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The Protein Surface as a Thermodynamic Frontier: A Fractal Approach

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1. Introduction

The objects in Nature cannot be simply described in terms of the Euclidean geometry. It is difficult to find natural objects that can be represented as perfect spheres, planes and stright lines. Unfortunatelly, when researchers are intent on describing Nature in detail, they usually fall in mathematical descriptions that are extremately complex, many times with no solution. The fractal geometry is a fascinating conceptual framework (Mandelbrot, 1982) as it possibilitates to characterize Nature irregularities with a single number, a really tempting idea *per se*. Moreover, this idea push us to a change in our minds when describing Nature, as we are used to the limited vision given by the Euclidean geometry. The word "irregular" itself remind us the idea that these objects do not fit into the Euclidean description of Nature. Fractals act as compression algorithms, as they contain minimal information and a reduced complexity (Abel & Trevors, 2006). Most of the objects in Nature are irregular, and the most fascinating thing is that irregular objects are the norm in the fractal geometry. Unfortunately, the application of the "fractal" label has been too extended, mainly because the mathematical law that rules this concept (a potential law) is versatile enough to allow that virtually all experimental data set fits the equation (Bryant et al., 1989).

The surface of a protein constitutes the spatial domain through which the proteins interact with the surroundings. A great number of processes depend on surface phenomena, which at the same time depend on a wide range of structures and geometrical patterns. The protein surface is determined by the packing achieved in the folding process. Hence, the study of the geometrical characteristics yields valuable information not only on the folding process itself, but also related to the proteins in their interactions with the surroundings. The folded structure of a protein determines two different but closely related characteristics: stability and functionality. The stability of a protein relates with the surrounding media, while its functionality relates with its capacity to interact with that media through the interfacial surface.

There is a consensus in considering the aqueous media surrounding the protein as the interacting media. In this regard, the protein surface is determined as the surface contacting the water molecules. However, this definition left behind other types of interactions that may take place, like protein-protein, protein-ligand, protein-DNA, or even, out of the natural context, protein-metallic surface. The immediate conclusion that one can take out is that the exact nature of the protein surface is highly dependent on the size of the interacting

media, which may vary from something very tiny (water molecule) to something relatively big (metallic surface). In other words, the surface of a protein depends on the scale considered.

Proteins have occupied a privileged status in the application of the fractal concepts: from the first work in 1980 (Stapleton et al., 1980) until mid 2010, almost 800 papers appeared. However, the eventual fractal nature of the proteins is an issue still not resolved: neither is proved that the fractal dimension is a property of the protein, nor exist a unique fractal dimension value that characterizes all the proteins. Thirty years after the first study, one can arrive to the conclusion that the point has not been firmly concluded. In this chapter, we will briefly review the main point of views in which protein structures have been treated by a "fractal approach", and we present a new view in which we will show that a specific protein, cytochrom *c*, is not a fractal object, but forms part of a materials distribution network of fractal nature.

2. The protein surface

2.1 Definitions

The surface of a protein constitutes the first level of communication with its sorroundings. It is already recognized that the global and local roughness of the protein surface affects this communication in terms of diffusion, molecule recognition, and physical properties.

Among the different definitions of protein surface, the following are the most commons (Fig. 1).
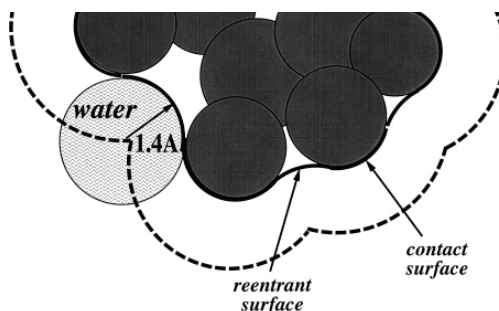

Fig. 1. Protein surfaces defined after rolling a rigid sphere along the protein. The full line represents the contour of the SES, and the dashed line represents the contour of the SAS

- **van der Waals surface (SVDW).** It is the surface resulting from considering each atom of the protein as a sphere with a van der Waals radii. The external surface results from the multiple superpositions between each spheres.
- **Solvent accessible surface (SAS).** This defintion was introduced by Lee and Richards (Lee & Richards, 1971) as the surface generated by the center of a rigid sphere of fixed radii (usually that of water, 1.4 Å) after let it roll all over the van der Waals surface.
- **Solvent excluded surface (SES).** Originally named as "molecular surface" (Richards, 1977), is the surface defined by two geometric domains: the contact surface resulting from the accessible van der Waals surface contacted by a rolling sphere of fixed radii (probe sphere), and the reentrant surface defined as the internal face of the rolling sphere when contancting simultaneously two or more atoms.

- **Hydration surface (HS).** It is defined as the second layer of water molecules sorrounding the protein (Gerstein & Lynden-Bell, 1993). This definition put the protein surface between the bulk solvent and the water molecules that strongly interact with the porotein. It is based on the fact that out of this surface, water molecules posses the same energy than those of the bulk, and are not oriented according to the dipole moment of the protein.

The protein surface definitions schematically depicted in Fig. 1, show the singular points resulting from the intersections of atoms in the SVDW. This singular points are also projected in the SAS, but resulted smoothed in the SES. The analytical and geometrical solution of these singularities are one of the main aspects in the development of algorithms for the calculation of the protein surfaces, because the singular points are not differenciables, and hence do not have analytical solution. An additional point of concern is the assessment of those atoms that do not participate of the exposed surface of the proteins, and that could be left out in the consideration in order to speed up the calculations. Hence, most of the algorithms which are continuously proposed differ in the way the singular points and the non-surface atoms are treated (Wodak & Janin, 1980, Goetze & Brickmann, 1992, Gabdoulline & Wade, 1996, Totrov & Abagyan, 1996, Flower, 1997, Can et al., 2006); their descriptions are beyond the scope of this chapter.

Among all algorithms proposed, MSDOT created by Connolly (Connolly, 1983) is considered the reference algorithm aginst which all the new ones are compared. The algorithm of Connolly calculates analytically the SAS. Of historical value is the algorithm developed by Lee and Richards (Lee & Richards, 1971), which was the first one that solved the singular points by designing a smooth surface coincident with the VDWS.

For the interest of the present work, it was necessary to have an algorithm capable of changing the size of the probe sphere. We have successfully employed the suit of programs ARVOMOL (Fernández Pacios, 1994) and SurfRace (Tsodikov et al., 2002), which were upgraded by their authors to suit the specific requierements of our work.

2.2 The protein surface and the thermodynamic stability of the folded state

The stability of the native state of a protein is governed by the sign and mangnitud of the Gibbs energy, $\Delta_{unf}G$, of the unfolding process:

$$\Delta_{unf}G = \Delta_{unf}H - T\Delta_{unf}S \qquad (1)$$

where $\Delta_{unf}H$ and $\Delta_{unf}S$ represent the change in the enthalpy and entropy of the process, respectively, and T is the temperature. Eq. (1) is applicable to every process involving some kind of phase transition, and protein denaturing is one of this processes. The change in the Gibbs energy depends on the temperature. For a given temperature T different to a reference temperature T_R, the change in the Gibbs energy is:

$$\Delta_{unf}G\,(T) = \Delta_{unf}H(T_R) + \Delta_{unf}C_P\,(T\text{-}T_R) - T[\Delta_{unf}S(T_R) + \Delta_{unf}C_P \ln(T/T_R)] \qquad (2)$$

where $\Delta_{unf}C_P$ is the net change in the heat capacity in the unfolding process. The thermodynamic stability of a protein can be obtained in calorimetric experiments. The interpretation of these changes in the thermodynamic properties requires the knowledge of the events taking place during the process. Of course, this does not imply that these values depends on the pathway taken during the process, as being state functions, the change in the thermodynamic properties G, H, S and C_P depends exclusively on their values in the

initial and final equilibrium states. Bearing this in mind, it must be recognized that proteins are folded in an aqueous environment, and most of the interactions that are broken in the unfolding process were between the chemical groups of the aminoacids and the water molecules of the solvent. Hence, the changes in the thermodynamic properties taking place during the unfolding process are due to a balance between the interactions of the folded protein within their own aminoacids and with the solvent, and the interactions between the unfolded protein and the solvent. This can be summarized as the addition of several contributions to the total change in the Gibbs energy:

$$\Delta_{den}G = \Delta_{gen}G + \Delta_{ion}G + \Delta_{tr}G + \Delta_{other}G \qquad (3)$$

where $\Delta_{gen}G$ includes all the generic contributions associated with the formation of the secondary and tertiary structures (van der Waals, H-bonds, hydration, conformational entropy), $\Delta_{ion}G$ takes into account all the electrostatic effects in the formation of the native structure, $\Delta_{tr}G$ includes the change in the translational degree of freedom produced in the native structure in the unfolding process, and $\Delta_{other}G$ includes all the specific interactions (prosthetic groups, metals, ligands) within the protein.

The major contribution to the enthalpy change comes from the formations of intramolecular interactions, like van der Waals and H-bond, together with the desolvation of the chemical groups involved in these interactions. The enthalpy change due to these interactions is included in a generic term, $\Delta_{gen}H$, which can be expressed as a function of the atomic contributions of the changes in the salvation degree produced during the unfolding process:

$$\Delta_{gen}H = \Sigma_i \, \alpha_i(\rho) \, \Delta SAS_i \qquad (4)$$

where the sum includes all the atoms of the protein, ΔSAS_i is the change in the solvent accessible surface for each atom and $\alpha_i(\rho)$ is a coefficient that depends on each atomic type and the mean packing density in the protein.

The change in the heat capacity of the proteins in the unfolding process depends on the change of hydration of the exposed groups in the native and unfolded structures. These changes are closely related to the solvent accessibility:

$$\Delta_{unf}C_P = \Sigma_i \, \alpha_i(T) \, \Delta SAS_i \qquad (5)$$

where $\alpha_i(T)$ is a coefficient that depends on each atomic type, and it is a function of the temperature. The factor ΔSAS_i depends on the atom considered: non-polar atoms contribute negatively to $\Delta_{unf}C_P$ upon loosing the interaction with the solvent, while polar atoms contribute positively (oxygen is an exception).

Finally, the calculation of the entropy change for the unfolding process includes two main contributions: one arising from the salvation changes ($\Delta_{sol}S$) and the other due to the conformational changes ($\Delta_{conf}S$). While $\Delta_{conf}S$ is practically independent of the temperature, $\Delta_{sol}S$ dependence of the temperature is a function of the type of aminoacidic residue (polar or non-polar) in accordance to:

$$\Delta_{sol}S \, (T) = \Delta_{sol}S_{non-polar} + \Delta_{sol}S_{polar} \qquad (6)$$

$$\Delta_{sol}S \, (T) = \Delta_{sol}C_{P,non-polar} \ln(T/T^*_{non-polar}) + \Delta_{sol}C_{P,non-polar} \ln(T/T^*_{non-polar}) \qquad (7)$$

where T^*_i corresponds to the temperatures at which the absolute entropies of hydration of polar and non-polar residues are zero. The dependence between $\Delta_{sol}S$ and $\Delta_{sol}C_P$ (Eq. 7)

establishes the dependence between the entropy changes for the unfolding process and the solvent accessible surface (viz. Eq. 5).

2.3 Calculation of the protein surface

There are several techniques to determine the surface of a protein. The method of the rolling ball yields surface area values which depend on the radius of the sphere probe. The dimension is calculated from the double logarithmic transformation:

$$D_s = 2 - (\partial \log S(r) / \partial \log r) \tag{8}$$

Another way to calculate the fractal dimension of the protein surface is based on the co-dimension rule. After rolling the probe sphere over the protein, the contour obtained after performing parallel cuts with planes are measured with a variable rule of size ε. The size of the contour is proportional to ε^{D_c}, where D_c is the fractal dimension of the contour. According to the co-dimension rule, the surface fractal dimension is (Pfeifer et al., 1985):

$$D_s = D_c + 1 \tag{9}$$

3. The fractal approach to the description of the protein structure

Proteins are heteropolymers with variable composition obtained by combination of a basic pool of 20 aminoacids. The aminoacid sequence constitutes the primary structure of the protein, and the information contained in such structure is enough to determine the three-dimensional folding of the protein. The structure of the folded protein reveals a variety of interactions that brings into contact aminoacids located far apart each other in the primary structure. Such interactions include covalent (disulfide bridges), electrostatics and weak forces. These long-range interactions lead to the formation of the compact structure of the proteins, which shape fall in the non-Euclidean geometry. Fractal geometry appears as the alternative concept that allows for the description of the protein structure.

3.1 Proteins as mass fractals

Let us consider concentric spheres of increasing radius around the centre of mass of a protein, and its mass M determined as a function of the radius r. The mass fractal dimension (D) is defined by the scaling relationship:

$$M(r) \propto r^D \tag{10}$$

If the mass fractal dimension is determined outside the centre of mass of the protein, i.e. near the surface, the values are greatly affected (Enright & Leitner, 2005). The mass fractal dimension of proteins gives values for the fractal dimension for proteins of 2.47 (Moret et al., 2005, Moret et al., 2006, Moret et al., 2009)

3.2 Proteins as line polymers

When the protein is considered from the point of view its primary structure, the fractal dimension of the protein backbone can be determined. The length of the backbone is measured by a stepwise connection of straight lines between alpha carbon atoms, being the intervals of increasing length ε. The number of steps N is then considered as a function of the variable length ε, and the fractal dimension is defined by the scaling relationship:

$$N(\varepsilon) \propto \varepsilon^{-D} \tag{11}$$

The value reported for the fractal dimension calculated according to Eq. 11 is 1.65 (Isogai & Itoh, 1984, Isvoran et al., 2001, Isvoran et al., 2008).

3.3 Proteins as roughened surfaces

A rigid sphere that rolls all over a protein is able to detect its surface irregularities. However, the degree of detail achieved in the description of the surface depends on the size r of the sphere. Let it $N(r)$ be the number of probe spheres needed to cover the whole surface. The dimension for this surface emerges from the following relationship:

$$N(r) \propto r^{D} \tag{12}$$

The measured surface for the object is a function of the sphere probe radii, $s = s(r)$, and it is defined by the number of sphere probes multiplied by the sectional area (Torrens et al., 2001):

$$s(r) \propto N(r)\,\sigma(r) \propto r^{2}r^{-D} \propto r^{2-D} \tag{13}$$

If the scale exponent is unique, the dimension D has a fractal nature. The surface dimension calculated in this way is between 2.06 – 2.17 (Craciun et al., 2009, Aqvist & Tapia, 1987, Pettit & Bowie, 1999).

3.4 But…Are protein fractal objects?

As Mandelbrot himself recognized (Mandelbrot, 1984), the fact of being him who coined the term "fractal", he cannot control its use. In fact, he does not use the term "fractal", and instead he prefers to use the term "fractal dimensionality" to refer to any anomalous dimensionality. We feel that it is important to be strict in maintaining the mathematical definition of fractal in order to discuss the eventual feasibility of a fractal description of the proteins.

Let us first consider the scale length. In order to assess the fractality of an object, the scaling behaviour should hold in several orders of magnitude. Avnir (Avnir et al., 1998) has been highly critic in this respect, questioning the wide use of the label "fractal". Most of the published works determined the fractal nature of different objects in a scale range spanning between 0.5 and 2 orders; the accomplishment of a potential equation by the data is a necessary but not a sufficient condition for applying the concept of "fractal" to that object. From a strict mathematical point of view, the arguments of Avnir are correct. However, the same author acknowledges that the practical limitation for considering an object as fractal lies on the cut off limits that exist in real objects, a fact considered by other authors in a well-known discussion on this topic held in the *Science* magazine (Mandelbrot et al., 1998). In the case of proteins, the lower cut off is ideally given by the size of an atom (1 Å), and the upper cut off by the size of the protein itself (few tens of Å), implying just two orders of magnitude in the scale. Facing this fact, Fernández-Pacios (1995) proposed the use of the term "effective dimension" to refer to the dimensionality of the proteins.

Some methodological aspects in the evaluation of the surface dimension of the proteins are now considered. The co-dimension rule gives unequivocally information of the protein surface, as strictly the surface contour is considered. Unfortunately, there is not enough

evidence to assess the quality of this method. For the case of lysozyme, the D_s obtained was 2.17. When the surface assessed through Eq. (4) is employed to calculate the fractal dimension, some discrepancies are notorious. Usually, values around 2.2 are obtained. In a single case, the calculated surface dimension of the lysozyme was 2.44 (Lewis & Rees, 1985). In this case, the radii range employed was 1 – 3.5 Å, may be so little that one can wonder if the probe sphere was able to get into the protein and evaluate its interior. In fact, when the mass fractal dimension is calculated in two different ways, one putting the increasing sphere in the centre of the protein, and the other putting the sphere towards the border of the protein, the value of the calculated surface dimension were 2.4 (Enright & Leitner, 2005, Moret et al., 2005) and 2.2 (Xiao, 1994, Enright & Leitner, 2005).

From the above discussion, arises an intuitive definition of *internal surface* and *external surface*. The interactions that take place across the internal surface are related to the intramolecular interactions between atoms and residues, while those established through the external surface would be related with the protein surroundings, like the solvent, ligands, etc. Hence, it is the external surface the one that is considered the thermodynamic surface.

4. An alternative view for the fractality of proteins

4.1 The West-Brown-Enquist theory of biological scaling laws

The biological scaling laws are known since a long time, and they were since their origin, of axiomatic nature. West, Brown and Enquist (1997) proposed a quantitative model that explain the origin of the ¼ power laws, and for the first time these scaling laws were demonstrated, based both on hydrodynamic (West et al., 1997) and geometrical considerations (West et al., 1999).

For the description of geometric forms, the authors are based on two conceptually different frameworks: the Euclidean and the fractal. In the framework of Euclidean geometry, the objects are described through an external surface S that keep inside a volume V, while in the fractal framework the objects have an exchange surface s and a total volume v, that correspond to biological active matter (Table 1). In this regard, the objects that belong to the last framework are called *biological objects*.

Variable	Euclidean framework	Fractal framework
Length	$L \propto S^{1/2} \propto V^{1/3} \propto M^{1/3}$	$l \propto s^{1/3} \propto v^{1/4} \propto M^{1/4}$
Surface	$S \propto L^2 \propto V^{2/3} \propto M^{2/3}$	$s \propto l^3 \propto v^{3/4}\, M^{3/4}$
Volume	$V \propto L^3 \propto M$	$v \propto l^4 \propto M$

Table 1. Scaling laws for length, surface and volume scaling laws for objects in the framework of Euclidean and fractal geometries. Adapted from (West et al., 1999)

For all cases, the proportional equations are resumed as $Y = Y_o\, M^b$, where Y is a scaling property, Y_o is a normalization constant, and b is the scaling exponent. The scaling exponents for biological objects are defined as:

distance: $b = 1 + \varepsilon_l$ $0 \leq \varepsilon_l \leq 1$

surface: $b = 2 + \varepsilon_s$ $0 \leq \varepsilon_s \leq 1$

volume: $b = 1 + \varepsilon_v$ $0 \leq \varepsilon_v \leq 1$ $(\varepsilon_v = \varepsilon_l + \varepsilon_s)$

where ε_l, ε_s and ε_v are the fractional scaling exponents for distance, surface and volume, respectively. These exponents correspond to the fraction above the Euclidean dimension giving the fractional exponent. According to these definitions, the following proportionalities hold between the geometrical variables:

$$l \propto s^{1/(2+\varepsilon s)} \qquad\qquad s \propto v^{(2+\varepsilon s)/(3+\varepsilon s+\varepsilon l)} \qquad\qquad l \propto v^{1/(3+\varepsilon s+\varepsilon l)}$$

The exchange surface of the biological objects is maximized in such a way that occupy as much as possible of the volume. This means that the exponent $b = (2+\varepsilon_s)/(3+\varepsilon_s+\varepsilon_l)$ must be maximum, condition achieved when $\varepsilon_s = 1$ and $\varepsilon_l = 0$. That is, the surface of a biological object may achieve a surface dimension of 3, and a volume dimension of 4. The theory explains that biological objects are shaped to optimize distribution of materials in a hierarchical structure.

One of the most interesting aspects of the West-Brown-Enquist (WBE) theory, more than 10 years after its proposal, is that is accomplished by an impressive 27 orders of magnitude for the mass of biological objects spanning from respiratory complexes to large mammals (West et al., 2002).

4.2 Proteins as biological objects

According to the studies of West, Brown and Enquist, the enzyme citochrome *c* oxidase is the smallest biological object proved to accomplish with the theory, and in this regard, it would be the smallest unity in the hierarchical network that share all biological objects. This network is of fractal nature, and this point is important in relation to proteins: *proteins are not fractal objects but biological objects, in the sense of the WBE theory, as part of a distribution network of fractal nature*.

Which are the results already obtained that support this proposal? Most of the research agrees that the structure of proteins seen from the mass fractal dimension coincides with a percolation system of dimension 2.5, and a surface dimension near 2.2. Other macromolecules, like dendrimers, show a mass dimension of 2.5 and a surface dimension of 2 (Pricl et al., 2003). It seems that large molecules show two different dimensions, depending on weather the surface of the object is considered or not. Hence, one could hypothesize that one of the internal dimension would be related to the structure of the protein, while the external dimension would relate to the protein surface, in the thermodynamic sense.

Some isolated results are in partial agreement with this view, when considering only the surface. For example, (Lewis & Rees, 1985) and (Fernández Pacios, 1995) showed that the surface of a protein displayed a high dimensional value (around 3) for a limited range of probe sphere radii (between 1.5 – 2.5 Å), and for larger radii, the show the usual value around 2.2. This high dimensional value could be related with the high dimension proposed by the WBE theory.

4.3 Test hypothesis: cytochrome *c* as a biological object

Allthough there is evidence that the surface of a protein is not fractal in the strict mathematical sense, proteins may display a fractal behaviour according to the WBE theory. This hypothesis is based on the results from (Lewis & Rees, 1985) and (Fernández Pacios, 1995), who showed that the protein surface evaluated by probe spheres of radii of ca. 2 Å display a dimensionality close to 3, the maximum value for a surface in a non-Euclidean framework.

Although these authors did not conclude about this specific value, it should be noted that the diameter of such probe sphere (4 Å) is of the order of the optimal values for interatomic distance inside the proteins. Hence, one could hypothesize that a probe sphere of such diameter would fill all the protein inside, thus "reading" a value corresponding to the maximum achievable occupancy.

The WBE theory postulates that the biological scaling laws base on the optimization of the materials distribution within the organism. This optimization is achieved when the exchange surfaces of the biological structure are maximum, and at the same time, the internal distances of the structure are minimum. The WBE theory predicts that biological objects, independently of their size, form a real distribution network of fractal nature. Is the independence from the object size that gives the fractal basis of the theory.

The accomplishment of the WBE theory by the different biological objects requires the measure of the relationship between the exchange surface and the real volume of the object. Usually, all the published works dealing with this problem employed the measure of the metabolic activity (proportional to the surface) as a function of the mass of the object, assuming a constant density (homogeneity) of the object. The smallest biological object accomplishing the WBE theory is the cytochrome *c* oxidase, being the enzymatic activity in this case the property evaluated. The fact that this enzyme is the smallest biological object measured, it was proposed as the "vital unit" of Nature.

Cytochrome *c* is the protein in charge of electron transport between the cytochrome *c* reductase and oxidase. According to the WBE theory, if what it is optimized is the materials transport in a hierarchical network, one should remember that are the electrons the ultimate material transported. Based on these considerations, cytochrome *c* would play the role of vital unit held by cytochrome *c* oxidase.

In order to probe that proteins accomplish the WBE theory, it is necessary to calculate both the surface and the volume of the protein, in order to obtain the values for ε_s and ε_l. It should be mentioned that this is the first time an object is analyzed for the accomplishment of the WBE theory by direct measure of the surface and the volume, and not employing other measures proportional to these properties.

5. Results and discussion

In order to apply the proposed methodology, it is necessary that the surface calculation programs allow for the evaluation of the surface and volumes in the widest possible sphere radii range. SurfRace and GEPOL were employed for the calculation of the SES of cytochrome *c*, and the results were compared to those of MS-DOT. The ratio of the surface obtained between GEPOL and MS-DOT was 1.01 ($r^2 = 0.98$), between SurfRace and MS-DOT was 1.09 ($r^2 = 0.97$), and between GEPOL and SurfRace was 1.06 ($r^2 = 0.99$), indicating that all three programs could be equally used.
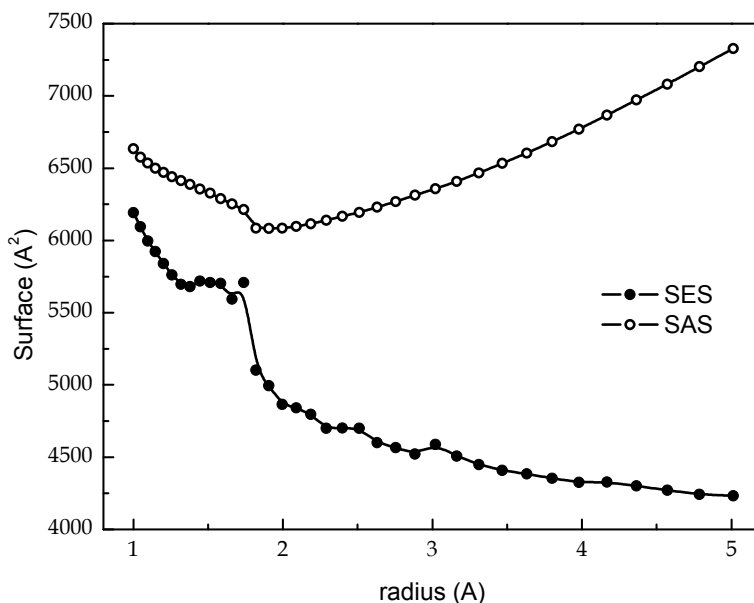
Fig. 2. Variation of the SAS and SES surfaces with the radius of the rolling sphere probe

The comparison of SAS and SES values (Fig. 2) show that for $r < 1.8$ Å, there is a monotonous decrease in the surfaces. However, at this sphere radius value, both surface show different behaviours: while SAS increases with r, SES decreases. This is because SES has less contact points with the protein as r increases, giving rise to a decrease in the surface, while SAS, due to its own definition, increases with the value of r. Below a radii of 1.8 Å, the decrease observed in SAS is due to the balance between the lesser number of contact points and the increase due to its own definition.

The divergence observed between SAS and SES for $r > 1.8$ Å suggest that below this value, the sphere probe is able to enter the protein and also evaluates the internal cavities of the protein. Let us consider a sphere of radius r which is able to enter the protein interior. If the internal distances (d) of the free spaces inside the protein are higher that the diameter ($2r$) of the sphere, it could be able to freely move within these spaces. As the sphere radius is increased, if the condition $2r < d$ is met, the contact points with the internal surface are lesser, and SES will progressively decrease. If the internal distances are homogeneous, a transition point at $2r = d$ will be verified, above which the sphere probe will not be able to enter the protein. Hence, those spheres that accomplish the condition $2r > d$ will be able to evaluate only the external surface of the protein, giving rise to a decrease in SES. In this way, the surface that can be considered as the thermodynamic surface is quantitatively defined.

The surface dimension calculated for cytochrome c show two clear values: one of ca. 3.0 for sphere probe radii of ca. 1.8 Å, and another in the range 2.05 – 2.20 for sphere probe radii above 2.5 Å (Fig. 3).
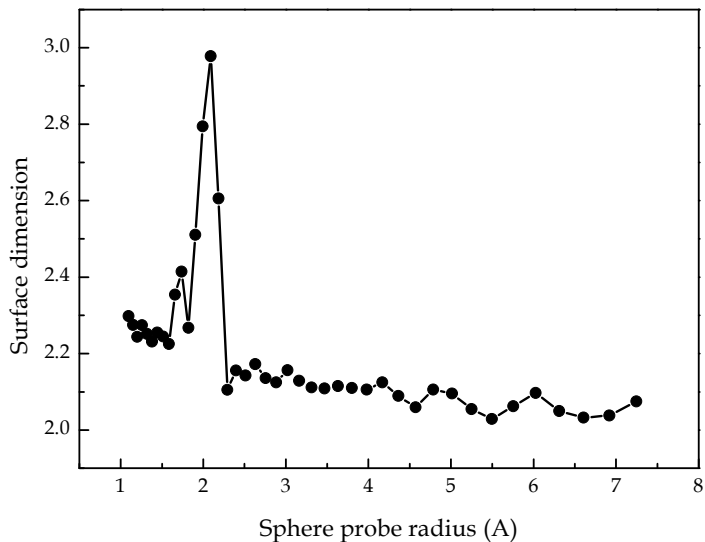
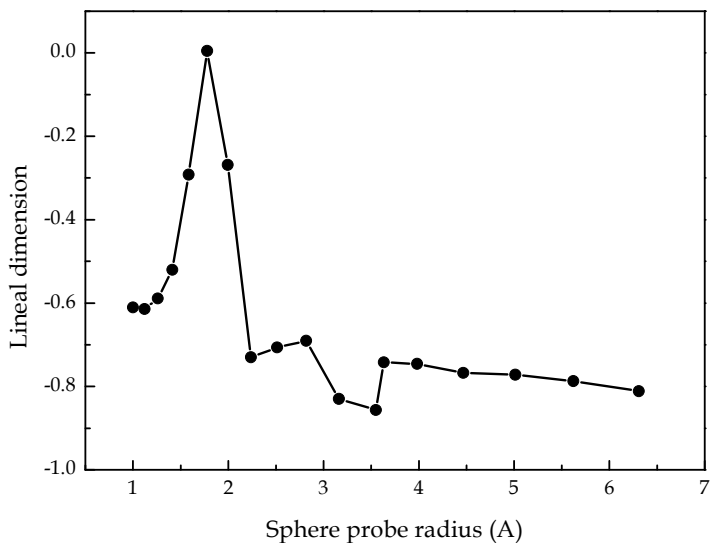Fig. 3. Surface dimension of cytochrome *c* as a function of the sphere probe radius

Fig. 4. Lineal dimension of cytochrome *c* as a function of the sphere probe radius

For the calculation of ε_l, the graphs of ε_l vs. r show the same behaviour to that of the surface dimension (Fig. 4). It is worth to note that the calculated values for ε_l are negative, that is, have no physical meaning. However, only for $r = 1.8$ Å, it is obtained the only value with physical meaning: $\varepsilon_l = 0$.

Hence, for cytochrome c it is demonstrated that $\varepsilon_s = 1$ and $\varepsilon_l = 0$. Notice that for establishing the accomplishment of the WBE theory for an object, it is necessary to measure the relationship between geometrical parameters, particularly surface and volume. In general, for biological objects (cells, animals), some proportional properties to these geometrical parameters are used, as the metabolic rate or enzyme activity (proportional to the surface) and the mass (proportional to the volume) (West et al., 1997). In our case, for the first time, it was possible to directly measure the surface and the volume of the object.

The results of the calculations indicate that for cytochrome c, a bimodal dimension was obtained. The existence of these two values is in agreement with the fact that proteins are not fractal objects in the strict sense of the definition, and accordingly, the dimension calculated is not a fractal dimension. Accordingly, it is more correct to name this dimension as *effective dimension* (Fernández Pacios, 1995). Besides, the value of the effective dimension of ca. 2.2 obtained for $r > 1.8$ Å has been extensively reported in the literature for proteins. Hence, it can be concluded that the thermodynamic surface of the proteins have an effective dimension of ca. 2.2.

The particular condition in which the sphere probe diameter equals the internal distances of the protein ($2r = d$), the sphere probe will completely fill all the free spaces inside the protein, it the internal distances are homogeneous. Under this condition, the maximum dimensionality is obtained, as correspond to a surface that fill all the volume of the object. The value of $2r = 3.6$ Å is a typical value of the internal distances between the aminoacidic residues in the protein, and at these separation the interacting energies (van der Waals, Hydrogen bonds) are optimum. These optimal distances have been related to the structure of a crumpled surface, which may be a good model for protein structure (Cassia-Moura & Gomes, 2006).

6. Conclusions and future research

We have explored another view of the fractal nature of proteins, particularly focusing on the possible role of cytochrome c as a step in the materials distribution network that scales along 27 orders of magnitude, according to the theory of West, Brown and Enquist. For the first time, the prove of the accomplishment of this theory is based on the direct measurement of the surface and the volume of the object. Our conclusions cannot be extended to all proteins, as it was only limited to cytochrome c. However, our initial insights into the behaviour of other proteins showed that many proteins have a higher dimensionality at a radius value of 1.8 Å, confirming our initial suggestion that this value represent common internal distances related to long-range interactions within aminoacid residues.

Another point of interest relies on the fact that the protein surface can be considered as a thermodynamic frontier. Our approach was directed to the use of proteins in the design of biosensors. In this regard, we assumed that the maximum interaction between two surfaces is achieved upon equality of their surface dimensions. For such purpose, we have designed a modified surface based on the adsorption of 2-thiobarbituric acid onto a gold surface, for which we obtained a surface dimension of 2.17, that is, of the same order to the protein surface (Méndez et al., 2008). Upon contacting both surfaces, we have obtained a high

protein coverage and a high electron transfer, indicating that indeed the surface of the protein behaves as a true thermodynamic frontier. Hence, we expect that these findings can bring new insights into biosensor design (Charcosset, 1998, Collier et al., 2001, Butala et al., 2003, Butala et al., 2004, Costanzo et al., 2004, Doke & Sadana, 2005, Doke et al., 2006, Doke & Sadana, 2006, Chodankar et al., 2007).

Finally, it is interesting to consider the reliability of the default value of 1.4 Å for the calculation of the protein surfaces. We have shown that this value may not be the best for this purpose, as the protein surface display large variations around this value. We think that, if the value of 1.8 Å is universal, this may be the best value to obtain information on the protein structure, and a value above 2.5 Å would be adequate to obtain information on the surface of the protein.

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8. References

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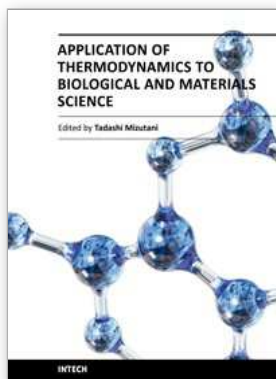
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