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An alternate description of two crystal structures of phospholipase A_2 from *Bungarus caeruleus*

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Reinterpretations of the space-group symmetry are reported for two crystal structures of phospholipase A_2 isoforms (PDB codes 1u4j and 1g2x). The two structures reported in space groups *R*3 and *C*2 are isomorphous with a third isoform with space group *R*32 (PDB code 1fe5). The original structure reports were interpreted in terms of different oligomeric forms of the isoforms, but these conclusions are not supported by the isomorphous structures.

Four crystal structures of phospholipase A_2 (PLA_2) isoforms from the common krait have been reported and deposited in the Protein Data Bank [PDB codes 1fe5 (Singh *et al.*, 2001), 1u4j (Singh, Gourinath, Sarvanan, Sharma, Bhanumathi, Betzel, Yadava *et al.*, 2005), 1g2x (Singh, Gourinath, Saravanan, Sharma, Bhanumathi, Betzel, Srinivasan *et al.*, 2005) and 1tc8 (Singh, Jasti *et al.*, 2005)]. A single PLA_2 molecule is found in the asymmetric unit in 1fe5 (see Table 1), two molecules are found in the asymmetric unit of 1u4j and three are found in 1g2x. Conclusions about the oligomeric status of PLA_2 in these crystal forms were based on the number of crystallographically independent molecules in each.

We re-examined these structures after surveying *WHAT_CHECK* results (Hooft *et al.*, 1996) for PDB entries with more than one molecule in the asymmetric unit. The three PLA_2 crystal forms are isomorphous and can all be described in space group *R*32 with one molecule in the asymmetric unit. The packing of the protein molecules is the same in each crystal structure and the unit cells of 1u4j and 1g2x can be transformed to that of 1fe5. The *R*3 unit cell for 1u4j is the same as the *R*32 cell, but is described in terms of a hexagonal lattice. The noncrystallographic twofold rotation axes relating the two molecules in 1u4j apply to the entire crystal structure and become crystallographic twofold axes when the structure is analyzed in space group *R*32.

A similar situation holds for the monoclinic 1g2x structure, but in this case the noncrystallographic threefold rotation axis relating the three molecules in the monoclinic asymmetric unit becomes a crystallographic axis when the structure and unit cell are transformed to *R*32. The crystallographic twofold rotation and screw axes in the monoclinic cell become one of the sets of crystallographic twofolds in *R*32, the others being generated by application of the threefold rotation operations.

No structure factors are available for the 1u4j structure, so further analysis of it is not possible. However, structure factors for the monoclinic structure (1g2x) are available from the PDB. The 1g2x data set can be expanded using the monoclinic symmetry operations to a complete sphere of data. Examination of this weighted reciprocal lattice confirms that it possesses the symmetry appropriate for space group *R*32. The deposited diffraction data were reindexed in terms of a rhombohedral unit cell (a = 57.10 Å, α = 89.75°) and averaging of the replicated measurements reduces the number of reflections from 12 745 to 4549. $R_{\rm merge}$ for the replicates is 0.049.

After transformation into the *R*32 unit cell, the *A* chain of the 1g2x structure was chosen as the contents of the asymmetric unit. No averaging of the three 1g2x chains was carried out. The structural model was refined using *REFMAC* v.5 (Murshudov *et al.*, 1997) from

Table 1
Summary of PDB entries for krait PLA_2.

PDB code	1fe5†	1u4j‡	1g2x§	1tc8¶
Space group	*R*32	*R*3	*C*2	$P4_12_12$
Unit-cell parameters				
a (Å)	57.98	80.36	80.949	53.79
b (Å)	57.98	80.36	80.572	53.79
c (Å)	57.98	99.44	57.098	82.50
α (°)	92.02	90.0	90.0	90.0
β (°)	92.02	90.0	90.35	90.0
γ (°)	92.02	120.0	90.0	90.0
No. of molecules per ASU	1	2	3	1

† Singh *et al.* (2001). ‡ Singh, Gourinath, Sarvanan, Sharma, Bhanumathi, Betzel, Yadava *et al.* (2005). § Singh, Gourinath, Saravanan, Sharma, Bhanumathi, Betzel, Srinivasan *et al.* (2005). ¶ Singh, Jasti *et al.* (2005).

Table 2
Refinement statistics for krait phospholipase (PDB code 2osn).

Values in parentheses are for the highest resolution shell.

Resolution range (Å)	30–2.5 (2.56–2.50)
R factor (working set)	0.213 (0.204)
R_{free} (test set, 4.6% of reflections)	0.288 (0.405)
No. of unique reflections in working set	4238 (290)
No. of unique reflections in test set	203 (15)
No. of protein atoms	897
No. of chloride ions	3
No. of water molecules	32
Wilson *B* value (Å^2)	33.3
Average *B* value (Å^2)	
Protein	19.1
Chloride ions	24.6
Water molecules	15.3
Ramachandran plot	
Most favored regions (%)	90.4
Additional allowed regions (%)	9.6
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.12

the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). R_{free} (Brünger, 1993) was calculated using 5% of the reflections. Water molecules and chloride ions were added to the model after examination of σ_A-weighted $|F_o| - |F_c|$ and $2|F_o| - |F_c|$ electron-density maps (Read, 1986) with *XtalView* (McRee, 1999). The thermal parameters for several water molecules refined to unreasonably low values and if they were largely acceptors of hydrogen bonds, they were reinterpreted as chloride ions. Several water molecules continue to have unreasonably low temperature factors, but no chemical evidence suggests larger atoms or ions present in the crystals and consistent with the binding environments. The final *R* and R_{free} values are 0.221 and 0.270, respectively. Additional details concerning the refinement are included in Table 2. Coordinates and structure factors for this reinterpretation of entry 1g2x have been deposited in the PDB and assigned identification code 2osn.

The major structural difference from 1g2x arising from this reinterpretation of the space-group symmetry is found in the non-protein portion of the structural model. The original monoclinic structural model included 258 water molecules. The rhombohedral model contains 35 water molecules and chloride ions. This could be a consequence of the use of slightly different contour levels to identify potential water molecules (3σ as opposed to 2.5σ in the original study). It could also be a consequence of the imposition of crystallographic threefold symmetry on the structure and a reduction in noise peaks in the electron-density maps.

The biological significance of this structure reinterpretation involves analysis of the oligomeric status of the PLA_2 isoforms in the rhombohedral and monoclinic unit cells. Based on the contents of the asymmetric units in PDB entries 1fe5, 1u4j and 1g2x, it was concluded that in solution the isoforms exist as monomers, dimers and trimers, respectively. Dynamic light-scattering experiments support these conclusions for 1u4j (Singh, Gourinath, Sarvanan, Sharma, Bhanumathi, Betzel, Yadava *et al.*, 2005) and 1g2x (Singh, Gourinath, Saravanan, Sharma, Bhanumathi, Betzel, Srinivasan *et al.*, 2005). However, the fundamental oligomeric unit in the crystals appears to be the trimer first described for the 1g2x structure (Singh, Gourinath, Saravanan, Sharma, Bhanumathi, Betzel, Srinivasan *et al.*, 2005). Because the three crystal structures are isomorphous, this trimer either exists in solution or is formed during crystallization. The crystal structures provide no evidence for the existence of different oligomers in solution.

Three of the four krait PLA_2 isoforms crystallized to date form the same trimer. The isoforms differ in sequence at 35 residues (out of a total of 120). Of these, only one (residue 54) lies at the subunit–subunit interface in the trimer. The side chain of this residue is close to the carbonyl O atom of residue 79 in a neighboring subunit. In the 1u4j and 1g2x molecules, residue 54 (Lys in 1u4j and Gln in 1g2x) donates a hydrogen in a hydrogen bond to the carbonyl. (In 1fe5, residue 54 is a glutamic acid and the close approach of the carboxylate to the carbonyl is not easily understood.)

The trimer is not seen in the 1tc8 structure. Crystals of all four isoforms are obtained from nearly identical conditions. Protein in 50 m*M* Tris–HCl pH 8.5, 1.4 *M* NaCl, 1 m*M* NaN_3 was equilibrated against the same buffer containing NaCl. The protein and final NaCl concentrations varied slightly for the isoforms (1fe5, 10 mg ml^{-1} protein, 3.5 *M* NaCl; 1u4j, 7 mg ml^{-1} protein, 2.8 *M* NaCl; 1g2x, 5 mg ml^{-1} protein, 2.8 *M* NaCl; 1tc8, 20 mg ml^{-1} protein, 2.4 *M* NaCl). These similar crystallization conditions suggest that the different crystal form for 1tc8 might be a consequence of the specific amino-acid sequence for that isoform. However, superposition of the 1tc8 monomer onto the subunits in the 1g2x trimer shows that none of the amino-acid sequence differences between the isoforms can account for the relative instability of the 1tc8 trimer. Presumably then, there are crystal-packing interactions between the 1tc8 monomers that favor that packing of monomers in that crystal form over the formation of trimers. This is supporting evidence that the krait PLA_2 trimer seen for the three isoforms crystallizing in the rhombohedral crystals is marginally more stable than the oligomers in solution.

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