Ab initio structure determination of the triple mutant (K53,56,121M) of bovine pancreatic phospholipase A_2 at atomic and high resolution using ACORN

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Atomic resolution (0.97 Å) data were collected for the triple mutant (K53,56,121M) of bovine pancreatic phospholipase A_2 at 100 K and data extending to 1.0 Å resolution were used for the present study. Accuracy of the data at high resolution allowed the structure to be solved using the program ACORN, with a random single-atom start in an *ab initio* manner. The phases obtained from ACORN are of good quality and revealed most of the amino acid residues. Single wavelength Anomalous Diffraction (SAD) data were also used to locate the position of the sulphurs and ACORN was run with these atomic positions as a source of phasing information. The effect of truncating the data to 1.4 and 1.45 Å for input to ACORN is also examined. Larger fragments are required to trigger successful phase refinement at these lower resolutions.

Keywords: *Ab initio* phasing, ACORN, bovine pancreatic phospholipase A_2, triple mutant.

USING conventional direct methods, small molecules containing less than 200 atoms, are routinely solvable through the estimation of phases consistent with relationships between the experimentally measured intensities. Conventional direct methods fail to solve macromolecules even at atomic resolution due to the weak restraints of probabilistic relations. SHELXD[1] and SnB[2,3], the direct method programs based on the dual-space refinement principle, consisting of the modification of phases in reciprocal space, combined with the discrimination of model atoms in direct space have proven to be successful in solving structures of macromolecules diffracting to atomic resolution. These programs have also been used to find the position of anomalous scatterers in Multi- or Single-wavelength Anomalous Diffraction (MAD or SAD) experiments[1,4]. More conventional direct methods are also successful, especially for metalloproteins[5,6].

ACORN is a program designed to solve protein structures *ab initio* at atomic resolution (AR)[7–9] using limited initial phasing information. Several test cases for the use of ACORN ranging from small proteins to larger proteins of molecular weight 34 kDa have been discussed[7,10–13]. This article examines the success of ACORN when the data are at atomic resolution and when resolution is around 1.4–1.45 Å. Detailed procedures for arriving at the final model under these situations using various types of seeding information to ACORN are also described. Since the present investigation is meant as a pure methodology paper on the application of ACORN to generate the initial phases, structural changes and their functional implications are not discussed. However, a detailed article on the atomic resolution (0.97 Å) refinement of the triple mutant has been published earlier[14].

Materials and methods

The protocols adopted for the construction and characterization of the present phospholipase A_2 (PLA2) mutant are described in the literature[15,16]. Crystallization and data collection details are described elsewhere[14]. Diffraction data were collected on beamline X9B (National Synchrotron Light Source, Brookhaven National Laboratory, NY, USA) using an ADSC Quantum 4 CCD detector. Data were processed and scaled using the HKL2000 suite of programs[17]. The relevant crystal data are given in Table 1. The CPU times quoted here correspond to 866 MHz, Intel Pentium III processor with 256 MB RAM (RedHat Linux 7.3 Operating System).

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Table 1. Crystal data and model details

Crystal data	Atomic resolution	SAS data
Intensity statistics		
Synchrotron source	BNL–NSLS	BNL–NSLS
Temperature (K)	100	100
Space group	P2	P2
Wavelength (Å)	0.97	1.35
Crystal size	0.40 × 0.30 × 0.30 mm	0.40 × 0.30 × 0.30 mm
Unit-cell parameters (Å, °)		
A	36.93	37.12
B	23.86	23.92
C	65.93	66.12
a	90	90
b	101.5	101.2
g	90	90
Resolution range (Å)	30.0–0.97 (1.00–0.97)	30.0–1.35 (1.40–1.35)
Number of observations	627,336 (44605)	210,652 (14723)
Number of unique reflections	67,307 (6666)	47,122 (4255)
Completeness (%)	100 (100)	96.1 (87.0)
R_{merge}	0.051 (0.486)	0.034 (0.099)
$I/s(I)$	39.3 (3.5)	36.3 (12.3)
Redundancy	9.3 (6.7)	4.5 (3.5)
Wilson *B*-factor (Å^2)	12.5	11.2
Model details		
Protein atoms	954	954
Bound calcium atoms	2	2
Bound chloride ion	1	1
MPD atoms	8	8

Description of the method

ACORN uses strong reflections with $E > 1.2$ in the phase refinement using the Dynamic Density Modification (DDM) and Patterson superposition procedures. Both strong and weak reflections ($E < 0.1$) are used in the multisolution Sayre-Equation Refinement (SER). Intermediate reflections ($0.1 < E < 1.2$) are used to calculate the CC between the magnitudes of the observed (E_o) and calculated (E_c) *E* values after each cycle of DDM. Here, CC values for the medium reflections strongly indicate a correct solution without ambiguity. The first part of ACORN, namely ACORN-MR, deals with finding the position of a fragment of the structure even when a single atom provides an initial set of estimated phases. This set is passed into ACORN-PHASE, where phase refinement by a number of real-phase processes is performed.

ACORN does little phase refinement in reciprocal space and works in real space by modifying the density according to empirical rules, rather than by peak picking and adding more atomic positions to the position list. The density is generated from the current phases and strong *E* values. Inspecting the CC between the medium observed *E* values and the calculated *E* values derived from the modified map, success can be monitored. If this increases rapidly during recycling, the phase set will be substantially correct.

For locating a single atom, this approach randomly generates thousands of positions in the asymmetric unit. For each random position, the normalized structure factor values and corresponding CCs are calculated for all reflections. Thousand sets with highest CCs are saved as starting points for further calculations. In most cases, the solution is normally found in the top 100 sets. This approach can be used to determine a native protein structure from the atomic resolution data, if the structure contains at least one heavy atom (sulphur or heavier).

Ab initio phasing using ACORN

Normalized structure factors (*E*) for the atomic resolution data of the triple mutant were calculated using the program ECALC, available in the CCP4 package[18]. ACORN was run with 5000 random single atom trials and the solution (nonorigin peak) with highest CC was selected. ACORN generated and refined the phases using DDM and converged to a solution after 60 cycles. Figure 1 shows a section of the superposition of the final structure on the electron density map obtained from the output phases of ACORN. This clearly shows the good quality of the output phases using ACORN, generated using a single calcium atom. Further details are listed in Table 2. Similar results were obtained starting from heavy atom sites positioned using the anomalous diffractions of SAS data.

Table 2. Details of ACORN, ARP/wARP and REFMAC results with calcium atom as seed input

	Ab initio		
Input resolution	1.0 Å	Atomic resolution data	
Program	Resolution limit	20–1.0	
ACORN–I	Total no. of reflections	60,600	
(Random atom trial)	Strong reflections with $E > 1.2$	13,940	
	Medium reflections with $0.1 < E < 1.2$	46,150	
	Weak reflections with $0.0 < E < 0.1$	510	
	Initial	R = 59.2 (%)	CC = 0.0108
	Final	R = 36.2 (%)	CC = 0.5343
	5000 random atom trials		
	No. of DDM cycles	50	676 atoms
ACORN–II			
(Calcium ion as starting atom)	Total no. of reflections	60,600	
	Strong reflections with $E > 1.2$	13,940	
	Medium reflections with $0.1 < E < 1.2$	46,150	
	Weak reflections with $0.0 < E < 0.1$	510	
	Initial	R = 52.7 (%)	CC = 0.0155
	Final	R = 41.7 (%)	CC = 0.4147
	Phase shift cut-off	0.2	
	No. of DDM cycles	60	676 atoms
ARP/wARP	Initial (%)	Rw = 21.0	Rf = 23.1
	No. of auto-building cycles	6	
	No. of REFMAC cycles in each auto-building cycle	5	
	Final (%)	Rw = 17.3	Rf = 21.0
	Connectivity index	0.98	
	No. of chains	1	
	No. of residues built	119	
	No. of dummy atoms	277	
The rms* deviation of C^a atoms		0.14 Å	

*The rms deviation of C^a atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

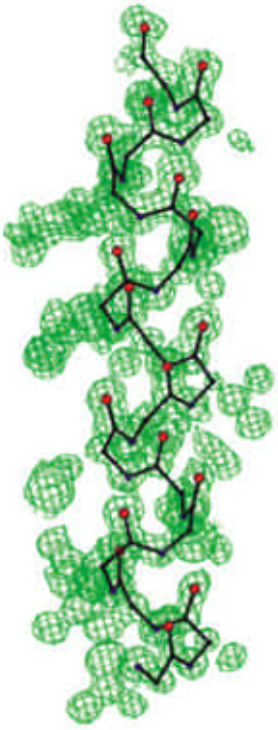

Figure 1. Section of the map calculated using the phases of ACORN (*ab initio* phasing with a single calcium atom as seed) along with the final model. Map is contoured at 1.0 s level.

Out of 18 heavy-atom positions given by SnB using SAS data at 1.35 Å resolution, peaks 3 to 7 (the first and second peaks correspond to calcium ions) are given as input to ACORN as 'seed phasing' and the generated initial phases are refined using DDM followed by SER. After 229 cycles of DDM, the CC increased from 0.0162 to 0.4227. The quality of the resultant map from the output phases of ACORN is good (Figure 2) and has been examined further by calculating the correlation between the ACORN map and the map corresponding to the final model. The resultant map correlation is about 69% and the relevant details are given in Table 3.

Structural fragments for seed phasing

PDB-id: 1UNE[19] corresponding to the recombinant PLA2 was subjected to molecular replacement by AMoRe[20] with the present AR data and the single and double helices from this model were used for seed phasing to ACORN.

Single helix as seed input: A single alpha helix with 18 residues (residues 40–57 as shown in Figure 3) has been fed as seed input to ACORN-MR with the 1.4 Å truncated data (from SAS dataset). Initially, the rotation and translation functions are calculated for the above set and ranked

Table 3. Details of ACORN, ARP/wARP and REFMAC results with five sulphurs as seed input

	Five sulphur peaks obtained from 1.35 Å SAS data using SnB		
Input resolution	1.0 Å	Atomic resolution data	
Program	Resolution limit	20–1.0	
ACORN	Total no. of reflections	61,483	
	Strong reflections with $E > 1.2$	14,146	
	Medium reflections with $0.1 < E < 1.2$	46,826	
	Weak reflections with $0.0 < E < 0.1$	511	
	Initial	R = 53.9 (%)	CC = 0.0162
	Final	R = 41.4 (%)	CC = 0.4227
	Phase Shift Cutoff [°]	0.2	
	No. of DDM cycles	229	673 atoms
ARP/wARP	Initial (%)	Rw = 38.5	Rf = 39.5
	No. of auto-building cycles	10	
	No. of REFMAC cycles in each auto-building cycle	10	
	Final (%)	Rw = 19.1	Rf = 23.6
	Connectivity index	0.98	
	No. of chains	1	
	No. of residues built	121	
	No. of dummy atoms	317	
The rms* deviation of C^a atoms		0.16 Å	

*The rms, deviation of C^a atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

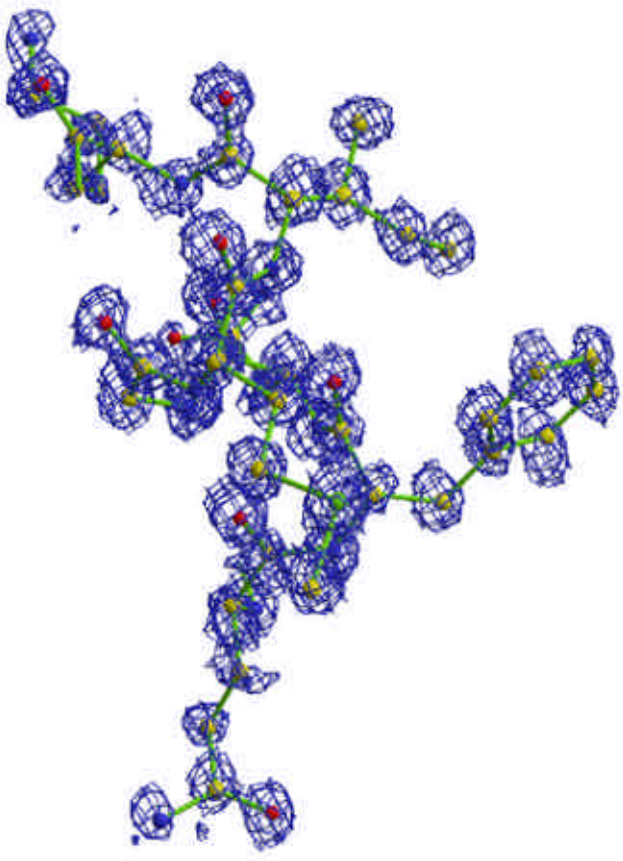

Figure 2. Five sulphur atoms (from 1.35 Å SAS data) as input to ACORN – 1.0 Å resolution AR data. A section of the ACORN map (1.0σ level) superimposed with the residues 4–10 of the final model.

with the CC. The top solution is fed into ACORN. After 44 cycles of DDM, the CC increased from 0.0506 to 0.1648. The resultant map from the output phases of ACORN is shown in Figure 4 *a*. Further, the quality of the ACORN phases has been examined by calculating the CC between the ACORN map and the final map using the program OVERLAPMAP[18]. The correlation for the ACORN map is about 66% with the final map and relevant details are presented in Table 4.

Data truncated to 1.4 Å from the atomic resolution are used and the above procedures are followed systematically. The single helix is given as input to ACORN and after 56 cycles of DDM, the CC increased from 0.0484 to 0.1699. The electron density map generated from the output phases of ACORN is shown in Figure 4 *b* and the values are mentioned in Table 4. The correlation between ACORN map and the map corresponding to the final model is about 67%.

With the truncated data of 1.45 Å resolution obtained from the atomic resolution data, ACORN has led to CC < 0.04 after DDM, which indicated that the input phasing power is not sufficient at this resolution. This prompted us to use two helices as input rather than a single helix at this resolution.

Two helices as seed input: The input sub-structure containing 37 residues (residues 40–57 and 90–108 as shown in Figure 3) in two long alpha helices from the PDB-id: 1UNE[19] has been fed into ACORN-MR with the 1.45 Å SAS truncated data. The top solution ranked by

Table 4. Details of ACORN, ARP/wARP and REFMAC results with single helix as seed input

Input resolution	Single helix (18 residues)				
	1.4 Å	Atomic resolution data (truncated)		SAS data (truncated)	
Program	Resolution limit	20–1.4		20–1.4	
ACORN	Total no. of reflections	22,677		22,342	
	Strong reflections with $E > 1.2$	5248		5203	
	Medium reflections with $0.1 < E < 1.2$	17,028		16,828	
	Weak reflections with $0.0 < E < 0.1$	401		311	
	Initial	R = 53.7 (%)	CC = 0.0484	R = 53.5 (%)	CC = 0.0506
	Final	R = 51.2 (%)	CC = 0.1699	R = 51.5 (%)	CC = 0.1648
	Phase shift cut-off [°]	0.5		0.5	
	No. of DDM cycles	56	275 atoms	44	257 atoms
ARP/wARP	Initial (%)	Rw = 42.1	Rf = 43.1	Rw = 42.0	Rf = 41.8
	No. of auto-building cycles	10		10	
	No. of REFMAC cycles in each auto-building cycle	5		5	
	Final (%)	Rw = 17.7	Rf = 22.7	Rw = 18.0	Rf = 22.7
	Connectivity index	0.98		0.98	
	No. of chains	1		1	
	No. of residues built	121		121	
	No. of dummy atoms	336		314	
The rms deviation of the search model to the final model (Å)		0.36		0.34	
The rms* deviation of C^{α} atoms (Å)		0.12		0.12	

*The rms deviation of C^{a} atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

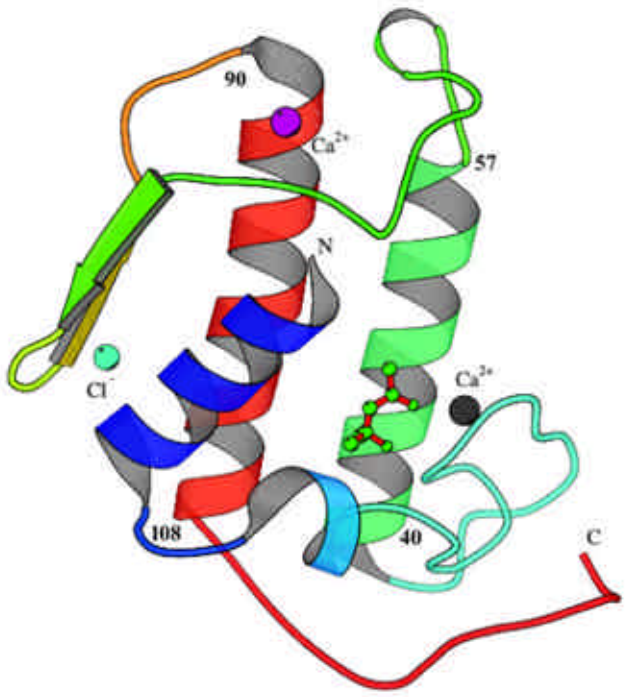

Figure 3. Cartoon of the final model showing primary, secondary calcium ions and MPD molecule. The structure is solved using ACORN with a single calcium atom as start.

the CC values after rotation and translation functions is fed into ACORN-PHASE. The CC increased from 0.1166 to 0.1553 for reflections with medium E values after 33 cycles of DDM. The quality of the ACORN phases is examined further by calculating the correlation (65%) between the ACORN map and the map of the final model. The electron density map (generated from the ACORN phases) of the final model is shown in the Figure 5 *a*. The corresponding values are listed in Table 5.

For the data truncated from the atomic resolution to 1.45 Å, the above procedures are repeated. Two helices are fed to ACORN and after 25 cycles of DDM, CC increased from 0.1103 to 0.1623. The quality of the resultant map from the output phases of ACORN is good (Figure 5 *b*) and the necessary values are given in Table 5.

N- and C-cap residues of the two helices as seed input: ACORN was run with the N-cap and C-cap residues (a total of 19 residues) of the two helices with the truncated data (from AR) at 1.4 Å resolution. After 61 cycles of DDM, CC increased to 0.1541, suggesting that a good model has been obtained. Further details are given in Table 6. A successful model was obtained after running ACORN with same input when truncated data at 1.4 Å resolution from SAS data were used. The rms deviation of C^{a} atoms in these two cases with that of the model corresponding to the isotropic temperature refinement is 0.1 Å.

Residues corresponding to the middle portion of the two helices as seed input: A total of 18 residues from the middle of the two helices were fed as seed input to ACORN with the truncated data (from AR at 1.4 Å resolution). After 30 cycles of DDM, CC increased to 0.1711 from an ini-

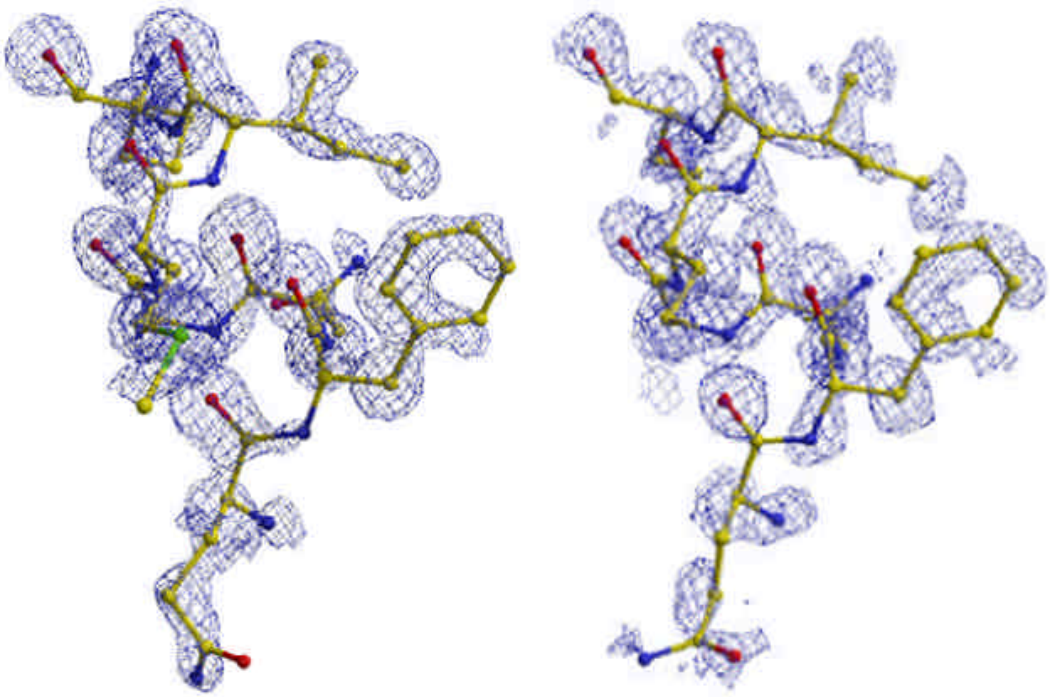

Figure 4. ***a***, ACORN map (contoured at 1.0*s* level) superimposed with the residues 4–10 of the final model and generated using a single helix (18 residues) as seed – 1.4 Å resolution SAS (truncated data). ***b***, Single helix input to ACORN – 1.4 Å resolution AR (truncated data). ACORN map superimposed with residues 4–10 from the final model and contoured at 1.0*s* level.

Table 5. Details of ACORN, ARP/wARP and REFMAC results with two helices as seed input

		Two helices (37 residues)			
Input resolution	1.45 Å	Atomic resolution data (truncated)		SAS data (truncated)	
Program	Resolution limit	20–1.45		20–1.45	
ACORN	Total no. of reflections	20,476		20,200	
	Strong reflections with $E > 1.2$	4738		4696	
	Medium reflections with $0.1 < E < 1.2$	15,367		15,208	
	Weak reflections with $0.0 < E < 0.1$	371		296	
	Initial	R = 52.9 (%)	CC = 0.1103	R = 53.1 (%)	CC = 0.1166
	Final	R = 51.4 (%)	CC = 0.1623	R = 52.4 (%)	CC = 0.1553
	Phase shift cutoff [°]	0.5		0.5	
	No. of DDM cycles	25	308 atoms	33	238 atoms
ARP/wARP	Initial (%)	Rw = 42.1	Rf = 43.9	Rw = 35.9	Rf = 41.3
	No. of auto-building cycles	10		10	
	No. of REFMAC cycles in each auto-building cycle	5		5	
	Final (%)	Rw = 18.0	Rf = 22.6	Rw = 17.4	Rf = 22.1
	Connectivity index	0.97		0.98	
	No. of chains	2		1	
	No. of residues built	119		121	
	No. of dummy atoms	344		301	
The rms deviation of the search model to the final model (Å)		0.35		0.34	
The rms* deviation of C^{α} atoms (Å)		0.13		0.16	

*The rms deviation of C^{a} atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

tial value of 0.0823. The use of the same residues as seed input to ACORN with the truncated data at 1.4 Å resolution from the SAS data also resulted in a successful model. Further details are given in Table 7.

Backbone atoms of the two helices as seed input: With the 148 backbone atoms of the 37 residues of the two helices, ACORN was run with the truncated data at 1.4 Å using AR data and SAS data respectively.

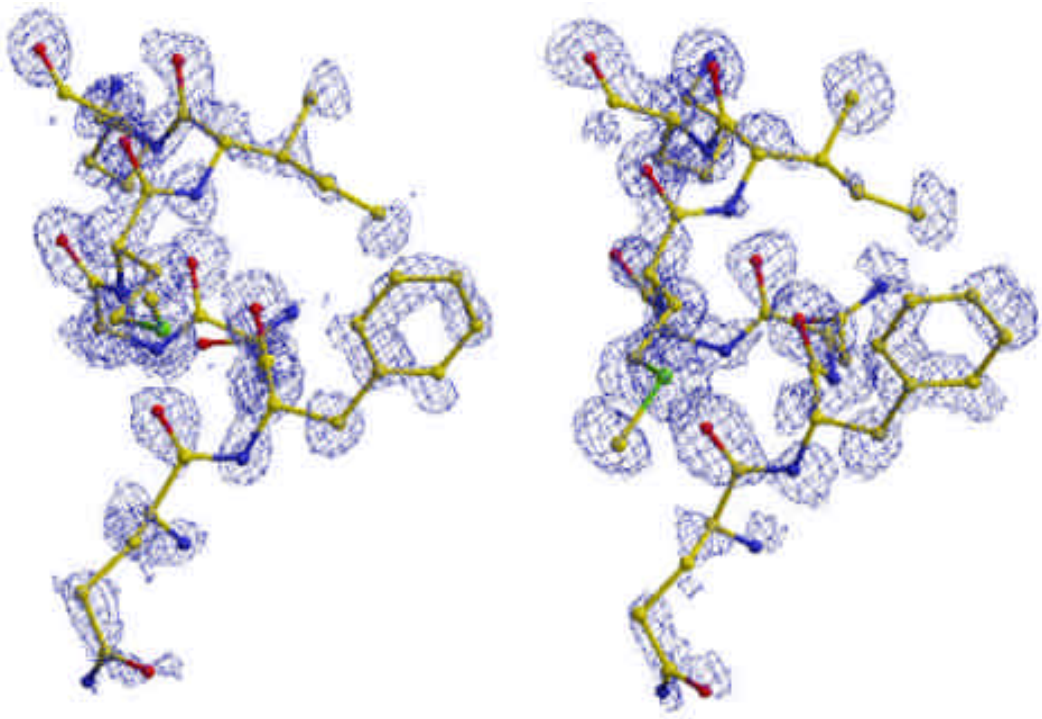

Figure 5. ***a***, Two helices as input (37 residues) to ACORN – 1.45 Å resolution SAS (truncated data) and ACORN map (contoured at 1.0 s level) superimposed with the residues 4–10 from the final model. ***b***, ACORN map (generated using two helices – 1.45 Å resolution AR (truncated data)) superimposed with residues 4–10 of the final model. Map is contoured at 1.0 s level.

Table 6. Details of ACORN, ARP/wARP and REFMAC results with N- and C-cap residues of the two helices as seed input, using SAS and AR data truncated at 1.4 Å

Input resolution	Nineteen residues				
	1.4 Å	Atomic resolution data (truncated)		SAS data (truncated)	
Program	Resolution limit	20–1.4		20–1.4	
ACORN	Total no. of reflections	22,677		22,342	
	Strong reflections with $E > 1.2$	5248		5203	
	Medium reflections with $0.1 < E < 1.2$	17,028		16,828	
	Weak reflections with $0.0 < E < 0.1$	401		311	
	Initial	R = 54.3 (%)	CC = 0.0432	R = 53.9 (%)	CC = 0.0515
	Final	R = 51.9 (%)	CC = 0.1541	R = 51.6 (%)	CC = 0.1605
	Phase shift cut-off [°]	0.5		0.5	
	No. of DDM cycles	61	271 atoms	44	253 atoms
ARP/wARP	Initial (%)	Rw = 42.8	Rf = 42.6	Rw = 41.9	Rf = 42.1
	No. of auto-building cycles	10		10	
	No. of REFMAC cycles in each auto-building cycle	10		10	
	Final (%)	Rw = 15.7	Rf = 19.9	Rw = 18.8	Rf = 24.0
	Connectivity index	0.98		0.97	
	No. of chains	1		2	
	No. of residues built	122		120	
	No. of dummy atoms	375		378	
The rms deviation of the search model to the final model (Å)		0.1		0.1	
The rms* deviation of C^a atoms (Å)		0.1		0.1	

*The rms deviation of C^a atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

Successful model building could be carried out with the phases after DDM. The C^a atoms of the final model have an rms deviation of 0.1 Å when superposed with those of the model corresponding to isotropic temperature refinement (AR data). Further details are given in Table 8.

Table 7. Details of ACORN, ARP/wARP and REFMAC results when the residues from the middle of the two helices were fed as seed input for the SAS and AR data truncated at 1.4 Å

Input resolution	Eighteen residues				
	1.4 Å	Atomic resolution data (truncated)		SAS data (truncated)	
Program	Resolution limit	20–1.4		20–1.4	
ACORN	Total no. of reflections	22,677		22,342	
	Strong reflections with $E > 1.2$	5248		5203	
	Medium reflections with $0.1 < E < 1.2$	17,028		16,828	
	Weak reflections with $0.0 < E < 0.1$	401		311	
	Initial	R = 53.3 (%)	CC = 0.0823	R = 53.5 (%)	CC = 0.0817
	Final	R = 50.8 (%)	CC = 0.1711	R = 51.6 (%)	CC = 0.1743
	Phase shift cuto-ff [°]	0.5		0.5	
	No. of DDM cycles	30	283 atoms	32	262 atoms
ARP/wARP	Initial (%)	Rw = 42.3	Rf = 41.9	Rw = 42.6	Rf = 41.2
	No. of auto-building cycles	10		10	
	No. of REFMAC cycles in each auto-building cycle	10		10	
	Final (%)	Rw = 17.5	Rf = 21.7	Rw = 18.7	Rf = 24.0
	Connectivity index	0.97		0.97	
	No. of chains	2		2	
	No. of residues built	117		120	
	No. of dummy atoms	396		365	
The rms deviation of the search model to the final model (Å)		0.1		0.1 Å	
The rms* deviation of C^a atoms (Å)		0.1		0.1	

*The rms deviation of C^{α} atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

Table 8. Details of ACORN, ARP/wARP and REFMAC results when backbone atoms of the two helices were fed as seed input for SAS and AR data truncated at 1.4 Å

Input resolution	Backbone atoms (148) of 37 residues				
	1.4 Å	Atomic resolution data (truncated)		SAS data (truncated)	
Program	Resolution limit	20–1.4		20–1.4	
ACORN	Total no. of reflections	22,677		22,341	
	Strong reflections with $E > 1.2$	5248		5203	
	Medium reflections with $0.1 < E < 1.2$	17,028		16,827	
	Weak reflections with $0.0 < E < 0.1$	401		311	
	Initial	R = 55.2 (%)	CC = 0.0683	R = 55.7 (%)	CC = 0.0728
	Final	R = 47.6 (%)	CC = 0.2449	R = 48.4 (%)	CC = 0.2260
	Phase shift cut-off [°]	0.5		0.5	
	No. of DDM cycles	31	284 atoms	30	250 atoms
ARP/wARP	Initial (%)	Rw = 42.3	Rf = 43.7	Rw = 41.8	Rf = 41.9
	No. of auto-building cycles	10		10	
	No. of REFMAC cycles in each auto-building cycle	10		10	
	Final (%)	Rw = 18.2	Rf = 21.2	Rw = 18.5	Rf = 22.1
	Connectivity index	0.98		0.98	
	No. of chains	1		1	
	No. of residues built	121		121	
	No. of dummy atoms	214		202	
The rms deviation of the search model to the final model (Å)		0.1		0.1	
The rms* deviation of C^a atoms (Å)		0.1		0.1	

*The rms deviation of C^a atoms when superimposed with the model corresponding to the isotropic temperature refinement (AR data) model.

Automatic model building (ARP/wARP) and refinement (REFMAC)

Model building was carried out in an automatic fashion using ARP/wARP[21] combined with REFMAC[18,22,23]. The phases from ACORN after DDM were given as input to ARP/wARP with the mode 'warpNtrace'. Further details can be found from Tables 2–8. Finally the missing residues, Ca ions, Cl ion and MPD molecule were manually added as densities were clearly present in these re-

gions and refinement using REFMAC was carried out in each case.

Description of the structure

The final model obtained using ACORN by *ab initio* phasing consists of 123 amino acid residues, two calcium ions, one chloride ion, one MPD molecule and 277 water molecules. A Ramachandran plot calculated using PROCHECK[24] shows 93.3% of the residues in the most favoured regions and the remaining residues in the additionally allowed regions. The overall fold is similar to that of the structures reported earlier[14,25]. The model shows well-defined electron density for the surface loop residues 60 to 70. Superposition of the backbone atoms of the present model with the model refined[14] anisotropically at atomic resolution (0.97 Å, PDB-id : 1VL9) shows the rms deviation of 0.07 Å. As observed earlier[14,26], the present model also features a second calcium ion, which is coordinated by six (three protein atoms and three water molecules) ligand atoms. The protein ligand atoms are the side chain atom O*d*1 of Asn 71, the backbone carbonyl oxygen of Asn 72 and one of the carboxylate atoms (O*e*2) of Glu 92. The corresponding coordinating distances are 2.28, 2.40 and 2.32 Å respectively. The three water ligand distances are 2.33, 2.35 and 2.52 Å. As found in the earlier investigations[14,25], a MPD molecule is located near the active site mouth. Additionally, the model also features a chloride ion in a similar position as in the earlier structures[14,25]. The chloride ion makes close contacts with the nitrogen atom (NZ) of the residue Lys 12 (3.11 Å), the backbone nitrogen atom of Ile 82 (3.17 Å) and NH2 of Arg 100 (3.42 Å).

Conclusion

The present work clearly demonstrates the usefulness of the direct method program ACORN for *ab initio* phasing of a 14 kDa protein at 1.0 Å resolution and the successful working of ACORN to good solutions at higher resolutions also, provided one inputs enough reliable seed (heavy atoms or structural motifs) to generate reasonable initial phases. It has also been proved that these seed helices can also be fed in parts (N-cap and C-cap regions or middle portion). Further, success can be obtained if 15% of the structure (a total of 18–19 residues in the present case) is fed as input fragment (in case of non-atomic resolution data). Further work in this direction on larger molecules and also on seed inputs with varying mean positional errors is in progress.

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