Corrections

The First Crystal Structure of a Thioacylenzyme Intermediate in the ALDH Family: New Coenzyme Conformation and Relevance to Catalysis, by Katia D'Ambrosio, Arnaud Pailot, Francois Talfournier, Claude Didierjean, Ettore Benedetti, Andre Aubry, Guy Branlant,* and Catherine Corbier*, Volume 45, Number 9, March 7, 2006, pages 9275−9279.

Editor's Note: The new data analysis presented in this addition was provided by the original authors in collaboration with Gérard Bricogne and Clemens Vonrhein, Global Phasing Ltd., Sheraton House, Castle Park, Cambridge CB3 0AX, U.K. [telephone, +44-(0)1223-353033; fax, +44-(0)1223-366889; e-mail, gb10@globalphasing.com].

Following the questioning of a reader, relayed to us by the editorial board of *Biochemistry*, who was concerned that electron density maps were not sufficiently convincing with respect to the presence of the intermediate and that the conclusions drawn might therefore be subjected to alternative interpretations, we have re-examined the initial data using programs different from those used originally. Two strategies have been used for this purpose.

(i) The initial diffraction data have been re-indexed and re-integrated using Wolfgang Kabsch's XDS package (*1*). This led to an extended data set with a resolution limit of 2.2 Å [number of unique reflections, 124 421 (overall) and 17 938 (last shell); R_{merge}, 0.087 (overall) and 0.393 (last shell); $I/\sigma(I)$, 17.4 (overall) and 3.9 (last shell)]. Monomer A from the initial structure was then used as a search model, and monomers B, C, and D were generated through the noncrystallographic symmetry (NCS) operations, thus suppressing any overfitting that might have arisen previously through model refinement in the absence of any NCS restraints. Refinements were conducted with the beta version of BUSTER-TNT 2.0 (Global Phasing Ltd.), a program which has been shown to be capable of accurately refining partial occupancies and to produce maps and difference maps that are known to be more informative than those from conventional programs in terms of both the quality of the final refined models produced and the amount of bias-free detail shown by the final $2F_o - F_c$ and $F_o - F_c$ maps (*2*−*4*). NCS restraints were imposed on all four copies of the protein molecules (excluding residues 2−4, 284, and 317), while the cofactor and substrate molecules were refined without NCS restraints to R and R_{free} values of 0.1912 and 0.2302, respectively.

(ii) As a check of the respective roles of the reprocessing of the original diffraction images and of the change in the

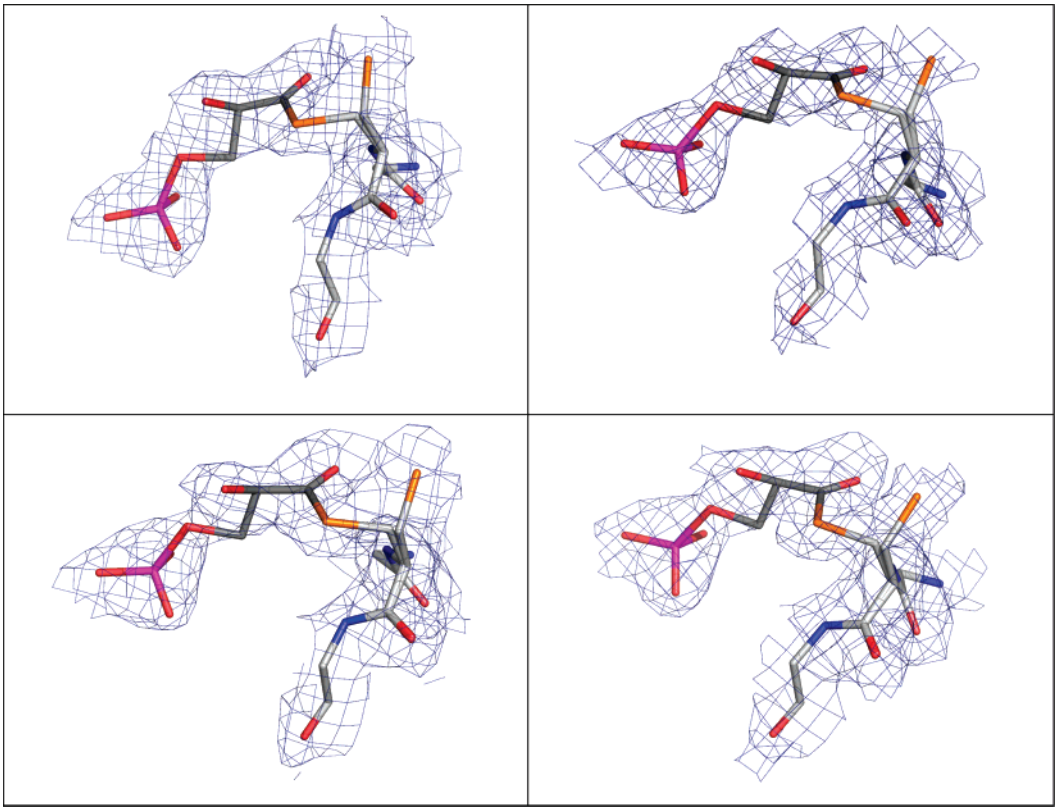

FIGURE 1: $2F_o - F_c$ electron density maps of the active site for the four monomers [contour level, 0.75 σ; monomer A (top left), monomer B (top right), monomer C (bottom left), and monomer D (bottom right)]. Considering a usual contour level of 1.25σ for a full occupancy, these maps have been contoured at 0.75σ to take into account the fractional occupancy (≈0.6) of the substrate. This figure was drawn with Pymol [DeLano, W. L. (2002) *The PyMOL Molecular Graphics System*, DeLano Scientific, San Carlos, CA].

Table 1: Comparison of Refinement Statistics for the Conformer in Which the Substrate Is Covalently Bound to the Catalytic Cys[a]

monomer	real-space correlation coefficient	B (Å^2)	fractional occupancy	connectivity level around the C3 atom
	Published Data (CNS refinement; $1\sigma = 0.185$ e/Å^3)			
A	0.8013	38.2	0.5	0.6σ
B	0.7955	49.7	0.5	1.0σ
C	0.9023	29.4	0.5	1.0σ
D	0.7751	45.8	0.5	0.6σ
	Re-Refinement (BUSTER) against the Original Data ($1\sigma = 0.225$ e/Å^3)			
A	0.9237	36.2	0.64	1.0σ
B	0.9190	46.5	0.58	0.9σ
C	0.9506	35.6	0.64	1.1σ
D	0.8524	54.5	0.65	0.8σ
	BUSTER Refinement against the Reprocessed XDS Data ($1\sigma = 0.243$ e/Å^3)			
A	0.8963	34.7	0.64	1.2σ
B	0.9033	38.6	0.58	1.1σ
C	0.9358	29.8	0.64	1.1σ
D	0.8810	41.9	0.60	0.8σ

[a] B values and connectivity belong to the C3 atom of the substrate, the electron density of which was observed to be the weakest one after refinement with CNS. Other values are for the complete G3P substrate molecule. The maps for the original CNS refinement were taken from the Uppsala Electron Density Server (*6*). Real-space correlation coefficients were calculated with OVERLAPMAP (*7*).

refinement package, re-refinement with BUSTER-TNT 2.0 was carried out against the original (deposited) data set (PDB entry 2esd; resolution limit, 2.55 Å; R, 0.193; R_{free}, 0.225). The same NCS restraints that are described above were used.

Detailed analysis of the refinements for the protein conformer in which the substrate is covalently bound to the catalytic cysteine is presented in Table 1. The four subunits exhibit similar partial rates of occupation (Table 1) for the covalently linked substrate. The density at the active site associated with the presence of thioacylenzyme in the crystals is stronger and better connected after refinements with BUSTER-TNT 2.0 than the one reported in the initial paper. It also reveals that monomers A, B, and C exhibit strong connectivity and an equivalent density quality (Figure 1) for the substrate covalently linked to the catalytic cysteine (Table 1) and a poorer density quality in monomer D, while initial refinement with CNS (*5*) led to density and connectivity that were both weaker in monomers A and D, a point that was not mentioned previously. The refined fractional occupancies for the substrate moieties covalently linked to the alternate conformer of Cys284 (Table 1) converge to values close to that fixed for the refinement carried out with CNS (0.5), and refinements converge to the same conformation of G3P, showing that our initial interpretation was correct from a crystallographic point of view. It is interesting to note that BUSTER-TNT 2.0 refinements against original published data increase the real-space correlation coefficients showing that the full information content of the original data was only realized through refinement with BUSTER-TNT 2.0, giving further weight to the correctness of the conclusions which had been drawn in the initial paper by consideration of lower-quality maps produced by CNS.

In conclusion, refinements with BUSTER-TNT 2.0 resulted in a significant improvement in the map quality and provide further support for the presence of the intermediate in the crystal.

Coordinates and structure factors have been deposited in the RCSB Protein Data Bank as entry 2QE0.

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BI701283C

10.1021/bi701283c

Published on Web 08/11/2007