Yeast chorismate mutase and other allosteric enzymes

William N. Lipscomb* and Norbert Sträter**

*Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA
**Present address: Institut für Mikrobiologie and Genetik Freie Universität Berlin, Takustrasse 6, D14195. Berlin, Germany

Abstract: Analysis of five crystal structures of dimeric yeast chorismate mutase include a T-state bound to the allosteric inhibitor Tyr, two R-state mutants and two super-R wild-type structures bound to the transition state inhibitor plus either Tyr or the allosteric activator Trp. Relative rotations of one subunit relative to the other are 0° (reference) for T, 15° for R and 22° for super-R states. A well defined pathway for conformational changes occurs from the active site to the subunit interface. Comparisons are made with other allosteric enzymes.

YEAST CHORISMATE MUTASE (YCM)

Function

This enzyme, which converts chorismate to prephenate in the pathway to Tyr and Phe, is inhibited at an allosteric site by Tyr and activated by Trp (ref. 1) (Fig. 1). Chorismate is also a substrate for anthranilate synthase which leads to Trp, and which is allosterically inhibited by Trp. Such regulatory processes achieve a balance between aromatic amino acids based on pyrmidine or purine rings. These enzymes are present only in archaebacteria, eubacteria, plants and fungi, thus providing an opportunity to design antibacterials, herbicides or fungicides which may be tolerated by humans.

Structure

The T-state dimer of YCM (ref. 2), 2 X 30k Da, consists mostly of helices and connecting loops (Fig. 2). The dimer axis lies in the plane of the paper, and the allosteric axis is perpendicular to the plane of the paper and...
intersects the dimer axis. The allosteric axis rotations described in the Abstract move the two helices H2 closer together at the subunit interface and make them more nearly parallel. Four helices H2, H8, H11 and H12 (Fig. 2) surround the active site and contribute binding and catalytic site chains. As the transition state is approached, the negative charge developing on the ether oxygen O2' (Fig. 1) is stabilized by Lys 168 and by a protonated Glu 246, thus stretching the C2-O2' bond and initiating the Claisen rearrangement of the enolpyruvate unit to yield prephrenate (Fig. 1).

The Transition

In the T to super-R transition, which is dominated by the substrate (here the transition state analogue), a series of changes propagate from the substrate site to the subunit interface (Fig. 3). As Arg 157 binds to the substrate the Arg 157...Glu 23 interaction is broken, and Glu 23 then moves to interact with Arg 204 and Lys 208 of H11. Also Lys 208 bonds to Asp 24 which binds to Tyr 212 near the interface, influencing Phe 28 (Fig. 3). These changes induce the relative rotation of subunits, and thus activate the active site of the other subunit.

The transition from the relatively tight T state to the looser R state is also promoted by the large aromatic ring system of the activator Trp, and inhibited by the smaller ring of the allosteric inhibitor Tyr. In addition the Trp inhibition involves the region of H4 plus the 80's loop (Fig. 2 and 4). In this region there is one hydrogen bond to the tyrosine OH group. This OH group of Tyr also donates a hydrogen bond to the hydroxyl group of Thr 145 of the H8 helix. The allosteric activator Trp cannot make these two hydrogen bonds, which favor the T state when Tyr is present at the allosteric site. Thus, Tyr favors the T state, whereas Trp favors the R state. These allosteric effectors have less influence than does the transition-state inhibitor (and therefore, presumably, the substrate).

COMPARISON WITH OTHER ALLOSTERIC ENZYMES

Rotations of subunits

Similar rotations of subunits in allosteric transitions (ref. 3) are seen in aspartate transcarbamylase (c4α6) where one c3 rotates 12° relative to the other c3, and each r2 rotates by 15° (ref. 4). Also, in fructose-1,6-bisphosphatase (α4) one α2 rotates by 17° (ref.5). In phosphofructokinase (α4) one α2 rotates by 7° (refs. 6

© 1998 IUPAC, Pure and Applied Chemistry 70, 527–531
and 7). In glycogen phosphorylase (α2) one monomer α rotates by 10° (ref. 8). In hemoglobin (α2β2) one αβ rotates by 15° (ref. 9-11). In allosteric bacterial lactate dehydrogenase (α4) rotations occur of α2 about two axes by 6° and by 4° (ref. 12). Except for the increase of 11Å between c3’s in aspartate transcarbamylase, relative translations are restricted to only a few Å. The generality of these rotations leads to a suggestion that detailed studies of torques in allosteric transitions may be worth investigation.

Fig. 3 Conformational changes of the T to R transition (arrows) between helices H2 and H11 extending from the active site to the dimer interface.

Preservation of organized structure

First, there is no significant change in the overall folding of the protein structures of allosteric proteins. Indeed, the transmission of conformational information requires organized secondary and tertiary protein
structure in all allosteric enzymes studied so far. There are small changes, for examples the loss of one turn of helix (155-162) in phosphofructokinase and the coil to helix transition of the N-terminal sequence containing the phosphorylated Ser 14 in glycogen phosphorylase. Structure is well preserved in yeast chorismate mutase, and the long helix H₈ connects the active site of one monomer to the allosteric site of the other monomer (ref.13). Although the symmetry before and after these allosteric transitions is largely preserved, partial occupancy of active or allosteric sites may lower symmetry during the transition.

Fig. 4. The regulatory site in the region of the inhibitor Tyr (left, T state) and the activator Trp (right, R state). Letters A and B identify subunits.

**Change of affinity or change of Vmax**

Allosteric chorismate mutase shows mixed behavior in these changes during the allosteric transition. For example, the allosteric inhibitor 8 lowers both [S]₀.₅ and Vmax (ref.14). In D-3-phosphoglycerate dehydrogenase Vmax is altered (ref.15). However, it is almost a generalization (ref.3) that substrate affinity is increased in the T to R transition, as it is in aspartate transcarbamylase, phosphofructokinase, glycogen phosphorylase, allosteric lactate dehydrogenase, and hemoglobin. In fructose-1,6-bisphosphatase it is the affinity for one of the catalytic metal ions that is altered.

**Sharing of ligand sites favors concerted transitions**

This sharing occurs between adjacent subunits in aspartate transcarbamylase, fructose-1,6-bisphosphatase, phosphofructokinase and allosteric lactate dehydrogenase. In yeast chorismate mutase, the allosteric site is shared between the two subunits (Fig. 4). Inhibitors and activators share parts of the same regulatory site in aspartate transcarbamylase, bacterial phosphofructokinase, glycogen phosphorylase b (nonphosphorylated), and allosteric chorismate mutase.

**Dominance of substrate or allosteric effectors**

Substrates induce the T to R transition in hemoglobin, aspartate transcarbamylase and glycogen phosphorylase, and dominate this transition in yeast allosteric chorismate mutase. On the other hand, an allosteric effector causes the allosteric transition in fructose-1,6-bisphosphatase, phosphofructokinase and allosteric lactate dehydrogenase.

**Flexible R-state structures**

The angle increase from T (0°) to 15° (R state) and 22° (super-R state) for yeast chorismate mutase is a kind of variation that has been observed in two other allosteric enzymes. In aspartate transcarbamylase, a further
expansion along the three-fold axis and further rotation of the catalytic trimers relative to one another has been observed in a low angle X-ray study in solution (ref.16). And, in hemoglobin the T to R equilibrium has been extended: T to R to R2. In the R2 structure there are two water molecules not previously seen in the α1β2 interface, and the R2 structure is proposed as the fully ligated state (refs. 17-20).

Acknowledgement

The authors thank the Organizers of the Conference, and the National Institutes of Health for Grant GM06920. We also acknowledge our collaboration with the research group of Professor G. Braus, including G. Schnappauf and R. Graf, for supplying the pure enzyme and the mutants.

REFERENCES