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SOME STRUCTURAL ASPECTS OF SUBINIT INTERACTIONS IN PROTEINS*

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SUMMARY

The self assembly of multi-subunit proteins frequently results in symmetrical aggregates. Attempts to relate the interprotomer interactions with biological activity were only partially successful. Several examples are discussed: phospholipase A_2 (atrox) and demetallized concanavalin-A both with a non-perfect internal 2-fold rotation axes, cholera toxin with 5-fold axis and giant particles of Δ^5-3-ketosteroid isomerase.

SOMMAIRE

Le processus d'assemblage de protéines multimérique conduit fréquemment à des agrégats symétriques. Les tentatives en vue d'établir une relation entre l'activité biologique et les interactions entre protomères n'apportent que des résultats partiels. Plusieurs exemples sont discutés : Phospholipase A_2 (atrox) et Concanavaline A démétallisée présentant un axe interne de rotation, non-parfait, d'ordre 2, Toxine cholérique (axe de rotation d'ordre 5) et les molécules géantes de Δ^5-3-Ceto-steroïde isomérase.

Many proteins exist as aggregates of subunits. In most cases these subunits have identical sequences that eventually may lead to identical three-dimensional conformations. However, there are several cases of proteins which consist of assemblies of more than one type of subunit. An example of this is the cholera-toxin, which will be discussed later.

The determination of the three-dimensional conformation of a protein by X-ray diffraction methods depends primarily on the availability of single crystals. These crystals are held together by packing forces, some of which are determined by the crystallization conditions whereas others may reflect specific functional interactions. For some multisubunit proteins the crystallographic symmetry coincides with the internal symmetry of a molecular aggregate in solution, which is very frequently important for biological activity. However, even for identical subunits there are cases where deviations from perfect symmetry have been observed. This may be a result of some functional requirements or of internal flexibility.

Unfortunately the correlation between the self assembly of the subunits and their function is not always clear. A well known example is hen egg white lysozyme, which is active as a monomer, and tends to dimerize in solution. In the tetragonal crystal form (Imoto et al 1973) the dimer interactions are conserved whereas in the triclinic crystal they are not (Moult et al 1976).

Concanavalin-A, a saccharide-bonding metal protein binds to cell surfaces of specific sugars such as α methy-L D-mannopyranoside (Goldstein et al, 1965). It is biologically active in solution only as a dimer or tetramer although there is one binding site for each protomer (Kalb and Lustig, 1968). Two metal ions are essential cofactors for its activity — a transition metal such as Mn^{++} and Ca^{++} (Summer and Howell, 1936). One ion of each metal binds to each protomer. In its crystal form (I222) there are three perpendicular crystallographic two-fold axes which coincide with the internal symmetry (Hardman and Einsworth 1972, Reeke et al, 1975). Thus, with a monomer in the asymmetric unit the tetrameric structure is conserved. In contrast, demetallized concanavalin-A, which has no biological activity, crystallizes in $P2_12_12_1$, with an asymmetric unit that consists of a dimer. (Jack et al 1971, Shoham et al, 1978, Reeke et al, 1978, Shoham et al 1979). The structure of the two forms is very similar ; however, they differ significantly in the metal binding region and in the interprotomer contact surface, although these two regions are about 20 Å apart. Since in the demetallized enzyme the two protomers are no longer symmetrically related, the structure of each protomer was determined, and deviations from identical conformation were observed. However, upon proper sequential exposure to metals the demetallized crystals are converted into the native form, and the perfect crystallographic symmetry is recovered.

Two mamalian phospholipases, from porcine and bovine pancreas function as monomers and crystallize with a monomer in the asymmetric unit (Dijkstra et al, 1978), whereas a fairly homologous enzyme from snake venum, phospholipase A_2 (Crotalus atrox), crystallizes in several space groups, all of which have a dimer in the asymmetric unit (Pasek et al 1975). For one of these forms ($P2_12_12_1$) there is a noncrystallographic, fairly distorted, two-fold internal rotation axis. This 28000 dalton enzyme catalyzes the speficic hydrolysis of the fatty acyl-2-glycerol ester in phospholipids. The activity is enhanced when the enzyme confronts an array of aggregated phospholipids, which are arranged in lammellar systems. The enzyme binds calcium ion as an essential cofactor, and it was suggested that the calcium ion is needed to maintain dimeric

association. Although it had not yet been proved, it was suggested that this enzyme, as well as phospholipase A2 from Crotalus adamnateus, are active as dimers (Wells 1971, Shen et al 1975, Hachimori 1971).

A model for steroid-protein interaction was constructed by developing a pseudomonas which metabolizes testosterone (Talaly & Wang 1955). The enzyme Δ^5-3-Ketosteroid isomerase, that catalyses the isomerization of a number of $\Delta^{5,6}$ and $\Delta^{5,10}$-3-ketosteroids to the corresponding $\Delta^{4,5}$-3-Ketosteroids, has been isolated from this organism (Talaly and Benson 1972). The enzyme, with a molecular weight of 13,400 has a high tendency to aggregate in solution and, although it has one active site per monomer, it has never been observed as a particle which is smaller than a dimer. (Benson et al 1975). The enzyme crystallizes in two space groups, both of which have very high symmetry ; monoclinic ($P2_1$) with 12 protomers in the asymmetric unit, (Westbrook et al, 1976) and hexagonal ($P6_122$) with four protomers in the asymmetric unit (Westbrook, 1976). In the latter form the protomers are probably related by two perpendicular non-crystallographic two-fold axes.

All the enzymes that were considered above are built of equivalent subunits. An example of a protein which consists of two species of protomers (A and B) is cholera toxin. The A subunit, of which there is one per molecule, has a molecular weight of about 27,000, and is actually composed of two parts (A_1 and A_2). Each B subunit, of which there are five per molecule, have a molecular weight of 11,400 daltons (Gill, 1977).

The interest in cholera toxin is due to its biological function and to its unusual structural properties. It serves as an effector of a specific intracellular function by binding to a specific receptor (the ganglioside GN_1) on the mucosal surface of certain secretory cells, and consequently exciting an intracellular adenylate cyclase which produces cAMP. This, in turn, stimulates the pumping of Cl^- ion through the cell membrane (Cassel & Pjeuffer, 1978, Gill and Meren 1978).

In consideration of the structure of cholera toxin it is important to note that the symmetry 2, 222 or 32 are greatly preferred to point groups of 5 (Liljas and Rossmann, 1974). In fact there are only few cases where 5-fold symmetry have been observed; among them are the viruses (Casper and Klug, 1962).

An analysis of the X-ray diffraction intensities obtained to 6 Å resolution from monoclinic crystals ($P2_1$; $a = 72.9$ Å, $b = 92.0$ Å, $c = 60.7$ Å, $\beta = 106°$; (Sigler et al, 1977) shows a substantial 5-fold axis of rotation normal to the $\underline{b}$ axis and at + 135° from the $\underline{c}$ axis, while the structure is virtually devoid of 4-fold or 6-fold axes (Zelano et al, 1978). This result indicates that a significant fraction of the molecule (presumably the 5 B subunits) are arranged with approximate 5-fold rotational symmetry. Thus the architecture of the "injection" mechanism of the cholera toxin is formally analogous to that of the quasi-5-fold symmetry vertex of an icosohedral virus. It is of importance to examine this result in terms of the molecular weights of the individual subunits. If the five fold symmetry is due to the five B subunits which are small compared to that of the A subunit, it is most likely that either the A subunit is disordered, or most of its surface has a pseudo 5-fold symmetry which provides a similar contact area for all the B subunits, and contributes to the total molecular symmetry.

It should also be mentioned that a 2-fold symmetry axis in the direction of the 5-fold axis was observed. As yet it is not clear whether it has structural significance.

The search for noncrystallographic symmetry was performed for all cases in the Patterson space. A rotation function (Rossman and Blow, 1962) was computed using Crowther's fast rotation function (1972) which was modified by Tanaka (1977) in a manner in which the Eulerian angles were transformed into polar coordinates. The search was performed in intervals of 5° on both polar (ϕ and ψ) and rotation (χ) axes.

For one case, demetallized concanavalin-A, an electron density map was constructed using observed intensities and phases calculated from the native protein, omitting the region of the metal binding site. The maps were interpreted for each protomer (Shoham et al 1979).

In addition a real space search for rotation and translation of both the 4 Å and the 2.5 Å maps of Phospholipase A_2 (atrox), using 3 derivatives (Hg, Re and Pt) had been carried out. The results of the two searches are in fair agreement.

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