

13 Homologous and Related Structures

13.1 Structures Homologous to Neu5Ac Lyase

The first specific step in the lysine biosynthesis in higher plants is the condensation of aspartate β -semialdehyde and pyruvate to dihydrodipicolinate (Kumpaisal *et al.*, 1987) which is catalysed by dihydrodipicolinate synthase (DHDPS). *E. Coli* Neu5Ac lyase has sequence identity over its entire length with DHDPS from *E. coli* (24%), *Brevibacterium lactofermentum* (28%), *Corynebacterium glutamicum* (27%) and *Bacillus subtilis* (30%). This sequence identity extends predominantly through the α/β barrel of Neu5Ac lyase, but it is also present through the extra three C-terminal α -helices, suggesting that this feature is also present in DHDPS. The structure of DHDPS has not been determined so far, but SDS/PAGE, gel filtration experiments and crystal density determination by the Bode-Schirmer Ficoll density-gradient method (Bode & Schirmer, 1985) have shown the *E. coli* DHDPS enzyme to be a tetramer of four identical subunits with a dimer in the crystallographic asymmetric unit (Laber *et al.*, 1992). Biochemical studies have established that in DHDPS pyruvate forms a Schiff base with the enzyme through Lys-161 (Laber *et al.*, 1992). Sequence alignment (Table 1.1) with Neu5Ac lyase identifies Lys-165 in Neu5Ac lyase as the homologous lysyl residue.

Based on the sequence alignment (Table 1.1), the amino acids in the three layered core of the barrel of DHDPS are Ala-8, Gly-76, Tyr-133 and Gly-186 (aligned with Neu5Ac lyase Ala-11, His-79, Tyr-137 and Gly-189), Val-40,

Table 14: Sequence alignment between Neu5Ac lyase (X033450001), *E. coli* dihydrodipicolinate synthase (DHDPS, M128440001) and the *mosA* gene product of *Rhizobium meliloti* (L170710002). The numbers in brackets refer to the GenBank protein translation database. Secondary structural elements of Neu5Ac lyase have been marked [α -helices (.) and β -strands (\hat{i} - \hat{j})]. Residues identical in the three sequences are marked (*) whereas residues in the putative active site are marked (+) and deletions as (-). The alignment was produced by CLUSTAL (Higgins & Sharp, 1988).

	10	20	30	40	50	60
	<-->		<-->	
lyase	MATNLRGVMAALLTPFDQQALDKASLRRLVQFNIIQQGIDGLYVGGSTGEAFVQSLSERE					
DHDPS	*---FT*SIV*IV**MDEKGNVCRAS*KK*IDYHVAS*TSIAIVSV*T***SATLNHD*HA					57
MosA	*---FE*SIT*LV**FAD-DRIDEVA*HD*VEWQIEE*SFGLVPC*T***SPTLSKS*HE					56
	+				++	
	70	80	90	100	110	120
	<--->	<--->	
lyase	QVLEIVAEEGKGIKLIHVGCVTTAESQQLAASAKRYGFDVSAVTPFYYPFSFEEHCD					
DHDPS	D*VMMTLDLAD*RIPV**GT*ANA***AISLTQRFNDS*IVGCLT*T*Y*NRPSQ*GLYQ					117
MosA	Q*VEITIKTAN*RVPV**GA*SNS***AIAFVRHAQNA*ADGVLI*S*Y*NKPTQ*GIYQ					116
	130	140	150	160	170	
	<--->	<--->	
lyase	HYRAIIDSADGLPMVVYNI PALSGVKLTLDQINTLVLT-LPGVGALKQTSGDLYQMEQIRR					
DHDPS	*FK** -AEHTDL*QIL**V*SRTGCDLLPETVGRL-AKVKNIIIGI*EAT*N*TRVNQIKE					175
MosA	*FK** -DAASTI*IIV**I*GRSAIEIHVETLARIFEDCPNVKGV*DAT*N*LRPSLERM					175
		+	+		+	+
	180	190	200	210	220	230
	.	<--->	<-->
lyase	EHPD-LVLYNGYDEIFASGLLAGADGGIGSTYNIMGW--RYQGI VKALKEGDIQTAQKL					
DHDPS	LVSDDFV*LS*D*ASALDFMQL*GH*V*SV*T*VAAR---DMAQMCKLAAEEHFAE*RVI					232
MosA	ACGEDFN*LT*E*GTALGYMAH*GH*C*SV*A*VAPALCADFQQAC-LNGD--FAA*LKL					232
		++				
	240	250	260	270	280	290

lyase	QTECNKVIDLLIKTGVFRGLKTVLHYMDVVSVPLCRKPFPGPVDEKYQPELKALAAQQLMQRG					
DHDPS	NQRLMPLHNK*FVEPNPIP*WACKELGLVATDTL*L*MTPI TDSGRETVRAALKHAGLL					292
MosA	QDRMLPLHRA*FLETNPAGA*YALQRLGRMRGD-L*L*LVTI SPSFQEEIDDAMRHAGDPFM					293
MosA	MDNARFAERIEMDLIGANNQRRKQGGTCMGLDSGEAPCTS					333

Leu-101, Lys-161 and Ile-203 (aligned with Tyr-43, Ser-104, Lys-165 and Ile-206) and Ile-6, Ile-74, Ile-131 and Leu-184 (aligned with Met-9, Ile-77, Val-135 and Tyr-187). Six of the twelve core residues of the β barrel (Ala-11, Ile-77, Tyr-137, Lys-165, Gly-189 and Ile-206) are invariant in the alignment between *E. Coli* DHDPS and lyase. Seven are also conserved within DHDPS sequences from *E. Coli* (Ala-8, Ile-74, Leu-101, Tyr-133, Lys-161, Gly-186 and Ile-203), wheat, maize and *Corynebacterium glutamicum* (Laber *et al.*, 1992).

While the substrate pyruvate in the DHDPS reaction mechanism is also a substrate in the aldol condensation of Neu5Ac lyase, the product dihydrodipicolinate in the DHDPS reaction mechanism and the substrate Neu5Ac in the Neu5Ac lyase reaction mechanism both have free carboxyl groups suggesting similarities in both active site structures. The sequence alignment in Table 1.1 shows five of the nine putative active site residues of Neu5Ac lyase (Ala-11, Thr-48, Tyr-137, Lys-165 and Gly-189) to be conserved in the two structures, while two catalytic residues are conservatively exchanged (Ser-47 \rightarrow Thr, Ile-139 \rightarrow Val) and two are different (Thr-167 \rightarrow Ala, Tyr-190 \rightarrow Asp). The histidine residue closest to the proposed active site in Neu5Ac lyase (His-79) is replaced by a glycine residue in *E. coli* DHDPS. The proposed catalytic Lys-165 forming a Schiff base with the substrate and Tyr-137, a possible hydrogen donor, are both conserved in DHDPS and could be important in similar reaction mechanisms.

The *mos* locus of *Rhizobium meliloti* encodes proteins involved in the syn-

thesis of rhizopine (Murphy *et al.*, 1993). One of these gene products, MosA, has 29% identity with Neu5Ac lyase and 44% with *E. Coli* DHDPS over its entire length. The function of MosA is unknown but it has been suggested that it may be involved in either a condensation of pyruvate to inositol or the addition of a methyl group to *scyllo*-inosamine in the production of rhizopine (Murphy *et al.*, 1988). The sequence alignment in Table 1.1 indicates that the same six conserved residues in the core of the barrel of Neu5Ac lyase and *E. coli* DHDPS (Ala-11, Ile-77, Tyr-137, Lys-165, Gly-189 and Ile-206) are also preserved in MosA. The proposed catalytic amino acids (Ala-11, Thr-48, Tyr-137, Ile-139, Lys-165 and Gly-189) are conserved in MosA, while Ser-47 is conservatively exchanged (\rightarrow Thr), and Thr-167 (\rightarrow Ala) and Tyr-190 (\rightarrow Glu) are different in MosA.

While one of the two salt links in Neu5Ac lyase between the α/β barrel and the C-terminal helical extensions, from Glu-50 (located on the loop following β -strand *b*) to Lys-256 (on the extra C-terminal helix *J*), is preserved in MosA and *E. coli* DHDPS, the other, from Glu-58 (on helix *B*) to Arg-271 (on the loop connecting the two extra C-terminal helices *J* and *K*), is preserved in MosA and DHDPS from *E. coli*, *C. glutamicum*, wheat and maize.

13.2 Comparison with Known Aldolase Structures

Several three-dimensional structures of fructose-1,6-bis-phosphate (FBP) aldolases have been determined: from rabbit skeletal muscle (Sygusch *et al.*, 1987), human muscle (Gamblin *et al.*, 1990) and *Drosophila melanogaster* (Hester *et al.*, 1991). FBP aldolase catalyses the reversible aldol cleavage of FBP to dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate through a Schiff base intermediate involving a lysyl residue with FBP (Brenner-Holzach & Zumsteg, 1982). The FBP aldolases have no significant sequence homology with Neu5Ac lyase. The polypeptide chain consists of 363 residues, compared to the 297 residues per protomer in Neu5Ac lyase. The insect enzyme has about 50% amino acid sequence identity with the mammalian enzymes. All structures are tetramers with point group 222 of α/β barrels, circular in cross section with a C-terminal extension in the form of a single additional α -helix running towards the C-terminal end (the catalytic center) of the barrel. A role for the C-terminus in modulating the catalytic activity has been postulated (Hannappel *et al.*, 1982; Sygusch *et al.*, 1987). The active site lysyl residue in these FBP aldolases is located on β -strand *f* (Lys-230) supporting the identification of Lys-165 on β -strand *f* as the active site lysine in Neu5Ac lyase. Thirty-six C_α atom positions of the β -strands in the barrel of Neu5Ac lyase can be superimposed onto the FBP barrel with an *r.m.s.* deviation of 1.1 Å (Figures 40 and 41).

The arrangement of the protomers in the FBP tetramer is similar to that seen in Neu5Ac lyase insofar as each subunit makes contacts with only two

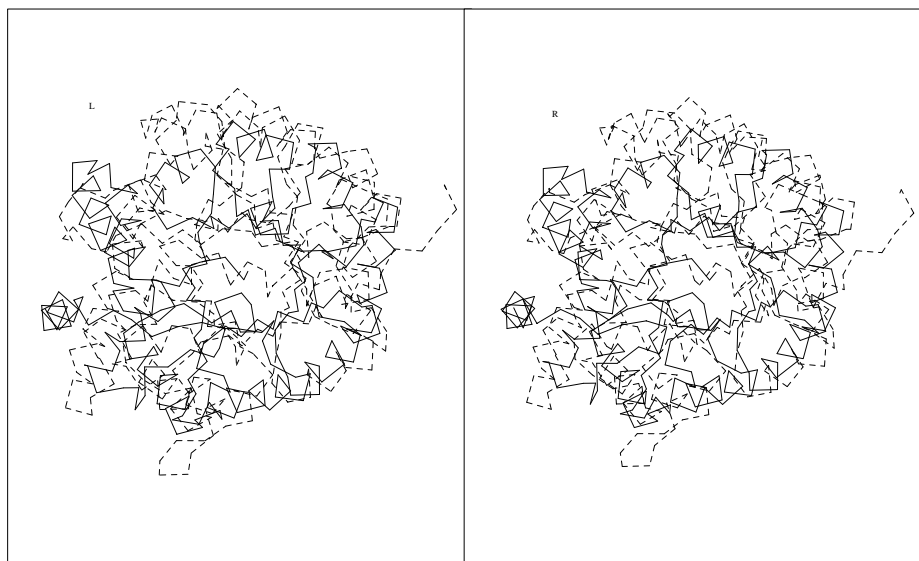


Figure 40: Stereo view of the C_α trace of Neu5Ac lyase (solid line) superimposed onto FBP aldolase from *Drosophila melanogaster* (dotted line; Hester *et al.*, 1991). While the thirty-six C_α atom positions of the β -strands in the barrel of Neu5Ac lyase can be superimposed onto the FBP barrel with an *r.m.s.* deviation of 1.1 Å, the helices and loops are oriented differently in both structures. This Figure was produced using the program MOLSCRIPT (Kraulis, 1991).

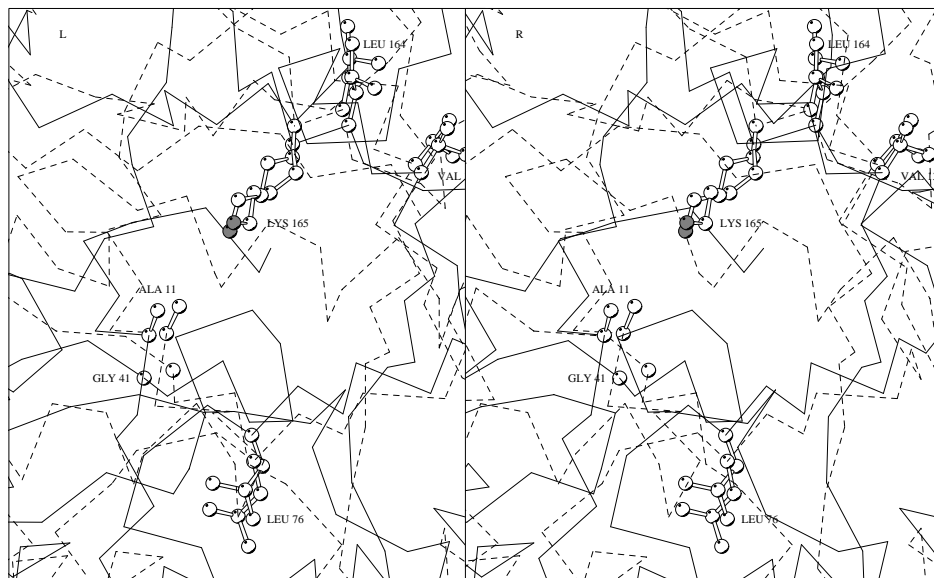


Figure 41: Stereo C_{α} trace of the barrel of Neu5Ac lyase (solid line) superimposed onto FBP aldolase from *Drosophila melanogaster* (dotted line; Hester *et al.*, 1991). The proposed catalytic lysine in Neu5Ac lyase and all identical residues in both structures are drawn in ball-and-stick representation (carbon atoms in white and nitrogen atoms in dark grey). These side chains enjoy very similar conformations. This Figure was produced using the program MOLSCRIPT (Kraulis, 1991).

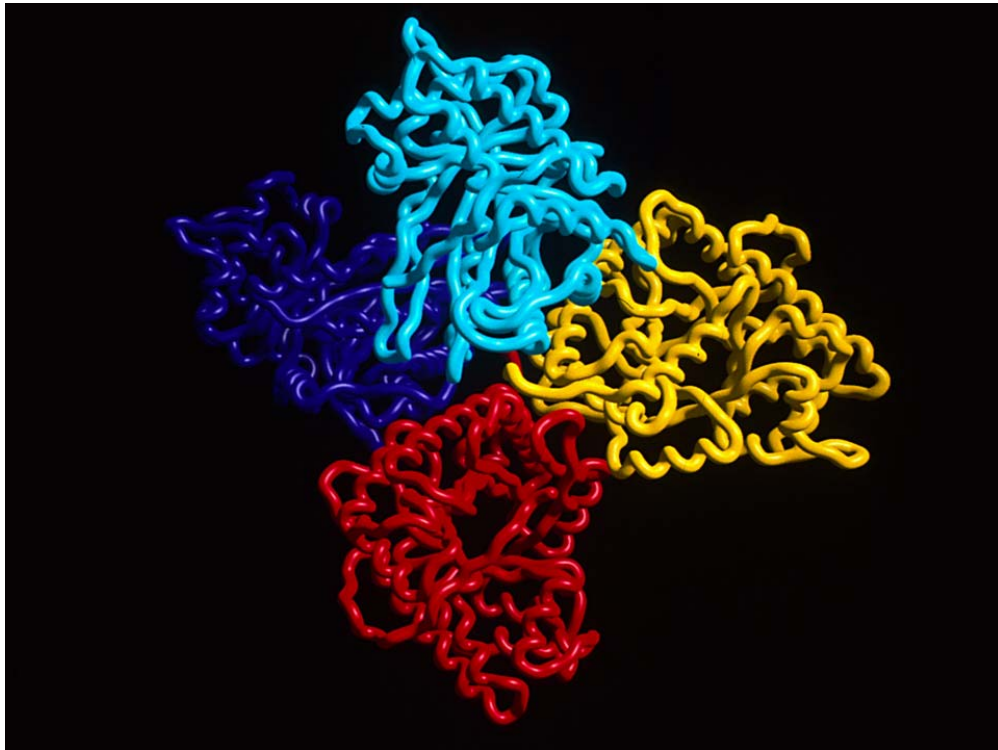


Figure 42: Backbone worm drawing of the *Drosophila melanogaster* FBP aldolase tetramer (Hester *et al.*, 1991). Like in Neu5Ac lyase, each protomer (coloured differently) makes contact with two other subunits only but they are packed more closely together about the local 222 axes. This figure was produced using HYDRASTER, written by S. Watowich and L. Gross, based on work by D. Bacon and W. Anderson (RASTER3D) and R. Hubbard (HYDRA).

others in the oligomer (Figure 42). While one of the two subunit interfaces in human muscle aldolase (Sygusch *et al.*, 1987) also involves residues from loop *c* and helix *F*, the orientation of the protomers within the tetramer is quite different to that found in Neu5Ac lyase. FBP (human muscle aldolase) and Neu5Ac lyase [as well as triose phosphate isomerase (TIM) and pyruvate kinase (PK)] all have a subunit interface involving the connecting region between β -strand *c* and helix *C* of the barrel. The flattened tetrahedral configuration of FBP packs the protomers more closely together about the local 222 axes compared to Neu5Ac lyase. The eight helices packed around the β -barrel have approximately the same orientation and position in both structures but helices *D*, *E* and *F* are longer in the structure of FBP. Neither the extra helices occurring in FBP as inserts after β -strands *a*, *b* and *h* (Sygusch *et al.*, 1987) nor the additional *N*-terminal helix seen in FBP are present in this structure. Whereas there are five charged side chains in the interior of the barrel in FBP, there is only one charged residue (Lys-165) in Neu5Ac lyase.

Mavridis (*et al.*, 1982) determined the three-dimensional structure of 2-keto-3-deoxy-phosphogluconate (KDPG) aldolase which is a trimer of α/β barrel and catalyses the cleavage of KDPG to pyruvate and D-glyceraldehyde-3-phosphate via a Schiff base formation with a lysine residue. Its reaction mechanism is similar to that of the FBP aldolases of higher animals. Like FBP aldolase, but unlike Neu5Ac lyase, KDPG aldolase has an additional *N*-terminal helix which is not present in Neu5Ac lyase. In the structure of KDPG aldolase, the catalytic lysine is located on the bend after β -strand *f* lying in a

positively charged cleft lined with hydrophobic residues (Mavridis *et al.*, 1982).