of the Enzymes

There are 83 charged amino acids presented on the surface of beta-fructosidase 1UYP and 85 amino acids of such type presented on the surface of beta-fructosidase 1W2T. It was determined that 96 and 95% of charged amino acids form clusters, respectively. We detected 36 hydrophobic residues on the surface of 1UYP enzyme and 33 residues of such type on the surface of 1W2T. It was showed than only 50 and 42% of hydrophobic residues form clusters on the surfaces of 1UYP and 1W2T, respectively. 6 clusters of charged amino acids on surface of beta-fructosidase 1UYP and 5 clusters on surface of beta-fructosidase 1W2T was detected Both types of beta-fructosidase have 3 clusters of hydrophobic amino acids on their surface. The composition of amino acid clusters is shown in Table 1.

Table 1. The composition of charged amino acids clusters.

	***Thermotoga maritima* 1UYP**	***Thermotoga maritima* 1W2T**
	Clusters of Charged Residues	
No. 1	Lys3, Arg37, Lys38, Glu40, His59, Asp67, Asp68, Glu69, Lys269, Arg270, Glu271	Lys3, Arg37, Lys38, Glu40, His59, Glu69, Asp94, Lys269, Arg270, Glu271
No. 2	Lys25, Lys27, Asp52, His56, Arg58, Glu80, Asp82, Lys84, Glu111, Asp115, Arg143	Lys25, Lys27, Asp52, His56, Arg58, Asp67, Asp68, Glu80, Asp82, Lys84, Arg93, Lys99, Glu101, Lys102, Glu111, Asp115, Lys118, Asp120, Lys127, Glu130, Glu131, His134, Arg143, Glu147, Asp157, Glu158, Lys159, Asp169, His173, Lys175, Glu177, Glu182, Glu184, Lys187, Lys224, Arg230

No. 3	Arg93, His97, Lys99, Glu101, Lys102, Lys127	Arg196, Glu199, Lys221, Glu222, Glu228, Lys229, Glu286, Glu289, Glu333, Arg351, Asp352, Glu353, Lys370, Glu374, Asp375, Glu376, Arg380, Asp396, Lys416, Lys421
No. 4	Glu130, Glu131, Glu147, Lys156, Asp157, Glu158, Lys159, Asp169, His173, Lys175, Glu177, Glu182, Glu184, Lys187, Arg196, Glu199, Lys221, Glu222, Lys224, Glu228, Lys229, Arg230	Asp296, Arg302, Lys303, Arg304, Lys305, Glu308, Asp318, Lys320, Glu321, Glu426
No. 5	Glu286, Glu289, Asp296, Arg302, Lys303, Arg304, Lys305, Glu308, Lys311, Asp318, Lys320, Glu321, Glu333, Arg337, Arg351, Asp352, Glu353, Arg369, Lys370, Glu374, Asp375, Glu376, Arg380, Asp396, Lys416, Lys421	Glu335, Arg337, Glu341, Arg360, Glu407
No. 6	Glu341, Glu344, Arg360, Glu407	
Clusters of Hydrophobic Residues		
No. 1	Ile11, Pro36, Pro61, Tyr65, Leu114, Val117, Trp431	Ile11, Pro61, Tyr65, Leu114, Val117, Tyr119, Gly121, Pro123
No. 2	Pro123, Pro128, Pro129, Gly132, Tyr176	Trp41, Trp260, Leu261, Gly264, Leu265, Val363
No. 3	Trp41, Trp260, Leu261, Gly264, Leu265, Val363	

Based on the above data, it can be assumed that the enzymes presented in this work may form stronger bonds with charged matrixes than with hydrophobic matrixes due to closer co-location of charged residues than hydrophobic. However, clusters of charged amino acids contain residues with opposite charge which may interfere with the binding of the enzyme to a matrix based on electrostatic interactions. These facts should be considered when choosing immobilization conditions.

3.2. Distribution of Amino Acids on the Surfaces of the Enzymes

As seen in Figure 1, there are clusters of amino acids in the region of the catalytic N-terminal domains of both types of beta-fructosidase. However, only such clusters were found for hydrophobic residues, which may indicate the probable binding of enzymes to the carrier in the region of the active center. Such binding may have a loss of enzyme activity as a result. Based on these data, it can be concluded that hydrophobic carriers are the least promising for the immobilization of fructosidase.

There are clusters of charged amino acid which are localized at sites remote from the active site in the region of the C-terminal domain. For 1UYP such clusters are No. 5 Glu286, Glu289, Asp296, Arg302, Lys303, Arg304, Lys305, Glu308, Lys311, Asp318, Lys320, Glu321, Glu333, Arg337, Arg351, Asp352, Glu353, Arg369, Lys370, Glu374, Asp375, Glu376, Arg380, Asp396, Lys416, Lys421 and No. 6 Glu341, Glu344, Arg360, Glu407. This may indicate the possibility of binding this enzyme to a charged carrier without significant loss of activity.

For beta-fructosidase 1W2T such clusters are No. 3 Arg196, Glu199, Lys221, Glu222, Glu228, Lys229, Glu286, Glu289, Glu333, Arg351, Asp352, Glu353, Lys370, Glu374, Asp375, Glu376, Arg380, Asp396, Lys416, Lys421, No. 4 Asp296, Arg302, Lys303, Arg304, Lys305, Glu308, Asp318, Lys320, Glu321, Glu426 and No. 5 Glu335, Arg337, Glu341, Arg360, Glu407. However, cluster 3 is localized in region of N- and C-domains both, which makes it possible to overlap the active center upon binding of this enzyme to a charged carrier. Nevertheless, it's still possible to bind this enzyme to a charged carrier without such an effect.

Based on these data, it can be concluded that the development of an immobilized biocatalyst based on these enzymes by its immobilization on charged carriers is promising.

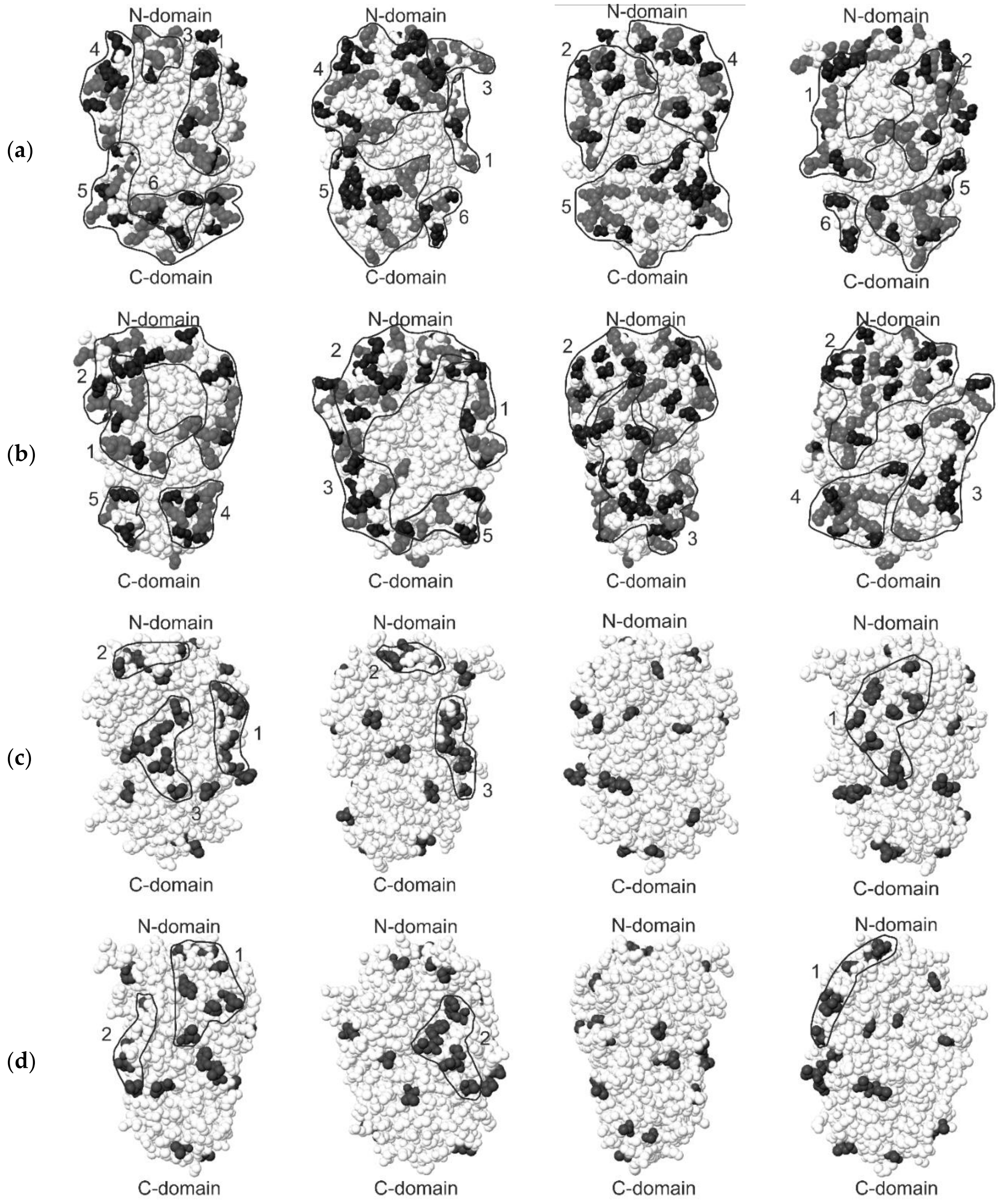

Figure 1. Distribution of amino acids on the surfaces of beta-fructosidases 1UYP and 1W2T (molecules rotated 90 degrees): (**a**) The distribution of charged amino acid clusters on the surface of beta-fructosidase 1UY; (**b**) The distribution of charged amino acid clusters on the surface of beta-fructosidase 1W2; (**c**) The distribution of hydrophobic amino acid clusters on the surface of beta-fructosidase 1W2T; (**d**) The distribution of hydrophobic amino acid clusters on the surface of beta-fructosidase 1UYP.

4. Conclusion

Insoluble biocatalysts based on immobilized enzymes are promising catalytic agents for industry and medicine due to high activity and stability. Immobilization based on weak interactions, such as hydrophobic and electrostatic interactions, make it possible to save more activity than covalent bonds.

It was showed that charged carriers are more promising for immobilization of beta-fructosidases 1UYP and 1W2T from *Thermotoga maritima* because of presence on its surfaces several clusters remote from active site. Binding of carrier's matrix with such clusters may lead to immobilization of the enzymes without significant losses of activity.

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