Progress in Nuclear Magnetic Resonance Spectroscopy 54 (2009) 278–289

Contents lists available at ScienceDirect

Progress in Nuclear Magnetic Resonance Spectroscopy

journal homepage: www.elsevier.com/locate/pnmrs

Probing protein surface accessibility with solvent and paramagnetic molecules

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ARTICLE INFO

Article history:
Received 16 September 2008
Accepted 28 October 2008
Available online 12 November 2008

Keywords:
Surface accessibility
Protein hot spots
Paramagnetic probes
Surface hydration
Nucleic acid recognition

Contents

1. Introduction

Protein interactions with other molecules in the crowded and confined environment typical of living systems are not governed solely by chance. Fig. 1 gives a pictorial view of the distribution of nucleic acids and large proteins inside an *Escherichia coli* cell. It is apparent that molecular diffusion and reciprocal interactions of biomolecules in their natural environment and those in a test tube are considerably different.

The way proteins, together with all other molecular ingredients of life, interact with one another is encoded in their surface accessibility, a dynamic parameter which is difficult to analyse. In any case, the accessibility of protein surfaces at atomic resolution

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0079-6565/$ - see front matter
doi:10.1016/j.pnmrs.2008.10.003

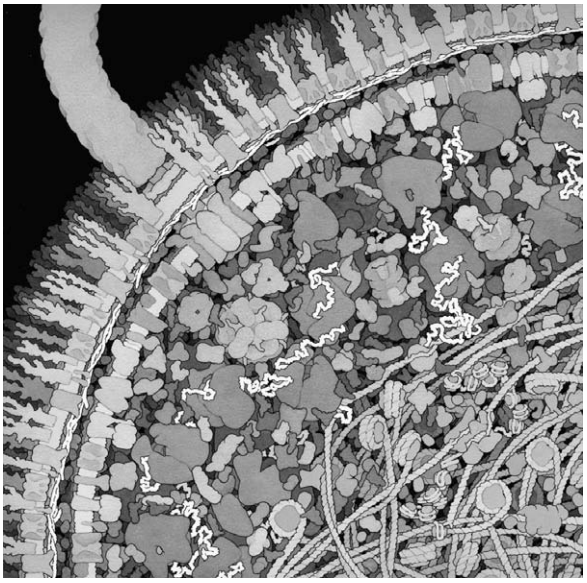

Fig. 1. Inside a *E. coli* cell. Illustration reproduced under permission of the author David S. Goodsell, the Scripps Research Institute, La Jolla (CA, USA).

needs to be investigated in detail in order to understand the mechanisms of the molecular interactions. In this respect, it is rather obvious that water, the most ubiquitous and abundant molecule of life, having influenced the evolution of shape, stability and function of proteins, as well as of all other biopolymers, should play a primary role in regulating molecular traffic.

Thus, understanding how particular surface regions of complex molecules can be preferential targets for binding ligands represents not only a step forward for interpreting natural processes at atomic resolution, but also for opening up new strategies for designing new drugs, materials and nanomachines.

2. Protein surfaces: hot and cold spots

The fact that proteins work through contacts of their surface with other molecules led to a detailed definition of this part of the macromolecule by using water, certainly the most important biomolecule, as a suitable probe for the estimation of protein static accessibility [1]. This accessibility, proportional to surface area, can be analytically calculated by applying the Connolly method [2] to the static snapshots of structures offered by X-ray or NMR methods. It follows that dynamic contributions from the approaching counterparts cannot be considered in these calculations and thus the obtained exposed surface area (ESA) refers only to static structural features. It is clear that protein surface accessibility, governing protein–protein and protein–ligand interactions, should be analysed by taking into account all the dynamic contributions which can modulate the extent and the orientation of the intermolecular approach.

2.1. Techniques for protein accessibility studies

A large dataset of unambiguous experimental results would be needed to include both static and dynamic aspects in a comprehensive view of the accessibility of protein surfaces and to develop new algorithms for its assessment. The way internal motions, hydration and surface accessibility of proteins are interconnected to give specific biological functions seems, indeed, to be very complex.

So far, it has been established that a protein surface presents some regions which are more prone than others to interact with other molecules. These regions have been named protein surface hot spots and represent regions where protein engineers should primarily focus their attention for designing new drugs. As an example, it has been recently pointed out that protein–protein interfaces incorporate hot spots, indicating that these protein moieties have huge pharmaceutical relevance. Binding of small molecules to protein surface hot spots, indeed, can disrupt protein–protein interactions, triggering therapeutic effects [3].

Data from alanine-scanning mutagenesis pointed out that occlusion of solvent is a necessary condition for the highly energetic interactions which are typical of protein hot spots [4]. Furthermore, cross-reactivity of protein binding sites has been frequently observed in protein–protein and protein–ligand interactions [5]. Multiple solvent crystal structure investigations (MSCS) proposed by Ringe and Mattos [6,7] have yielded similar results. In the case of porcine pancreatic elastase, organic solvent molecules have been found clustered in the active site, even where there is no structural similarity between the solvent molecules and the protein substrate. For the interpretation of these MSCS results, three conditions have been proposed for defining a protein surface patch as a genuine binding site: these are the presence of (i) local plasticity, (ii) water molecules that can be easily displaced contributing an entropically favourable term to the binding and (iii) hydrophobic surface hot spots [7].

Several NMR experiments have been developed to obtain direct information on the binding of small molecules to protein. For instance, lysozyme binding to organic solvent molecules has been investigated by observing magnetisation exchange among proton nuclei belonging to the protein active site and small molecules [8]. One molecule of DMSO bound to the substrate binding site of FKBP12, a small protein involved in several biochemical processes, was observed by using a modified ePHOGSY experiment. Also in this case, a similarity between DMSO and the protein substrate could not be found [9].

All the results reported in this section support the fact that small molecules, characterised by low reactivity and the absence of a net electric charge, can localise protein hot spots. Thus, organic solvents can be typical examples of such surface accessibility probes, but unambiguous hot spot mapping can be obtained only at high probe concentrations. This aspect can be critical in the case where such co-solvents modulate the molecular structure and in such cases alternative probes of surface accessibility should be devised.

2.2. Designing surface accessibility probes

The existence of protein surface hot spots implies that there are other surface regions where protein/protein or protein/small molecules approaches are not favoured. Experimental detection of such protein surface *cold* spots is a fundamental step in the understanding of the mechanisms of intermolecular interactions and cannot be achieved with the use of organic solvents. Because of the short-range nature of proton/proton dipolar interactions, NOE-based methods for detecting organic molecule/protein interactions are quite insensitive, and structural information is limited to the areas where stable intermolecular adducts do form. By contrast, paramagnetic analogues of the organic molecules are much more sensitive probes for use in surface accessibility studies. The strong electron/proton dipolar coupling allows, indeed, detection of transient interactions and sparsely populated conformers [10,11], providing a more accurate picture of the dynamic molecular surface.

In surface accessibility studies, both diamagnetic and paramagnetic probes are used in water solution (see Section 2.2.2). Thus, information on water/protein interactions is important for a correct interpretation of the accessibility profiles, giving detailed in-