Protein structure

New light on old defects

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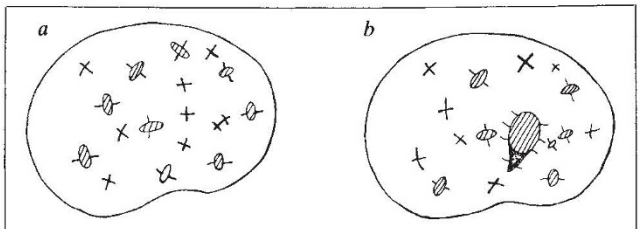
MYOGLOBIN, the 'hydrogen atom of biology', shares with other globular proteins the characteristic that its 1,261 non-hydrogen atoms are remarkably well packed (although not as closely as a box of tennis balls). As I have discussed previously in a News and Views article[1], this relaxed packing is associated with compulsive motion of the atoms in a manner which, like the chemical and physical nature of the protein interior, is anisotropic and which is believed to contribute to the biological function of these molecules. Two quite different experimental approaches, solution compressibility and X-ray crystallography, have now been used by Gekko and Hasegawa[2] and by Frauenfelder *et al.*[3] to shed more light on these basic attributes of protein structure.

There are three kinds of free space within a protein structure: the inevitable vacuum left if and when the atoms are packed ideally to the limit of their van der Waals' radii; spaces of greater than atomic size frequently occupied by water molecules and termed cavities; and defects where atoms and groups are on average separated by subatomic distances. Such defects, or regions of poor bonding interconnected by protein fabric, can provide a high cooperative mobility of free volume and its associated potential energy. These have been called 'mobile defects'[4] and can provide pathways for the penetration of small molecules to the interior of the folded protein (see figure). The question arises as to whether the defects are the result of the forces that lead to condensation of the polypeptide chain being opposed by thermal agitation, or whether they represent the optimal packing of each particular chain.

Gekko and Hasegawa[2], using measurements of sound velocity, extend existing data on compressibility to 25 proteins, including myoglobin. All the globular proteins so far studied (with the exception of subtilisin) show a positive adiabatic compressibility β_s, suggesting that the cavities and/or defects *can* be reduced in size, although quantitation is subject to the uncertainty of the negative contribution of hydration to β_s. This finding is strengthened by the demonstration that values of β_s correlate reasonably well with the independently measured volume occupied in solution by unit mass of protein, V_0. In other words, the larger the volume occupied by a given length of folded polypeptide chain, the more the molecule can be compressed.

Because bulk properties must have their origin in the detailed molecular structure it was hoped that it might be possible to identify important features by correlating compressibility with various features of the 25 different proteins. Proteins with a high content of helical structure seem to be more compressible than those with low helix content, although Gekko and Hasegawa knew that correlations could reflect other properties — in this case multi-subunit and multi-domain structure. Their result could, however, mean that the rather densely packed helical structures are prevented, perhaps by their own rigidity, from packing together in a way that minimizes the defect space. The acid test should be a careful study of the X-ray structures of helical and non-helical proteins.



A rough two-dimensional projection of channel production by rearrangement of the free-volume increments associated with mobile defects. The channel is shown in *b*. Crosses, regions of good binding which are potential defects; shaded ellipsoids, defects showing broken hydrogen bonds (re-drawn from ref.4).

The correlation of compressibility with the paraffin-like nature or hydrophobicity of the component amino-acid residues reveals a tantalizing result that is not easy to translate into detailed structural terms. Overall hydrophobicity of the protein correlates positively with compressibility whereas, when the content of individual residues is examined, correlation of β_s is observed with only two out of the six major hydrophobic residues. Clearly, some other, unknown property of these residues, possibly associated with their 'packability', is involved.

It is possible to draw conclusions about the dynamic fluctuations of a protein using statistical thermodynamics as originally discussed by Cooper[5]. Gekko and Hasegawa show that the extent of the volume fluctuation is on average 0.3 per cent of the total volume in many proteins. As they point out, this is more than enough, if the increased volume is localized at a few points within the folded protein, to accommodate extraneous molecules in the manner proposed in the mobile defect hypothesis.

Frauenfelder and co-workers[3] approach the question of the basis of defect and cavity space in a different way by examining in detail the atomic coordinates of myoglobin obtained from X-ray crystallographic analysis at 300 and at 80 K. They observe a general shrinkage of the protein crystal for this reduction in temperature, expressed as a 5 per cent reduction in volume of the crystallographic unit cell and a 3 per cent decrease in that of the protein molecule. The fact that cooling was achieved without use of a cryosolvent suggests that the shrinkage is associated primarily with the decrease in thermal energy. Taken together with the compressibility studies, this suggests that a proportion at least of the free space in a protein structure is the result of thermal 'expansion' energy and not just limitation of folding.

Perhaps the most interesting aspect of the work of Frauenfelder *et al.* is the finding that the shrinkage has little effect on the dimensions of the helical cylinders and, similarly, that the proportional shrinkage of the larger cavities is small. In consequence the largest volume change must be occurring in the small packing defects. It is intriguing to postulate, therefore, that it is these defects that are most susceptible to deformation by thermal energy, and by extension that they, more than other regions, are subject to thermal fluctuations in the protein interior. This could lead on a longer timescale to mobility of the associated potential energy to create transitory larger defects in specific locations into which an extraneous molecule such as oxygen, water or acrylamide could fit[1]. Subsequent redistribution of the energy would allow the molecule to move through the protein as easily as a fishmonger through a debutantes' garden party, as proposed[4] in the mobile defect hypothesis.

Boyles' law has been redefined as "the greater the external pressure the greater the volume of hot air". The fact that the formidable amount of work involved in the new studies[2,3] relating temperature, pressure and volume has not led to instant understanding of protein dynamics shows how unready the protein molecule is to reveal its secrets. But the results do promise fresh insights from the combination of thermodynamic and structural experimentation. □

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2. Gekko, K. & Hasegawa, Y. *Biochemistry* **25**, 6563–6561 (1986).
3. Frauenfelder, H. *et al. Biochemistry* **26**, 254–261 (1987).
4. Lumry, R. & Rosenberg, A. *Colloques Internationaux du CNRS* No. 246, 53–61 (1975).
5. Cooper, A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2740–2741 (1976).

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