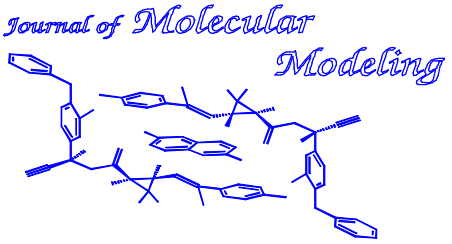
J. Mol. Model. **1995**, *1*, 46 – 53



Variation of Surface Areas and Volumes in Distinct Molecular Surfaces of Biomolecules

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Received: 14 December 1994 / Accepted: 14 March 1995

Abstract

The different surfaces usually defined in molecular modeling are explored focusing the attention on comparing the two common definitions which incorporate the presence of the solvent: the Accessibility surface and the Richards surface. The type of information contained in these surfaces is analyzed by studying the changes associated to the systematic variation of the radius of the solvent, represented by a probe sphere. An approximation to the effective fractal dimension associated with the Richards molecular surface is also obtained. Four biomolecules, hyaluronic acid and three proteins with sizes ranging from around 300 to 1500 atoms, have been chosen for this study. Graphical ray-traced renderings of these biomolecules, obtained by adapting public domain ray-tracing software for PC's are presented for illustration.

Keywords: Molecular Surfaces, Molecular Volumes, Fractality of Proteins

Introduction

The great progress in molecular modeling in the recent years has been partially motivated by the enormous development of computer capabilities and programming techniques, as well as the parallel reduction of the economical costs associated. Most laboratories involved in molecular research have currently some type of computer equipment to perform molecular modeling works insofar as many modeling features are implemented in commercially available software packages. It is also worth mentioning that many computer tasks regarding molecular modeling which demanded sophisticated machines a few years ago, can nowadays be accomplished almost routinely on a simple PC.

However, the kind of information provided by particular models is often not sufficiently explicit to general users in many applications. As a particular example, the attention is here focused on molecular surfaces because of their central importance in molecular modeling. When one tries to work out any spatial molecular representation, some type of limiting surface (envelope) has to be firstly settled. From a physical point of view, the surface is an important aspect of the structure of any molecule as it is through the surface that molecules interact with their surroundings and other molecules. Most applications regarding molecular surfaces are intended to study the aqueous environment surrounding the molecule, and thus the surface is normally traced using a probe sphere which represents a molecule of water. However, the variation of the surface area with the probe sphere viewed like a measurement yardstick provides more general information about the surface complexity of the molecule, especially in large biomolecules like proteins, and thus the concept of *fractality* has recently arisen in this field [1-5]. Several arguments relating the building principles of protein structures with this surface complexity have been presented in the past [4,5].

The main purpose of this work is to analyze comparatively the information provided by the most representative molecular surfaces when the probe size is systematically changed. The molecular surfaces are determined by means of a new

algorithm (GEPOL) which improves some slight anomalies found in the only method (MSDOT) available until very recently for computing the more complex surface. The comparative study presented in this work may help to gain more insight into the complexity of surfaces of biomolecules and to realize the type of information contained in every surface. The last point can be relevant to discuss the *fractal dimension* of proteins introduced in structural studies from different points of view in the past. The paper is organized as follows. The next section introduces the usual definitions of molecular surfaces and the computer methodology utilized. The biomolecules selected for the study are also presented in this section and some graphics issues are briefly discussed. The surfaces obtained with different probe sizes are analyzed in the third section where the main conclusions are also presented.

Methodology

Figure 1 illustrates the common definitions of molecular surfaces. To define any surface, the molecule is usually represented as a set of overlapping spheres centered on the atomic coordinates, each with its van der Waals radius. The external surface for this set of spheres defines the well-known van der Waals molecular surface, WMS, sketched as the dashed internal contour in Figure 1a. It is over this surface that usual molecular properties (equidensity contours, electrostatic isopotential regions, atomic charges, etc.) are represented, normally in the form of dot surfaces with some color coding for the property studied. Insofar as this is the obvious surface of the set of interlocking spheres, no ambiguities arise from its definition except for the atomic radii assigned. It must be remembered that various atomic scales have been proposed over the years and that some noticeable discrepancies still remain in some elements (see for example Ref.6a). The van der Waals scale proposed by Gavezzotti [6b] has been used in this work.

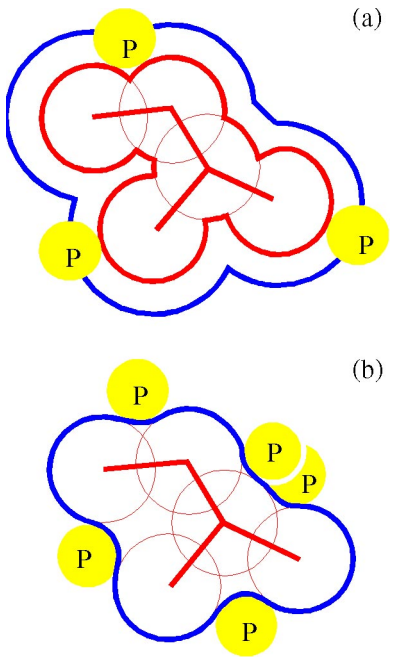

Fig. 1 *Definitions of molecular surfaces for a set of interlocking atomic spheres. a) Accessibility Molecular Surface AMS, solid line traced by a probe sphere P (the internal dashed contour is the van der Waals Molecular Surface WMS). b) Richards Molecular Surface RMS for the same probe sphere.*

The easiest way to incorporate the solvent into the molecular surface is through the accessible molecular surface, AMS, drawn as the outer solid line in Figure 1a and defined as the surface generated by the centre of the solvent represented like a rigid sphere ('P' in Fig.1) when it rolls over the WMS. It is easily obtained by just adding to every atomic sphere the radius of the probe sphere P. The third surface accounting for the solvent is sketched in Figure 1b and was initially proposed by Richards [7], therefore it is often called Richards Molecular Surface, RMS. This surface has two parts: the contact surface and the reentrant surface: the contact surface is the atomic part of the WMS which is accessible to the probe sphere. The reentrant surface is defined as the inward-facing part of the probe sphere when it is in contact with more than one atomic sphere.

Some confusion still remains when speaking about solvent surfaces; for instance, it is frequent to use 'accessible surface' for the RMS, or the name 'Molecular Surface' for denoting one particular of the above definitions. Very recently, Silla et al. [8] have proposed the more specific and descriptive name Solvent-Excluding Molecular Surface for the Richards surface.

Although both solvent surfaces have their uses, the kind of information about the own molecular geometrical complexity conveyed by AMS and RMS is rather different. It is the purpose of this work to study the variation of surface areas and volumes when the probe sphere viewed as a yardstick to measure surfaces changes its size. All the calculations have been performed with the program ARVOMOL [9,10] developed by implementing into only one executable module five of the most widespread methods to obtain molecular surfaces and volumes, ranging from numerical Monte-Carlo estimates to analytical algorithms [9]. This program permits the control of many useful options as for example the choice of different atomic radii scales, generation of auxiliary files for further analyses or displaying, graphical plots of contours of the molecule, transformation of coordinates to the molecule-fixed axes system computing the moments of inertia and rotational constants, etc. However, the most appealing feature of ARVOMOL is that it allows obtaining surface areas and volumes by distinct methods in one single run, permitting thus more precise determination of these properties. While virtually every existing approach to determine

molecular surfaces is able to obtain WMS and AMS, the Richards surface can currently be computed by only two methods: GEPOL by Silla et al. [8,11] and the well known MSDOT algorithm by Connolly [12], both implemented in ARVOMOL. The obtention of the RMS becomes computationally very demanding if the parameters controlling the precision are set to higher levels.

In order to explore the two surfaces, AMS and RMS, four biomolecules with sizes ranging from around 100 to 1500 atoms have been selected. The smaller non-protein biomolecule is the hyaluronic acid, hereafter named HYA, with 129 atoms including hydrogens. This compound is present in the extracellular matrix of animal connective tissues and contains alternating units of D-glucuronic acid and N-acetylglucosamine. The second molecule is the small plant seed protein crambin, CRN, with 46 residues and 327 non-hydrogen atoms. The third one is the hen egg white protein lysozyme, LYZ, with 129 amino acids and 1000 non-hydrogen atoms. The last molecule chosen is the sweet tasting protein thaumatin-I, THI, with 207 residues and 1551 non-hydrogen atoms. The atomic coordinates for these molecules have been taken from the Brookhaven Protein Data Bank [13], entries 1HYA, 1CRN, 7LYZ and 1THI. This last entry gives just the 207 α-carbon coordinates of a preliminary unrefined set obtained from a 3.2 Å resolution map. The whole set of coordinates for the complete protein structure of THI has been generated by means of PROGEN [14], a modeling algorithm recently proposed for constructing the complete structure from only the α-carbon coordinates and which produces for thaumatin-I a total of 1551 atomic positions [15]. This choice is intended to include in the test a molecule 'artificially modelled' from partial information.

Color Figures 2-5 present ray-traced renderings of different stages of visualization of a protein structure (in this case, thaumatin, the larger molecule studied in this work) starting at its bare backbone up to the successively more complex molecular surfaces. Although ray-tracing techniques are nowadays standard in many commercially available packages, these pictures have been obtained with the freeware program *Persistence of Vision Raytracer*, POVRAY Version 2.2 [16]. The syntax file needed to adapt POVRAY for rendering molecular graphics has been automatically generated with an auxiliary program developed at this laboratory which generates a machine-independent ASCII file used as input by the ray-tracer program. Figure 2 displays the backbone α-carbon chain of THI from the coordinates given in the original PDB file; the first carbon is colored in cyan and the last one (207th) in red. The WMS of this protein is given in Figure 3 for the structure generated by PROGEN with the following color coding: blue=C, red=O, green=N, yellow=S. The AMS is depicted in Figure 4 whereas an approximation to the Richards molecular surface of this protein is displayed in Figure 5. This last representation has been obtained by adapting some special features of POVRAY for rendering connected curve surfaces so it must be regarded as an approximation to the RMS.

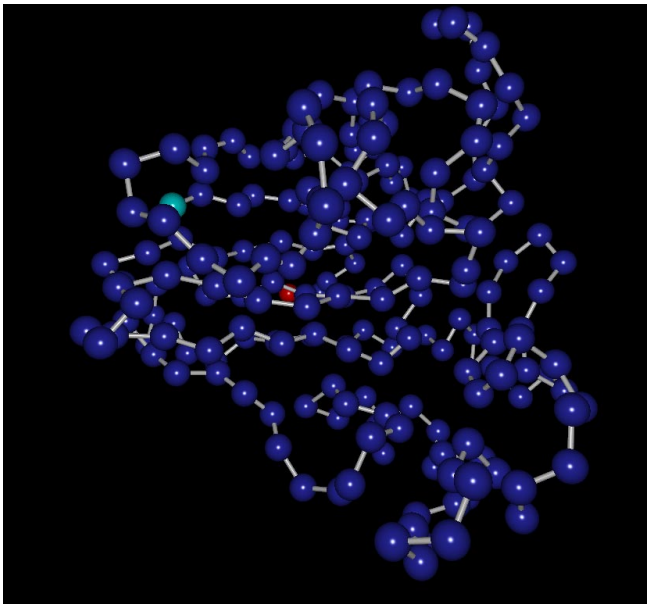

Fig. 2 *Ray-traced rendering of the α-carbon chain of the thaumatin-I protein with coordinates taken from the Brookhaven Protein Data Bank (entry PDB1THI). The α-carbon in the first residue is colored in cyan and the last one (207) in red.*

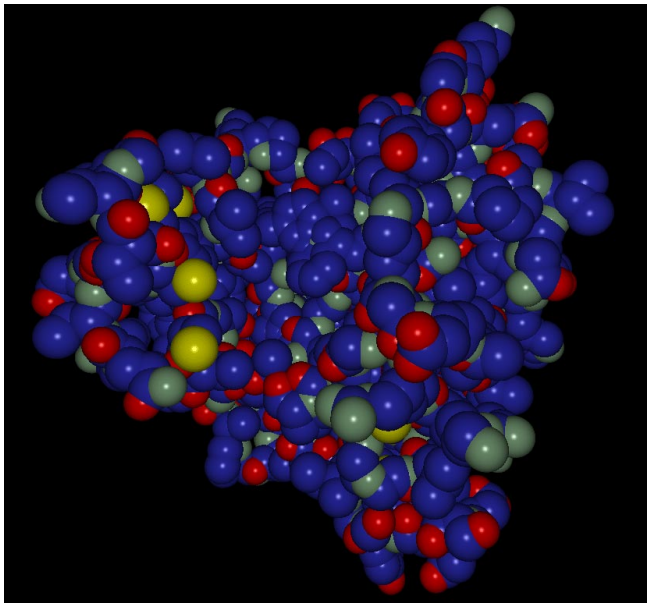

Fig. 3 *Ray-traced rendering of the van der Waals surface of thaumatin-I for the atomic coordinates generated with the program PROGEN from the α-carbon positions shown in Figure 2 (see text). Color coding: C = blue, N = green, O = red, and S = yellow.*

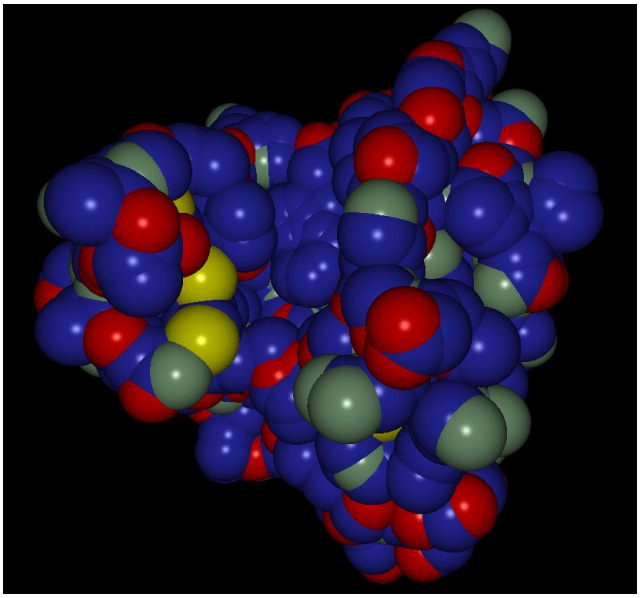

Fig. 4 *Ray-traced rendering of the accessibility molecular surface of thaumatin-I for the structure shown in Figure 3 with a probe sphere of radius 1.4 Å.*

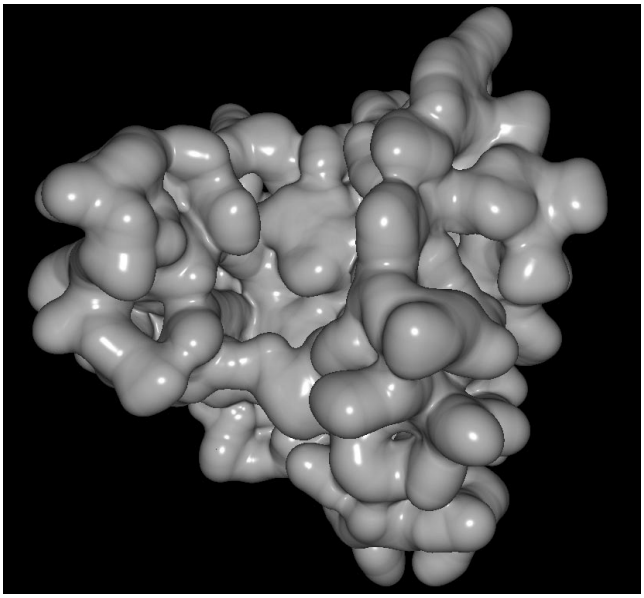

Fig. 5 *Ray-traced rendering of an approximation to the Richards molecular surface of thaumatin-I for the structure shown in Figure 3 with a probe sphere of radius 1.4 Å.*

Discussion

The great majority of studies concerning molecular surfaces until GEPOL was available in 1990 were obtained with the successful program by Connolly [12,17] (formerly named MSDOT) and his procedure is deservedly recognized. However, MSDOT results exhibit a somewhat oscillatory behaviour under variation of its internal parameters, as it was initially noted by Meyer [18] and checked afterwards [11c] . Figure 6 shows the logarithm of RMS surface areas plotted against the probe radius (with constant logarithmic step) computed with GEPOL and MSDOT for HYA and CRN molecules in the 1.2 - 3.0 Å range. Although the internal parameters of both methods are left unchanged in this calculation, MSDOT surface areas oscillate with the probe radius while GEPOL results show remarkable smooth variations. If one tries to analyze the complexity of large biomolecules on the basis of computed surface areas, it is mandatory to guarantee the numerical stability of results when one variable, i.e. the probe size, is systematically changed. Moreover, GEPOL93 (the version included in ARVOMOL) allows for systematic improvement of surface areas and volumes tending to limit values by changing two internal parameters in a consistent manner [8].

After selecting GEPOL results for the study, let us analyze the information provided by the two surfaces accounting for the solvent. Figure 7 shows a double logarithmic plot of RMS and AMS areas against probe radii for a range covering two orders of magnitude for the four biomolecules chosen. This analysis is intended to explore the geometrical irregularity features of the surface, therefore the probe must be viewed as a yardstick for this surface rather than as a representation of a particular solvent. Various interesting features are revealed by this comparison.

(i) While the smaller non-protein HYA molecule displays curves diverging from the beginning, the three proteins roughly share common Richards and accessibility sur-

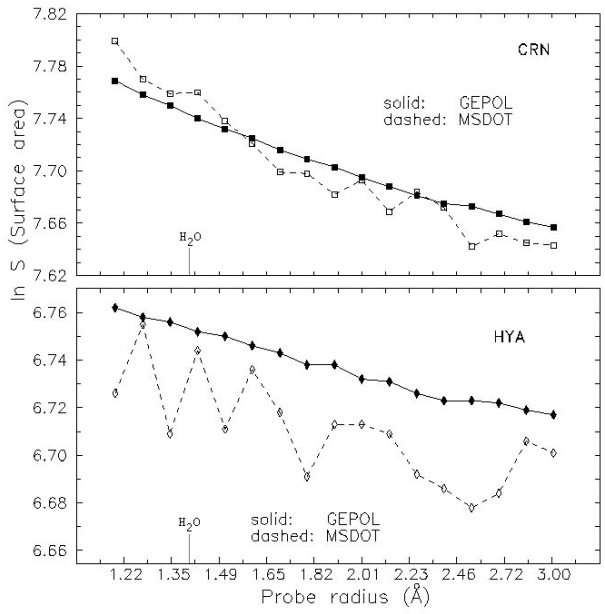

Fig. 6 *Logarithm of the RMS area against the logarithm of the probe radius in the interval 1.2 - 3.0 Å for crambin (CRN) and hyaluronic acid (HYA) computed with GEPOL and MSDOT algorithms (see text).*

face areas until the probe radius reaches a value which seems to depend on the size of the protein, 0.7 Å for CRN, 1.1 Å for LYZ and 1.4 Å for THI.

(ii) AMS areas continuously increase with large probes after passing through a minimum probe radius which again happens to depend on the protein, around 1.2 Å for CRN, 1.7 Å for LYZ and 2.4 Å for THI.

(iii) RMS areas, on the contrary, tend to stabilize around a constant value as soon as the probe radius reaches a sufficiently large value between 3 Å in CRN and 5 Å in THI.

(iv) The slopes of RMS curves approach zero as the probe size becomes small and large so that it is in the intermediate region where RMS rapidly changes revealing thus a surface sensitivity to the probe radius.

(v) The RMS area for the HYA molecule changes very smoothly albeit with a shape slightly reminiscent of protein curves.

(vi) As expected, AMS and RMS curves converge at probe radius zero to the van der Waals surface area which is 1005 $Å^2$ for HYA, 4204 $Å^2$ for CRN, 12558 $Å^2$ for LYZ, and 16476 $Å^2$ for THI. These values are the WMS results obtained with the computational method being employed (GEPOL) within the set of internal parameters chosen along all the study as optimized in a previous exploratory work.

Molecular volumes are also a fundamental physical property useful for understanding not only the structure of the molecule but also its interactions [19]. Density variations in regions of the protein interior for example, have been related to conformational fluctuations and protein folding [20]. Figure 8 depicts the variation of molecular volumes computed for RMS and AMS in a representation analogous to Figure 7.

The following remarks can be made.

(i) Proteins exhibit again a qualitative common behavior distinct from HYA molecule: while the slope of the protein volumes curves changes noticeably around probe radii between 0.7 and 2 Å, the volume for hyaluronic acid increases steadily.

(ii) Unlike surface area curves, the probe size region where slope changes come out seems to be essentially the same for the three proteins.

(iii) Even at very small probes, AMS volumes are clearly larger than RMS volumes, so that there is no common region of both quantities at any probe size interval.

(iv) AMS volumes rapidly increase from the beginning avoiding thus any sensitivity to the molecular structure.

Table 1 gives data directly related with the molecular volume for both types of solvent surfaces. The first row is the molecular weight and the second the density computed from van der Waals volumes. These WMS densities, slightly larger than 2 g/cm^3 in all cases, may then be regarded as the

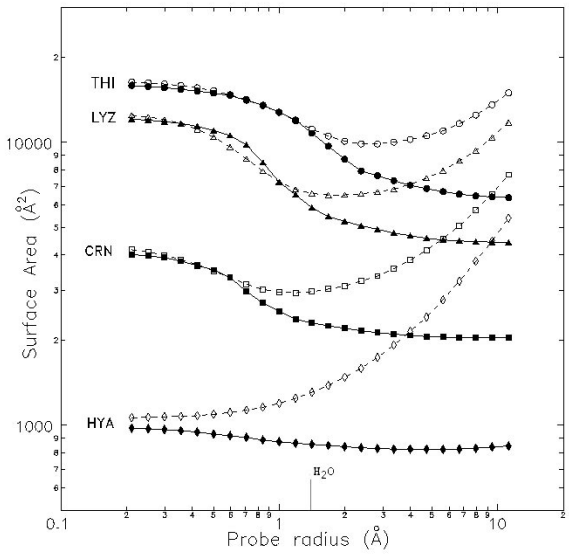

Fig. 7 *Surface area against probe radius in the interval 0.2 - 11.0 Å for thaumatin-I (THI), hen egg lysozyme (LYZ), crambin (CRN) and hyaluronic acid (HYA). Filled points, solid lines: Richards Molecular Surface RMS; unfilled points, dashed lines: Accessibility Molecular Surface AMS*

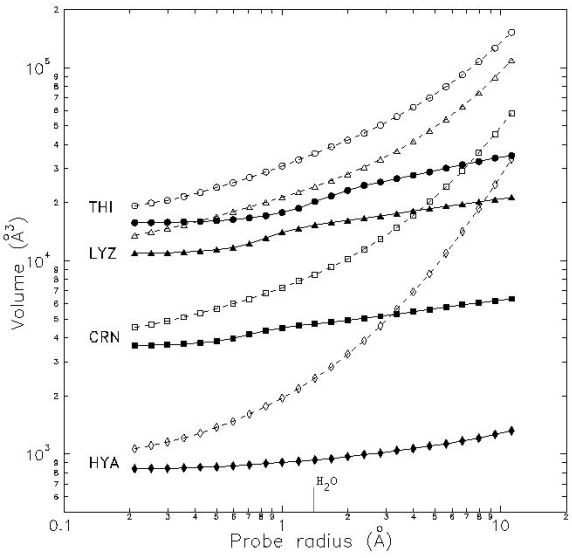

Fig. 8 *Volume for RMS (filled points, solid lines) and AMS (unfilled points, dashed lines) against probe radius in the interval 0.2 - 11.0 Å for THI, LYZ, CRN and HYA molecules.*

densities for the isolated molecules. When the solvent is introduced by means of a probe sphere simulating water (1.4 Å), proteins show a common behavior qualitatively different from the HYA molecule: the RMS density represents around 75% of the isolated density in the three proteins while it is 90% for the non-protein molecule. The van der Waals volume is then

Table 1 *Molecular masses M (g/mol), densities ρ (g/cm^3) for van der Waals volumes, ρ_{WMS}, accessibility volumes, ρ_{AMS}, and Richards volumes ρ_{RMS}, and percentage of van der Waals volumes respect to accessibility volumes, $(\%)_A$, and respect to Richards volumes, $(\%)_R$ for probe spheres of radius r_p 1.4 Å and 3.0 Å*

	HYA	CRN	LYZ	THI
M	1122	4410	13315	20693
ρ_{WMS}	2.227	2.028	2.033	2.192
r_p = 1.4 Å				
ρ_{AMS}	0.755	0.862	0.912	0.957
ρ_{RMS}	2.000	1.553	1.441	1.696
$(\%)_A$	34	43	45	44
$(\%)_R$	90	77	71	77
r_p = 3.0 Å				
ρ_{AMS}	0.375	0.537	0.636	0.657
ρ_{RMS}	1.819	1.402	1.284	1.327
$(\%)_A$	17	26	31	30
$(\%)_R$	82	69	63	61

HYA: hyaluronic acid (nonprotein molecule); CRN: crambin; LYZ: hen egg lysozyme; THI: thaumatin-I (proteins)

obviously around 75 % of the RMS volume for proteins while it represents the 90 % for HYA. When the probe sphere is incremented up to 3.0 Å, these percentages fall to 65 % for proteins and 82 % for HYA. If accessibility molecular surfaces are now considered, the influence of the much larger volumes associated is apparent in the larger decreases in density or, equivalently, in the lower percentages of van der Waals volumes: around 44 % for proteins and 34 % for HYA when the probe radius is 1.4 Å and 28 % for proteins and 17 % for HYA with probes of 3.0 Å.

Table 2 *Effective fractal dimensions D in equation (1) for the Richards Molecular Surface computed (a) from linear fits to the points in Figure 9 (D_fit), and as averages of the numerical derivatives for probe sizes (b) in the interval 1.2 - 3.0 Å (D_num1) and (c) in the interval 0.2 - 11 Å (D_num2).*

	HYA	CRN	LYZ	THI
D_fit	2.05	2.12	2.28	2.54
D_num1	2.05	2.12	2.32	2.53
D_num2	2.04	2.18	2.26	2.24

HYA: hyaluronic acid (nonprotein molecule); CRN: crambin; LYZ: hen egg lysozyme; THI: thaumatin-I (proteins)

Summarizing, it seems evident that increasing the probe sphere used as yardstick to trace the molecular surface conveys information on the own geometrical irregularity only in the RMS definition. On the contrary, the accessibility molecular surface provides information dealing with bulk features of the molecule as seen by the solvent. Only the Richards's definition gives a procedure sensitive enough to analyze the geometrical complexity of molecular surfaces. It is therefore interesting to test RMS results for the small/medium biomolecular systems treated in this work when applied to determine the *fractality* of the molecular surface.

As pointed out by Pfeifer et al. [2], the geometrical complexity can be quantified only for surfaces showing *self-similarity* properties, customarily in the form of the *fractal dimension*, D. One practical procedure to estimate D for an object of surface area S measured with a probe size r_p is to make use of the relation [5,21]

$$\frac{d(\ln S)}{d(\ln r_p)} = 2 - D \qquad (1)$$

As the surface becomes more irregular, D increases from the value D = 2 in a smooth surface and thus, the logarithmic plot of log S against log r_p must present a negative slope. The variations of RMS areas displayed in Figure 7 for proteins are globally indicative of self-similarity features. In fact, the slopes of these plots approach zero in the limit of both small and large probe radii. However, no straight lines are found between these limits, as expected from equation (1): only in narrow intervals of probe sizes it is possible to find linear correlations for determining D. Figure 9 displays plots of log S versus log r_p in a probe radii range (1.2

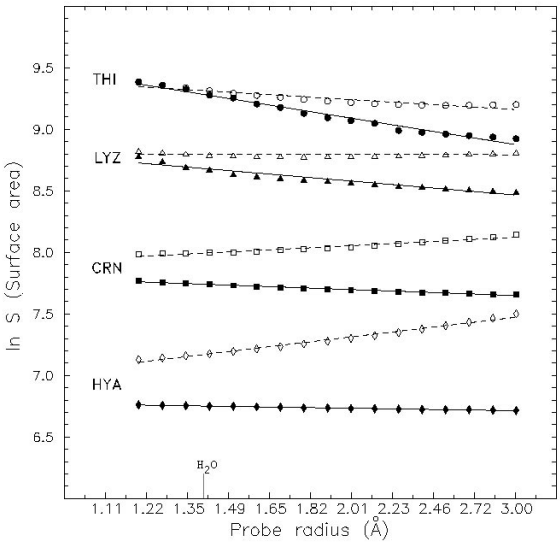

Fig. 9 *Logarithm of the surface area against the logarithm of the probe radius in the interval 1.2 - 3.0 Å for THI, LYZ, CRN and HYA molecules. Filled points, solid lines: RMS; unfilled points, dashed lines: AMS.*

- 3.0 Å) of the order of magnitude of water molecule and interatomic close distances: in this interval roughly straight lines may be found. Note that only RMS curves decrease with larger probes while AMS curves increase in all the systems except the larger molecule.

In their pioneering work on fractal surfaces of proteins, Lewis and Rees [5] followed this approach to calculate D although they showed curves similar to those plotted in Figure 7. They computed the fractal dimension by averaging the numerical approximations to the derivative in eq.(1) in the interval 1.0 - 3.5 Å and proposed values around 2.4 for three proteins studied (lysozyme, ribonuclease A and superoxide dismutase). This result is in apparent contradiction with more recent studies lending support to fractal dimensions in proteins around 2.2 from very distinct approaches to estimate D [2b,3,4]. If the well studied lysozyme protein is taken for example, Lewis and Rees [5] found D = 2.44, Pfeifer et al.[2b] 2.17, Åqvist and Tapia [4] 2.19 and very recently, Zachmann and Brickmann [3] 2.20. Moreover, against earlier suggestions on a unique degree of geometrical complexity characterized by a fractal dimension around 2.2 in proteins, recent work on self-similarity in biological and synthetical macromolecules seems to support the existence of variable degrees of such a complexity [3].

Some light may be shed on this controversy by analyzing the information on the effective fractal dimension extractable from the surfaces being investigated here. Table II gives estimates of D computed by following two alternative approaches: (1) from the slope of linear fits to the RMS points in Figure 9, and (2) from averages of the numerical derivatives in eq.(1). Linear fits to RMS log/log plots in Figure 9 show relatively poor correlation. For HYA, CRN, LYZ, and THI molecules the correlation coefficients are, respectively, 0.993, 0.994, 0.965, and 0.989. The set D_num1 has been calculated averaging the numerical derivatives in eq.(1) for the same interval selected in Figure 9 (1.2-3.0 Å). Three different well known approximations have been employed to obtain this derivative, namely, the forward difference formula, the central difference formula, and the Newton advance formula. These three estimates , which agree reasonably well, are then averaged to determine the final D value given in Table II. The set D_num2 is obtained with the same procedure but on the complete interval plotted in Figure 7, i.e. 0.2-11 Å.

Several interesting conclusions may be extracted from these results. For the reduced interval where the probe has a size of the same order of magnitude than water (1.4 Å), the molecular surface investigated seems to present an effective D dimension which depends on the protein size (result which agrees with the same conclusion reached from a different investigation on Hausdorff dimensions published on 1992 [22]). However, when analyzed over the whole interval spanning two orders of magnitude in probe radii, the three proteins present an effective dimension D around 2.2, in good agreement with the suggestions made from rather different point of views [2b,3,4]. Although not shown in Table II since the results are almost identical, the whole numerical work in this Table has been also carried out with the MSDOT method. However, it is worth mentioning that two out of the three numerical estimates of the derivative in the interval 1.2-3.0 Å for lysozyme happen to be equal to 2.40. While Lewis and Rees did not state the approach followed to calculate the numerical derivatives, they used the Connolly program to calculate surface areas in an interval rather similar to that included in the D_num1 set. Their value for lysozyme, 2.44, could then be explained in terms of the procedure followed and the small interval chosen, while still considering understandable the other value, 2.2, as a more global property underlying the molecular surface and the probe sphere approach. Finally, notice that the smaller non-protein HYA molecule has the same effective dimension around 2.05, irrespective of the probe size interval considered, as expected from the proofs on its lack of self-similarity before presented.

For surfaces not presenting clear linear correlations in log S / log r_p plots, the dimension D computed from equation (1) may not termed strictly fractal dimension. It must be viewed as an effective geometrical parameter related with the only molecular surface definition which is sensitive to the irregularity features of large molecules. Nonetheless, the reasonable agreement between the values of this effective dimension D and various estimates of the fractal dimension available in the literature, lends support to the validity of standard molecular surface calculations for extracting useful information on the geometrical complexity of large biomolecules like proteins.

Acknowledgements: I thank my son Alberto for his valuable help in rendering the POVRAY images as well as programming the interface between PDB coordinates and the input required to get these ray-traced molecular renderings.

This work was supported by the Spanish Dirección General de Investigación Científica y Técnica (DGICYT), project PB92-0331.

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