Supplementary Materials

Mechanistic Diversity in the RuBisCO Superfamily: The "Enolase" in the Methionine Salvage Pathway in *Geobacillus kaustophilus*

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Secondary structural elements of the polypeptide. The monomer of the "enolase," like the monomer of RuBisCO, is composed of two domains, an N-terminal α+β domain (residues 1-120) and a C-terminal $(\beta/\alpha)_8$-barrel domain (residues 121-413). Using the secondary structure labeling suggested for Rubisco (*1*), the N-terminal domain contains four antiparallel β-strands, βB (1-11), βC (47-58), βD (70-81), and βE (104-111), and three α-helices: αB (16-28), αC (88-101), and αD (113-119); this domain also contains two additional short α-helical segments, 39-43 and 61-66. The C-terminal domain contains eight β-strands, β1 (140-147), β2 (170-175), β3 (205-214), β4 (234-239), β5 (260-265), β6 (295-300), β7 (329-336), β8 (353-359), and eight α-helices, α1 (154-169), α2 (186-203), α3 (220-231), α4 (245-253), α5 (281-291), α6 (310-321), α7 (340-350) and α8 (369-384). An additional α-helix αE (127-135) is located at the N-terminal end of the barrel domain, and a C-terminal extension contains two additional α-helices, αG (388-395) and αH (397-406).

The monomer of the "enolase" activated with bicarbonate and Mg^{2+} and complexed with the alternate substrate DK-H 1-P (structure 4) can be superimposed on the monomer of subunit L of spinach RuBisCO activated with bicarbonate and Mg^{2+} and complexed with 2CABP (8RUC)

(*2*) with an rmsd of 1.72 Å for 369 Cα-pairs. However, the details of the structures of the monomers differ. The N-terminal segment 1-33 is missing in the "enolase." The BC loop, between αB and βC in the N-terminal domain, has different conformations and orientations in the two structures. The CD loop, between βC4 and βD in the N-terminal domain, is shorter in RuBisCO (90-96) than in the "enolase" (56-72); in the "enolase" this loop includes a short helix (60-66) which is missing in RuBisCO. The loop between αC and βE is longer in RuBisCO. In RuBisCO the loop between α6 and β7 in the $(\beta/\alpha)_8$-barrel is a long β-hairpin and includes residues 352-370; in the "enolase" this loop includes only residues 323-326. The loop between α8 and αG, located at N-terminal end of the $(\beta/\alpha)_8$-barrel domain, is longer in RuBisCO (430-443) than in the "enolase" (residues 386-387). The C-terminal segments (463-475 in RuBisCO) and (407-413 in the "enolase") have different orientations.

The monomers are packed as tight dimers, as found in the structure of RuBisCO; the structures of the dimers of the "enolase" and RuBisCO are compared in Figure 10 (panel A, "enolase"; panel B, spinach RuBisCO). The dimer interface is formed by three regions of interpolypeptide contacts: between the N-terminal domains of both monomers, between the N-terminal domain of one monomer and the C-terminal domain of the second monomer, and between the C-terminal domains of both monomers. The first region involves interactions of the CD loops from the N-terminal domains of both monomers across the local two-fold axis. The second region involves interactions between the L1, L2 and L3 loops at the ends of the first, second, and third β-strands of the $(\beta/\alpha)_8$-barrel domain of one monomer and helices αB, αC and the BC loop of the N-terminal domain of the second monomer; this interface forms the active site. The third region involves interactions between the L5 loops at the ends of the fifth β-strands of the $(\beta/\alpha)_8$-barrel domains in both monomers.

Dimer interface. The dimer of the "enolase" activated with bicarbonate and Mg^{2+} and complexed with DK-H 1-P (structure 4) can be superimposed on the dimer of spinach RuBisCO activated with bicarbonate and Mg^{2+} and complexed with 2CABP (8RUC) with an rmsd of 2.18 Å for 720 Cα-pairs. The interface between the N-terminal domains of both subunits is absent in RuBisCO because the CD loops differ in conformation. The interfaces between the N-terminal domain of one monomer and C-terminal domain of adjacent monomer differ in the two structures. The BC loop from N-terminal domain in one subunit is much closer to the active site in C-terminal domain of adjacent subunit in RuBisCO; also, these loops have different conformations. In RuBisCO the "extra" N-terminal extension (1-33) additionally closes the active site and is positioned behind the BC loop. As a result of these differences, the interface between the N-terminal domain of one subunit and C-terminal domain of the second subunit is tighter in RuBisCO than in the "enolase."

(1) Knight, S., Andersson, I., and Branden, C. I. (1990) Crystallographic analysis of ribulose 1,5-bisphosphate carboxylase from spinach at 2.4 A resolution. Subunit interactions and active site. *J Mol Biol 215*, 113-60.

(2) Andersson, I. (1996) Large structures at high resolution: the 1.6 A crystal structure of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase complexed with 2-carboxyarabinitol bisphosphate. *J Mol Biol 259*, 160-74.