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THE SINGLY-WOUND PARALLEL β BARREL: A PROPOSED STRUCTURE FOR 2-KETO-3-DEOXY-6-PHOSPHOGLUCONATE ALDOLASE

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SUMMARY

Two short local reconnections in the backbone chain tracing of 2-keto-3-deoxy-6-phosphogluconate aldolase suffice to make it an 8-stranded parallel β barrel whose size, shape, topology, and connection handedness match those of triose phosphate isomerase and of the first domain of pyruvate kinase. It is proposed that this singly-wound parallel β barrel is in fact the tertiary structure of the aldolase subunit.

The x-ray crystal structure of 2-keto-3-deoxy-6-phosphogluconate (KDPG)* aldolase from Pseudomonas putida has been determined by Mavridis and Tulinsky (1) at 3.5Å resolution. It is unusual in being a trimer, with rather large solvent channels around the molecule. The electron density map showed unambiguous connectivity in most places, and a consistent chain tracing was assigned. The subunit was found to contain nine α-helices and a small amount of parallel β sheet, but not organized in any readily describable overall pattern. More recently the amino acid sequence has become available, and tentative side chain assignments have been made (Tulinsky, personal communication). Alpha-carbon coordinates are available through the Brookhaven Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, N.Y. 11973.

For the purposes of classifying the structure and making a schematic drawing, I examined the KDPG aldolase α-carbon coordinates on the interactive molecular graphics system developed by Richard Feldmann at the National Institutes of Health. When the aldolase subunit was turned into one particular orientation, it showed a "rose" pattern strikingly similar to the end-on view of the singly-wound parallel β barrels of triose phosphate isomerase (2) (E.C. 5.3.1.1) and the first domain of pyruvate kinase (3) (E.C. 2.7.1.40). Figure 1

* Abbreviation: KDPG aldolase for 2-keto-3-deoxy-6-phosphogluconate aldolase.

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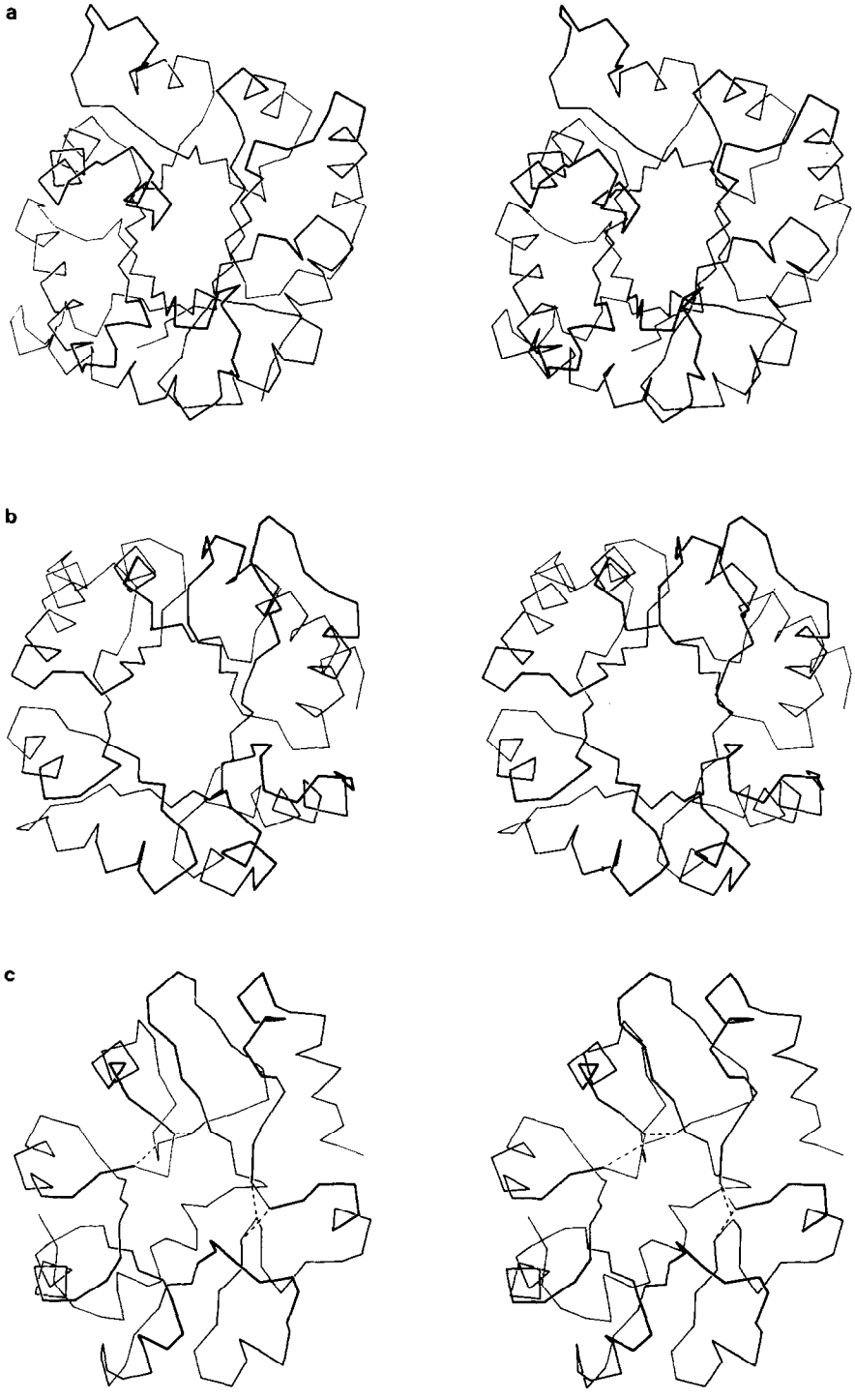

Fig. 1 - α-carbon stereo views down the β barrel axis for a) a triose phosphate isomerase subunit; b) the first domain of pyruvate kinase; c) a subunit of KDPG aldolase. The proposed reconnections in aldolase are shown dotted.

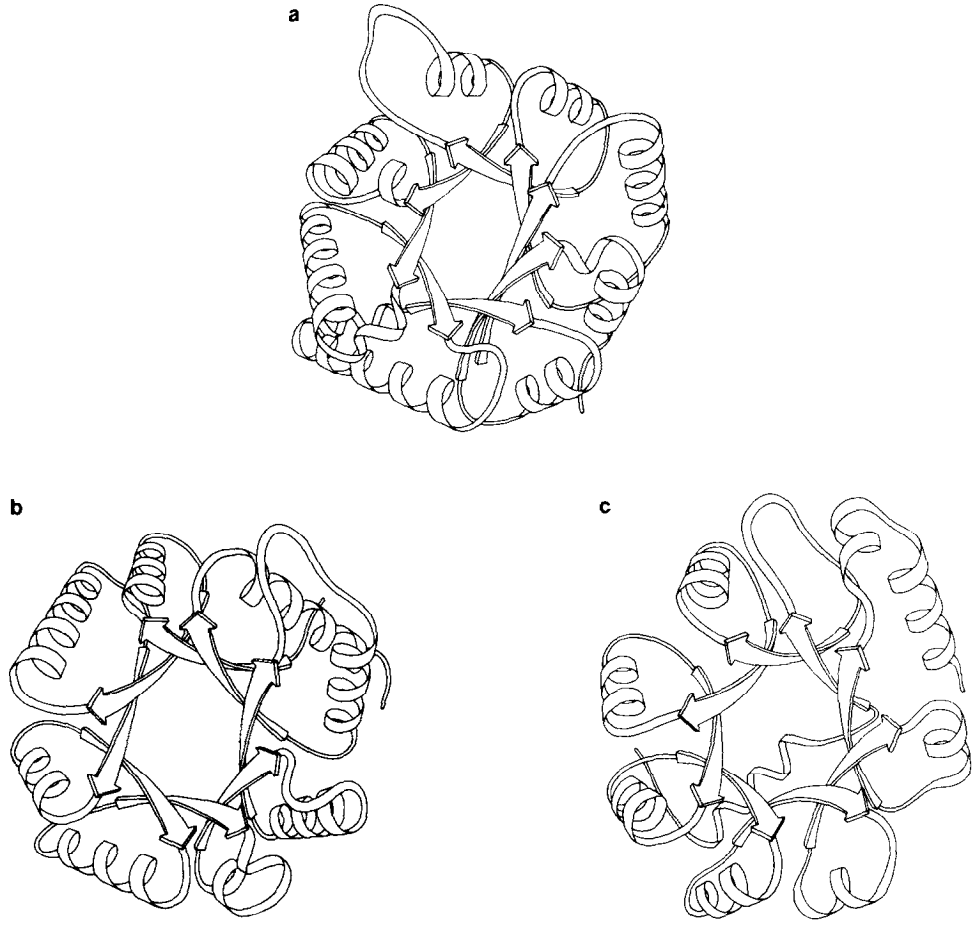

Fig. 2 - Schematic drawings from the same viewpoint as Fig. 1, for a) a triose phosphate isomerase subunit; b) the first domain of pyruvate kinase; c) a reconnected KDPG aldolase subunit. Copyright by Jane S. Richardson.

shows backbone stereos of these three structures in end-on view, and Figure 2 shows schematic drawings from the same viewpoint.

In the Mavridis and Tulinsky (1) chain tracing of KDPG aldolase, four and a half adjacent strands of a parallel β barrel are in place, with α-helices in the usual righthanded +1x crossover connections (4) joining them. Opposite that set of strands is an isolated strand in extended β conformation, at the correct distance and angle to belong on the opposite side of a barrel, but without any other main chains within hydrogen-bonding distance. In the center of the proposed barrel there is a large opening which at 3.5Å resolution ap-

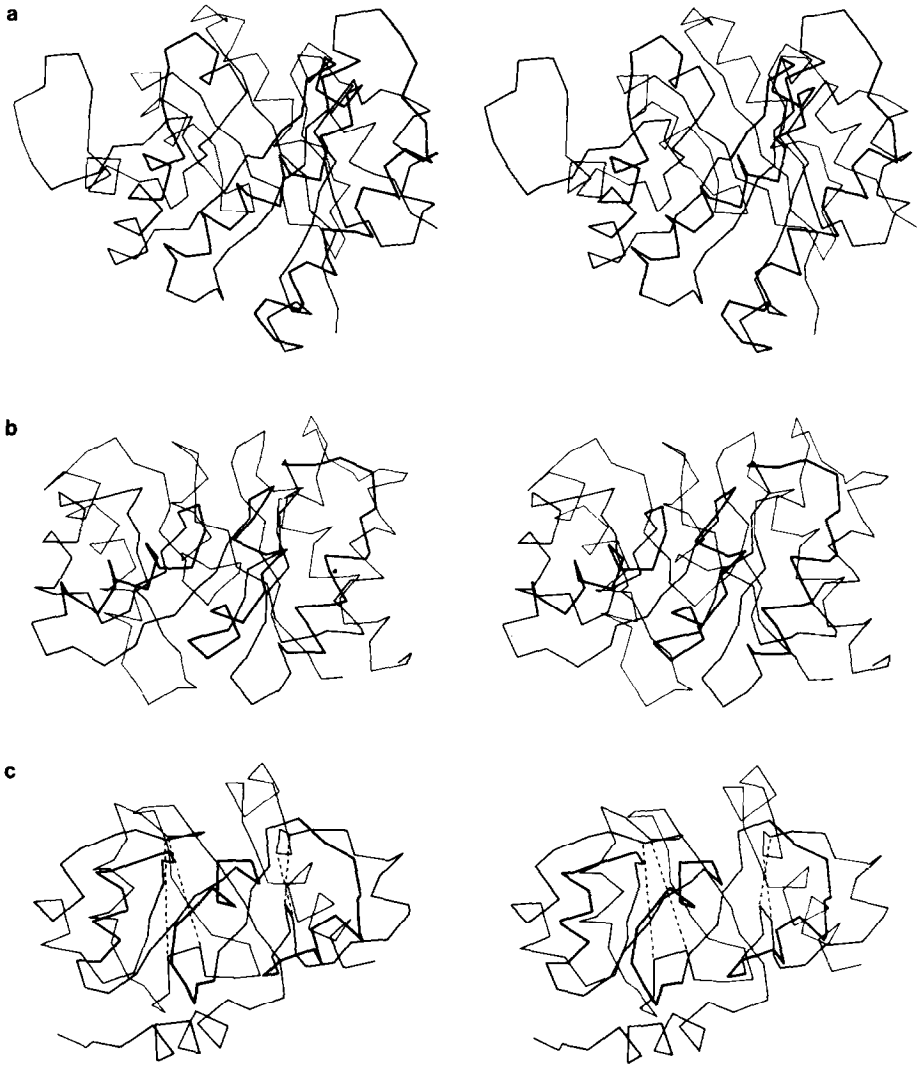

Fig. 3 - α-carbon stereo views perpendicular to the β barrel axis for a) triose phosphate isomerase; b) domain A of pyruvate kinase; c) KDPG aldolase. The proposed reconnections in aldolase are shown dotted.

pears to be empty. In the outer cylinder of the structure all the necessary connecting strands for a parallel barrel are in place; 7 of them are α-helical and one is irregular. Two short reconnections of the backbone (shown dotted in Figures 1c and 3c) provide the central portions of the missing parallel β strands and transform the aldolase structure into a singly-wound, 8-stranded, parallel β barrel with +1x righthanded crossover connections all around. Both triose phosphate isomerase and the first domain of pyruvate kinase are singly-

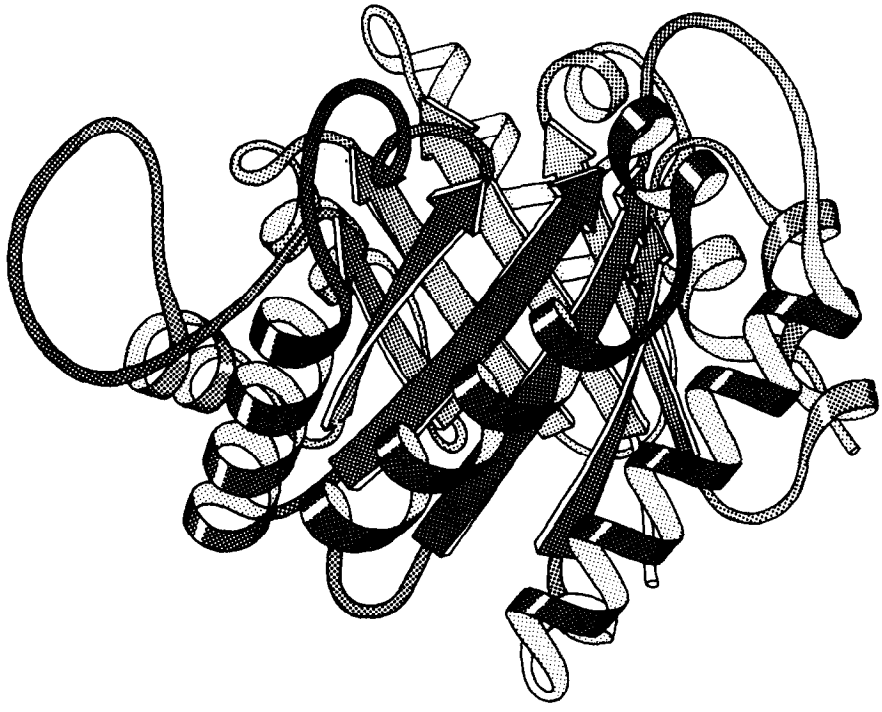

Fig. 4 - A schematic drawing of a triose phosphate isomerase subunit from the same viewpoint as Fig. 3. Copyright by Jane S. Richardson.

wound, 8-stranded, parallel β barrels with +1x righthanded crossover connections all around. The reconnected aldolase barrel matches them in cross-sectional area (as seen in Figure 2) and in strand twist (seen in Figure 3), as well as in overall topology.

The two sites of proposed connectivity change have been reexamined on the electron density map (Tulinsky, personal communication). At both sites the new backbone would cross gaps in the electron density, so that the 3.5Å resolution map does not support the proposed new tracing. However, the map cannot rule out the new tracing, because at resolutions lower than 3Å it is often necessary for the polypeptide backbone to jump gaps in electron density. In previous x-ray protein structures of 2.7-3.5Å resolution, a frequent type of incorrect chain tracing has been false connections running perpendicularly between a pair of true β strands (see references 5 vs 6, 7, 8 vs 9, and 10 vs 11). In pyruvate kinase domain A, only 7 of the 8 β strands were seen at 3.1Å resolution (12) in

spite of the recognized similarity to triose phosphate isomerase. Details of backbone connectivity are notoriously unreliable at 3.5Å resolution, while overall organization and relative placement of secondary-structure features is dependable. In KDPG aldolase the latter features match a singly-wound β barrel quite exactly; also, the singly-wound barrel topology is highly constrained by handedness, unknottedness, and packing requirements (13). Therefore, it seems likely that the detailed connectivity of KDPG aldolase will also turn out to be that of a singly-wound parallel β barrel. The reconnected aldolase structure provides neighboring β strands for the isolated extended chain, assigns the apparent central cavity to the relatively low density of hydrophobic side chains packed inside the barrel, and provides the entire subunit with a simple and familiar structure. Fortunately, a KDPG aldolase map at higher resolution will soon be available (Tulinsky, personal communication) to settle this question.

ACKNOWLEDGEMENTS

I am greatly indebted to Alexander Tulinsky for entertaining the reconnected aldolase structure as a serious possibility and for reexamining the electron density map. I also thank Richard Feldmann for the use of his molecular graphics system and Hilary Muirhead for providing the pyruvate kinase coordinates used to make Figures 1b and 3b.

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