Eklund et al., 1976). Models of the alkylcyclohexanols formed as products were placed into the active-site pocket such that their hydroxyl oxygen atom appears in the position where the water oxygen atom was observed in the apoenzyme. Within the freedom of motion allowed by the geometric constraints of the active-site pocket the alkylcyclohexanol models were allowed to change their orientation. This fitting process was carried on until full agreement was achieved between the interactions observed in the model and those determined kinetically. In the resulting model the cyclohexanol rings of all products are superimposed and therefore show the same orientation relative to the nicotinamide moiety of the coenzyme.

In applying the described method it had been assumed that the intermolecular interactions in the enzyme–product complex, which are studied in the model, are not significantly different from those in the enzyme–substrate complex and in the transition state, which are relevant in the kinetic considerations. The excellent agreement between the two types of information suggests that this assumption is acceptable and that the resulting model of the enzyme–product complex discloses all the interesting structural features of the enzyme–substrate complex.

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PROTOMER STRUCTURE OF OLIGOMERIC PROTEINS:
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Protomer Structure of Oligomeric Enzymes: Symmetry and Allosteric Interactions in Yeast Hexokinase

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Oligomeric protein symmetry and subunit interaction

The protomers of virtually all of the few dozen oligomeric proteins whose quaternary structure is known are related by point-group symmetry as was expected (Monod et al., 1965). The point-group symmetries of the known oligomers are either cyclic, dihedral or isometric. In general, protomers are far more commonly related by dihedral symmetry than by cyclic symmetry in those proteins that consist of more than two subunits. For example, all tetramers of known structure have D2 symmetry rather than having subunits related by a fourfold rotation axis, and hexamers tend to show D3 symmetry rather than containing a sixfold axis of symmetry. Thus for most oligomeric proteins, most symmetry-related residues of each protomer are in identical environments and should show identical properties.
However, examples are now known from several crystal structures in which not all of the amino acid residues of symmetrically related subunits have the same structure. In the cases of insulin (Blundell et al., 1971), glyceraldehyde 3-phosphate dehydrogenase (Moras et al., 1975) and α-chymotrypsin (Tulinsky et al., 1973), portions of the oligomer are not related by a twofold axis, although most of the molecule is in fact related by the twofold axis. Although the function, if any, of the less-than-perfect point-group symmetry is not obvious for the cases of α-chymotrypsin and insulin, the small asymmetry that appears to exist in crystals of lobster glyceraldehyde 3-phosphate dehydrogenase may account for the half-site reactivity observed with this enzyme.

The subunit interactions that have been observed can be classified into at least two categories. One kind of contact involves the interaction of β-pleated sheets either by hydrogen-bonding between the sheets of two subunits or by the stacking of one β-sheet on another. A second category of subunit-subunit interaction involves α-helices stacked on each other or the interaction of loops at the ends of chains or α-helices. The oligomers containing β-sheet interactions holding subunits together do not usually show allosteric interactions between subunits, whereas those proteins showing the second kind of subunit interactions often show co-operative behaviour. This may be due to the ease with which subunits can move with respect to each other.

Yeast hexokinase

Yeast hexokinase is a 104000-mol.wt. dimer of identical subunits whose activity can be increased by as much as sixfold by a number of anionic metabolites, including its substrate ATP (Kosow & Rose, 1971). We have determined the structure of hexokinase at high resolution in both dimeric and monomeric forms (Fletterick et al., 1975; Steitz et al., 1976). From these structural studies we have concluded that: (1) unlike other proteins whose structures have been solved thus far, the two subunits of hexokinase are not symmetrically related; (2) there is a binding site for ATP that lies between the two non-symmetrically arranged subunits, and further, this site appears to be an allosteric activator site; (3) there is a major conformational change in the enzyme which is produced by the binding of glucose and which results in one lobe of the monomer moving several angstroms closer to the other lobe; (4) its tertiary structure is not homologous to the structures of any of the glycolytic enzymes, kinases or dehydrogenases whose structures are currently known.

The two subunits of the hexokinase dimer [which has been solved at 0.35 nm (3.5 Å) resolution] are not in equivalent environments, since they are related by a 156° rotation and a 1.38 nm (13.8 Å)-translation along the rotation axis (Steitz et al., 1976). This non-symmetric interaction between the two subunits involves intersubunit contacts between loops of chain and ends of helices, as is found with some other proteins showing allosteric interactions (Fig. 1). This non-symmetric dimer does not polymerize further because of the potential binding domains are sterically blocked from interacting with a third subunit. Thus it appears that it is indeed possible to have a non-symmetric or heterologous interaction of subunits and still form a closed oligomer. The existence of such non-symmetric oligomers may well bear on the molecular mechanisms of half-site reactivity.

Tertiary structure of hexokinase

The hexokinase monomer is divided into two lobes by a deep cleft. One lobe is virtually all α-helix, whereas the other lobe consists of both helix and β-structure (Fletterick et al., 1975; Steitz et al., 1976). A model of the monomer has been constructed from a 0.25 nm (2.5 Å)-resolution electron-density map, and the co-ordinates have been refined to a crystallographic R-factor of 0.26 at 0.23 nm (2.3 Å) resolution. In the absence of a chemical amino acid sequence, the identity of many of the side chains has been guessed from the electron-density map. The identity of these side chains is being continuously reassessed during the course of the refinement procedure in the hope of converging to the correct amino acid sequence.
Fig. 1. Schematic drawing of the yeast hexokinase dimer with its ATP and glucose substrates superimposed

Regions of polypeptide in $\alpha$-helical conformation are represented by tubes and those in $\beta$-sheet structures are indicated by arrows. The non-symmetric interaction of subunits can be most easily appreciated by looking at specific regions. For example, the amino end of helix D' is interacting with the neighbouring subunit, whereas the corresponding part of helix D is not even near the subunit interface. Similarly, the environments of helices B and B' are strikingly different. The three binding sites for nucleotide are shown. The one lying between the two subunits is a unique site on the dimer and is presumably responsible for the allosteric activation of this enzyme. The other two nucleotides are near the active site and are probably involved in the phosphoryl transfer. The two glucose sites per dimer lie in the centre of each subunit. It is the helices C and D and the $\beta$-structure around residues 95 and 120 that are as much as 0.6 nm (6 Å) closer to the glucose and the helical lobe in the glucose complex than in the native enzyme.

Rossmann and co-workers have suggested that all nucleotide-binding proteins have evolved from a common ancestral gene (Rossmann et al., 1974). To test this hypothesis we have compared the structure of the portion of hexokinase that binds adenosine with the portion of lactate dehydrogenase that binds NAD (Steitz et al., 1976). In the case of both of these enzymes the nucleotide-binding domain consists of a $\beta$-pleated sheet flanked on both sides by $\alpha$-helices. However, the $\beta$-sheet in lactate dehydrogenase consists of all parallel $\beta$-structure, whereas that in hexokinase contains an anti-parallel strand. Further, if these two nucleotide-binding domains are optimally aligned, the adenosine bound to hexokinase lies about 1.2 nm (12 Å) from the analogous position of the adenosine moiety of NAD. Thus it seems unlikely that these two proteins have evolved from a common precursor protein. Indeed, this seems to be a common structure observed in proteins.
Substrate binding

Glucose binds at the bottom of the deep cleft and promotes a major conformational change in the enzyme. When the structure of the A-isoenzyme crystallized in the presence of glucose is compared with that of the B-isoenzyme crystallized in the absence of glucose, it appears that in the glucose complex a major portion of the β-structure lobe is about 0.6nm (6Å) closer to the α-helical lobe, thereby closing the deep cleft into which the glucose is bound. That is, the two lobes can move relative to each other much like the subunits of a dimer. It is most likely that the structural difference is due to the binding of glucose rather than the isoenzyme difference, though this point is not yet established. This is consistent with the prediction of Koshland's (1959) induced-fit hypothesis, which suggested that the binding of glucose to hexokinase should produce an essential conformational change in the enzyme.

Thus far we have not been able to bind ATP to the active site without breaking up the crystals. However, adenosine binds to both the monomer and dimer crystal forms of the B-isoenzyme at a site near the glucose (Fig. 1). From model-building, it is possible to place the γ-phosphate group of an ATP molecule about 0.5nm (5Å) from the 6-hydroxyl group of glucose. A conformational change in the enzyme may be required to bring the two substrates into contact. The B-isoenzyme has now been crystallized in the presence of xylose, a five-carbon analogue of glucose, and the transition-state analogue, CrATP. These crystals are not isomorphous with any of the four-crystal forms that have been solved, consistent with the possibility that xylose and CrATP have produced yet another major conformational change in the enzyme.

Allosteric binding site

In the presence of glucose there is an additional binding site for nucleotides on the dimer (Fig. 1). This binding site lies between the two subunits and is presumably an allosteric activator site. The binding of glucose to the dimer promotes the binding of nucleotide to this site and the binding of nucleotide promotes the binding of glucose. This intersubunit site exists only in the dimer, and results from the asymmetric association of the two subunits. Indeed, the formation of this binding site may be the reason for this rather unusual arrangement of subunits.


Protomer Structure of Glycoprotein Hormones

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Glycoprotein hormones are secreted by the anterior pituitary gland, by the primate placenta, and by specific endometrial cells in the pregnant mare. The names of the hormones which have been most extensively studied, their source and their biological activities are given in Table 1. The pituitary glycoprotein hormones available in largest

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