Review Letter

INTER-DOMAIN MOBILITY IN PROTEINS AND ITS PROBABLE FUNCTIONAL ROLE

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1. Domain mechanism of protein folding

In 1973 [1,2] we suggested (see also [3]) that a polypeptide chain of a globular protein can fold by the independent initiation and growth of two 'crystallization centers' of its tertiary structure. The compact structure of a protein as a whole is formed at the last stage of folding by the pre-existing structure merging of the two halves of a protein molecule. In the same year Wetlaufer [4] noticed that large proteins usually consist of two (or several) domains. He suggested that each of the domains folds independently.

Wetlaufer [4] defined domains as distinct structural compact regions which could be completely encircled by a closed surface. Later Rossman and Liljas [5] proposed a simple method of locating domains by distance maps (matrices of the distances between C_α-atoms of all monomer residues [6]). They noticed that different domains of some proteins have different functions (e.g., NAD-binding and catalytic functions in dehydrogenases). Later the functional role of domains was thoroughly considered in the review [7].

The term 'domain' as used, for example, in papers [4,5] has not only a geometrical but also an energetical meaning implying that inter-domain interactions are weaker than intra-domain ones. This is undoubtedly the case at least in some proteins (e.g., in phosphoglycerate kinase [8] and hexokinase [9]) which are evidently divided into two lobes and very probably it may be a general feature of domain-containing proteins. This makes the hypothesis about the independent folding of domains rather convincing. This hypothesis explains the high cooperativity of the folding of large proteins [10] because the folding of each domain is a cooperative process and the stability of separate domains (before their merging into the structural entity) is usually low.

However in some cases (e.g., in immunoglobins [11] and in troponin C [12]) separate domains are stable enough to remain in the folded state even after the enzymatic cleavage of the protein into two or more domains. Recent calorimetric data show that domains of some proteins, e.g., immunoglobin [13] or papain (E. I. Tiktopulo and P. L. Privalov, to be published) can unfold and refold independently. This suggests that the most stable intermediate states of partially folded large proteins are separate folded domains [10]. Very recently this hypothesis was directly confirmed for hen-egg lysozyme. It was shown by direct X-ray analysis [14] that partly refolded lysozyme (in 0.35 M sodium dodecyl sulfate) differs from the native one mainly in that the two domains of this protein move away from each other. The main features of the internal structure of both domains are retained.

2. Inter-domain mobility in protein molecules

If inter-domain interactions are in fact relatively small, one can anticipate the existence of domain displacements not only under denaturing influences but also under other ones. Indeed, X-ray data show the existence of minor but measurable relative domain displacements in hen-egg lysozyme after the binding of the competitive inhibitor [15] or in chymotrypsinogen after its activation into α-chymotrypsin [16]. Model calculations for lysozyme [17] and subtilysin [18] also demonstrated that their domains can be relatively easily displaced. Much more

pronounced (up to 5–6 Å) relative domain displacements were shown recently at glucose binding to yeast hexokinase [19,20] and at NAD^+ binding to D-glyceraldehyde 3-phosphate dehydrogenase [21]. From these results it seems that Koshland's induced-fit hypothesis [22] is valid not only for displacements of functioning groups in active centers but also for relative domain displacements.

Therefore it is natural to make a subsequent step and to assume that native proteins maintain a certain lability of relative domain positions *even in the absence of external influences* due to thermal fluctuations. At first sight this assumption contradicts the X-ray data in protein crystals which clearly show that the relative domain positions are fixed rigidly. However the widely spread belief on the identity of protein structure in crystal and in solution has never been checked before by a method sensitive to minor displacements of large regions of the protein (e.g., to the displacements of protein domains).

Such a method has been developed recently in our laboratory [23,24]. It consists in the comparison of an experimental large-angle X-ray diffuse scattering in solution with a curve calculated from the crystalline atomic coordinates of this protein. Using this method we have started a systematic comparison of protein structures in crystal and in solution [23,25–28]. The essential differences between crystal and solution structures (in a distance range of $\geqslant 10$ Å) were not observed for ribonuclease [25] or for the complexes of ribonuclease [25] and hen-egg lysozyme [23] with their inhibitors. However, the experimental curve for the hen-egg lysozyme without the inhibitor differs slightly from the theoretical one in a Bragg distance interval ~20 Å which was interpreted in terms of a fluctuation widening of the substrate-binding cleft between two domains of this protein [23]. Marked differences between the experimental (H.B. Stuhrmann, personal communication) and the theoretical [27] curves for myoglobin have been interpreted as a result of relative fluctuations or displacements of the GH hair-pin and the other part of the molecule [27]. Finally, very marked differences between crystalline and solution structures at Bragg distances of up to 20 Å were observed for phage T4 lysozyme [26] and have been interpreted to result from relative fluctuations or displacements of the C-end tetrahelical complex and the other part of the molecule [28]. These results of a direct comparison of protein structures in crystalline state and in solution show that at least some proteins in solution have marked fluctuations or displacements in the relative positions of domains or other large regions of the protein molecule.

It should be noted that relative domain lability was revealed long ago in immunoglobins presenting a classical example of domain-containing proteins [11].

3. The probable functional role of inter-domain mobility

The act of enzymic catalysis itself may involve the displacements just of small functional groups in an active center of a protein molecule. However the attachment of a substrate to an enzyme and the detachment of reaction products might involve relative displacements of large regions of a protein molecule [29].

It is known now (see, e.g., the review [30]) that active centers of all or nearly all enzymes with a domain structure are positioned between the domains. In conditions of functioning it may be assumed that the enzyme has an inter-domain mobility. The substrate-binding cleft dividing the domains may fluctuate continuously between its locked and unlocked states. The substrate could penetrate into the enzyme when the cleft is open thus permitting the formation of the enzyme–substrate complex without overcoming great potential barriers. The formation of this complex induces the closing of the cleft. Upon this closing, the enzyme enfolds and stretches the substrate creating a dense packing of the enzyme–substrate complex and a 'rack effect'. Then the act of enzymic catalysis occurs. (In general this scheme of enzyme functioning resembles Spirin's hypothesis of ribosome functioning according to which the work of the ribosome is induced by the locking and unlocking of its subparticles [31].)

Many examples are known where a change of the protein state owing to ligand binding or to minor environment alterations can be well tested by protein functions, by the ability to be digested by proteases, etc., but are not observable by conventional structural methods (particularly by optical ones). In all these cases the change of the protein state may occur due

to an increase or a decrease in the mobility of large regions of the protein. Therefore it does not affect the routine structural characteristics of protein though it may influence the large-angle diffuse X-ray scattering or the dynamic characteristics of a protein molecule.

4. Interconnection between functional mobility of proteins and their denaturation

In 1940 Nasonov and Alexandrov [32] assumed that the functioning of proteins is connected with their 'partial denaturation'. Later Alexandrov [33,34] showed the existence of a correlation between the body temperatures of organisms and the denaturation temperatures of their proteins, this correlation remaining also in the cases where the denaturation temperatures are much higher than the body temperatures. To explain this correlation, Alexandrov assumed [34] that protein functioning requires a definite lability level of its molecule determined by the interactions within globular proteins. The lower the body temperature of the organism, the less must be these interactions to preserve the given lability level and the lower must be the denaturation temperatures of its proteins.

The results of a detailed X-ray study of the structure and functioning mechanism of crystalline enzymes did not seem to be in accord with this idea. According to X-ray data there are only local displacements of functioning groups in active centers which have nothing to do with the unfolding of a protein molecule. However, the disclosure of inter-domain mobility in lysozyme and myoglobin in solution [23,26–28] and the experimental evidences of the domain mechanism of lysozyme folding [14] permit a structural interpretation of the above-mentioned suggestion of Alexandrov.

The functioning of many proteins may involve a definite relative lability of great regions (domains) of the protein molecule. The greater the inter-domain mobility, the smaller is the inter-domain interaction. On the other hand, the unfolding of a protein molecule begins with the breakdown of its structure into separate domains. Therefore the smaller the inter-domain interaction, the more easily does the protein unfold. If the interaction between the domains would remain unchanged, their relative mobility would decrease with a decrease of temperature. Thus, to have a similar inter-domain mobility, the protein which works at low temperatures must have a smaller inter-domain interaction than the protein which works at high temperatures. Therefore the 'low temperature' protein will unfold at a lower temperature than the 'high temperature' one. This is the reason for the correlation between the body temperatures of organisms and the denaturation temperatures of their proteins.

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