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A BIOINFORMATICS STUDY OF THE SURFACE PROPERTIES OF HUMAN AND BACTERIAL ALDOLASES

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SUMMARY

This study reveals that human and bacterial class I and class II aldolases, despite their low sequence similarity, present similar global fractal characteristics of their surfaces. Their surface fractal dimensions correspond to those reported in specific literature for globular proteins. There is one exception, bacterial aldolase class I, which shows a higher surface roughness confirming the dissimilarity of its active sites in comparison to other investigated aldolases and also its distinct evolution. The electrical properties of surfaces for the investigated proteins show that class II of aldolases have higher dipole moments and quite larger contact potentials, explaining the presence of active sites for Zn^{2+}, K^{+} and NH_4^{+} ions.

Keywords: aldolase; fractal surface dimension; electrostatic surface properties.

INTRODUCTION

Glycolysis is the metabolic pathway that converts glucose into pyruvate and it is thought to be the archetype of a universal metabolic pathway. It occurs, with variations, in nearly all organisms, both aerobic and anaerobic. The glycolysis mechanism can be divided in ten stages. At stage four, the sugar molecule is primed and the cell is ready to start breaking it up. The enzyme, fructose 1,6-bisphosphate aldolase, cuts the molecule in the middle, producing two similar pieces, each with a single phosphate attached.

Aldolase or fructose-bisphosphate aldolase are group of enzymes of lyases family, with molecular weight about 40 kDa per monomer which contains approximately 350 amino acid residues [1]. Usually aldolase biological unit is a dimmer or a tetramer. There are two classes of aldolases [2, 3]: aldolase class I (present mostly in animals and plant) forming a protonated imines with carbonyl group and aldolase class II (present in fungi and bacteria) increasing electron attraction by polarizing of the carbonyl group with metal ion as cofactor, usually zinc. In animals, aldolase can be found in muscles (type A), in liver (type B) and in brain (types C and A).

The aim of this study is to perform a bionformatics characterization of surfaces of animal and bacterial aldolases in correlation with their biological functions. It is useful to obtain such kind of information because the surface properties of proteins are important for protein-protein interactions, diffusion of metabolites, the absorption of drugs, DNA linkages, enzymatic catalysis and so on. On the other hand, considering that many of the proteins are loaded with electric charge and electrostatic forces have greater range of action, their electrostatic properties are also important because they may accelerate the combination of proteins and have a significant contribution to molecular recognition, protein-ligand and protein-protein interactions.

MATERIALS AND METHODS

We investigated fructose-bisphosphate aldolases, both animal and bacterial, with known crystallographic structures. In order to avoid studying similar sequences we performed a sequences alignment procedure using the free on-line accessible tool, ClustalW [4]. Thus we chose a set of five aldolases 2 human and 3 bacterial, whose sequential similarity is less than 70%. Considered aldolases are presented in Table I.

In order to investigate the surface properties of these enzymes we use their known crystallographic structures that are retrieved from Protein Data Bank [5]. The protein

surface area can be computed starting from various models, Van der Waals surface, molecular surface, solvent accessible area, etc, a detailed description of these methods being presented elswere [6]. Over the years, several algorithms have been developed based on these models for computing surfaces for proteins with known structure. There are some free accessible on-line tools to predict the protein surface cavities, such as CASTp [7]. For each of these aldolases we determined global and local surface properties.

Table I. The investigated fructose-bisphosphate aldolase

Aldolase Class	Type	Organism	glycolytic enzyme Swiss code	PDB code
Aldolase Class I	muscular type A	human	ALFA_HUMAN P04075	1ALD
	liver type B	human	ALFB_HUMAN P05062	1QO5
	bacterial	Escherichia coli	ALF1_PORGI P60053	2IQT
Aldolase Class II	bacterial	Escherichia coli	ALF_ECOLI P0AB71	1B57
		Mycobacterium tuberculosis	ALF_MYCTU P67475	3EKL

We investigate also the surface roughness measuring the surface fractal dimension. In order to determine this quantity we calculate the solvent accessible area SA of each protein for different redii of the rolling probe using the on-line tool GETAREA[8]. The surface fractal dimension is determined using the scaling law between the surface area *(SA)* of the protein and the radius of the rolling probe molecule *(R)*

$$SA \sim R^{2-D_s} \tag{1}$$

from the slope of the double logarithmical plot of *SA* versus *R* [6]. Surface area of the protein has been calculated using the probe radii of 1, 1.2, 1.4, 1.6, 1.8 and 2 Å respectively. In the case of dimmers, the surface fractal dimension has been calculated for the monomeric subunit A.

Another way to characterize the texture of the studied surface depending on the scale is to determine its lacunarity [9], which actually gives us a measure of how that fractal object fills the space region. This factor may differentiate from this point of view two objects of the same fractal dimension depending on texture. WE have determined the lacunarity coefficient by dividing the number of the surface cavities obtained for a rolling probe with radius of 1.4 Å to the number of amino acids of investigated structure.

The qualitative electrostatic properties of the surfaces of investigated enzymes is made using PyMol software [10]. This software can provide information on the local charge density (within 10 angstroms), regarding how positive, negative or neutral a region of the protein surface is relative to the rest of the protein. As this tool does not offer a quantitative measurement of electrical properties of the protein, one can use a very simple overall property, the dipole moment, and to analyze how it lines up in comparison with key structural features of the protein [11].

RESULTS

In Figure 1 we illustrate the determination of the global fractal dimension of muscular type of human aldolase surface.

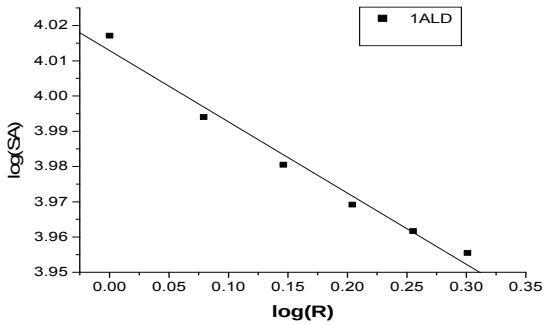

Figure 1. Determination of the global fractal dimension of muscular type of human aldolase (PDB entry code 1ALD)

Using this algorithm we have determined the global surface fractal dimension for all the aldolases considered in this study. We also determined, for every aldolase, the protein dipole moment, the protein contact potential, the number of cavities on the surface, largest cavity area and its lacunarity. The calculated parameters of the investigated proteins are presented in Table II.

Table II.The values of the investigated parameters for considered aldolases

PDB code	Dipole moment (D)	Protein contact potential	Protein Surface ($Å^2$)	Surface fractal dimension	No. of cavities	Largest protein cavity Surface ($Å^2$)	Lacunarity
1ALD	171	-58.006 58.006	9561.39	2.202±0.014	41	658.2	0.113
1QO5	189	-57.674 57.674	8912.28	2.257±0.012	45	562	0.125
2IQT	222	-71.099 71.099	7987.27	2.437±0.005	45	561.1	0.16
1B57	570	-66.093 66.093	9400.25	2.245±0.01	35	509.2	0.101
3EKL	733	-62.258 62.258	8803.13	2.28±0.015	42	301.2	0.126

Figure 2 illustrates the larges cavity for the muscular type of human aldolase (dark spheres in Figure 2). The surface of this cavity, computed using the same algorithm as presented in figure 1 is 2.877 ±0.153, significantly higher than 2.202 which corresponds to the global surface and this quantity reflects the higher degree of roughness for the cavity in comparison to the global surface. It also underlines that there are distinct local and global surface properties for the investigated proteins.

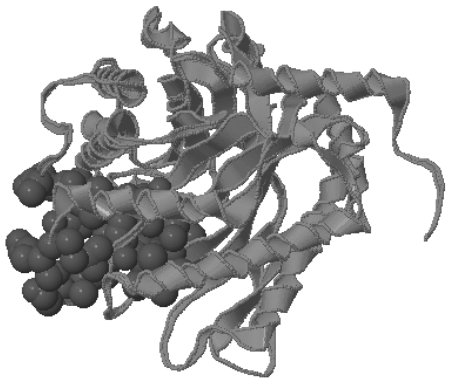

Figure 2. The largest pocket (dark spheres) for the muscle type of human aldolase A (PDB entry code 1ALD) calculated with probe radii R=1.4 Å and visualized using CASTp [7] software

Figure 3, obtained using PyMol software[10], reveals the electrostatic potential mapped on molecular surface for the muscle type of human aldolase. Dark regions are positively charged, gray ones are negatively charged and light ones are neutral. The local charge density on the surface of protein may influence how and where various substrates, inhibitors, cofactors, and other proteins bind. If these ligands have a large net charge or dipole, this effect might significantly increased.

Such a picture, in addition to other determined surface properties of the molecule and structural features of ligands, allows us to predict possible regions for charged ligands binding.

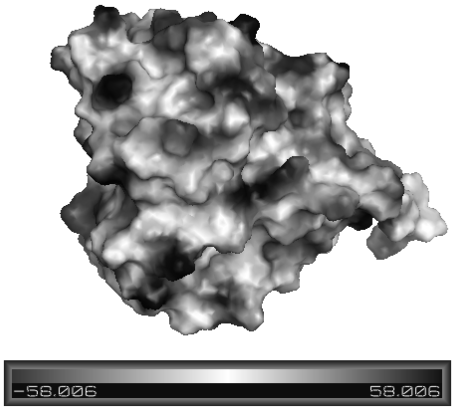

Figure 3. Electrostatic potential mapped on molecular surface for human aldolase, PDB code 1ALD

The average value of the dipole moment obtained for 14960 proteins is 542.66 [11]. For the investigated proteins, the dipole moments are presented in table 2 and we notice quite small values for this quantity for the class I of aldolases and higher ones for the class II.

DISCUSSIONS AND CONCLUSSIONS

Analysis of results presented in this paper reveal quite similar characteristics of surfaces for human and bacterial alsolases, despite their low sequence similarity. Student test shows that there are not significant differences between the global surface fractal dimensions and contact electric potential respectively for the two distinct classes of aldolases, class I and class II. This observation is important from the biological point of view. Being well known that glycolysis is one of physiological mechanisms that occurs in all types of organisms, we

interpret our results by the fact that, despite their low sequence similarity, aldolases developed such a folding in every organism to be able to perform their biological function.

The values of the global surface fractal dimensions obtained for investigated aldolases correspond to other values presented in specific literature for globular proteins [12] and this result underlines the applicability of the concepts of fractal geometry in structural characterization of proteins. Also, the fact that surface of aldolases have a fractal nature is important because it is known that the rate of substrate trapping by the active sites is largest when the protein surface has a dimension close to 2.2 [13].

There is one exception, bacterial aldolase class I (PDB code 2IQT), which presents a higher global surface fractal dimension and a more negative global surface electric potential than the other investigated aldolases. This aldolase has the lowest sequence similarity with the other aldolases considered in this study, between 2% with the human aldolase type A and 40% with human type B aldolase that may explain its different folding and distinct surface properties. Both class II aldolases considered in this study have higher dipole moments than class I ones, showing larger electrical properties. Our observation is in good agreement with other published data revealing that, although class I and class II aldolases catalyse identical reactions, their active are structurally dissimilar, and from sequence comparisons appear to be evolutionary distinct [14]. Also, aldolases belonging to class II present active sites for positive ions (Zn^{2+}, K^{+} and NH_4^{+}) binding [2] and it is in good correlation to their electric properties.

This study shows similar properties of the human and bacterial aldolases: the fractal nature and electrical properties of their surfaces in good correlation to their biological functions. The results obtained here may be used for other computational studies concerning ligands binding and/or drug design for aldolases. They also suggest that it is possible to perform experimental studies on class II of bacterial aldolases with a great probability to transfer them for humans

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