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Use of Molecular Replacement to Determine the Phases of Crystal Structure of Taq DNA Polymerase

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Abstract: Taq DNA polymerase from *Thermus aquaticus* has been shown to be very useful in the polymerase chain reaction method, which is being used for amplifying DNA. Not only does Taq DNA polymerase have high commercial value commercial value for the polymerase chain reaction application, but it is also important in studying DNA replication, because it is apparently an homologue to *E. coli* DNA polymerase I, which has long been used for DNA replication study (Lawyer *et al.*, 1993). The crystal structure determination of Taq DNA polymerase was initiated. An X-ray diffraction pattern breaks down a crystal structure into discrete sine waves in a Fourier series. The original shape of a crystal object in terms of electron density may be represented as the sum of those sine waves with varying amplitudes and phases in three dimensions. The molecular replacement method was initially employed to provide phase information for the structure of Taq DNA polymerase. The rotation search using the program MERLOT resulted in a solution peak with 5.4 r.m.s. PC-refinement of the X-PLOR program verified the result and also optimized the orientation angles. Next, the translation search using the X-PLOR program resulted in a unique solution peak with 7.35 r.m.s. In addition, the translation search indicated $P3_121$ to be the true space group out of two possible ones. The phase information from the molecular replacement was useful in the MIR phasing experiment.

Key words: crystal structure, crystallography, molecular replacement, polymerase chain reaction, Taq DNA polymerase.

Taq DNA polymerase from *Thermus aquaticus* has been shown to be very useful in the polymerase chain reaction (PCR) method, which is being used for amplifying DNA. It shows an optimum reaction temperature at 75℃ and keeps up activity for about one hour at 94℃. The high optimum polymerization temperature provides unique advantages when comparing Taq DNA polymerase to mesophilic DNA polymerase such as *E. coli* DNA polymerase I. The PCR method takes advantage of the high specific binding of a primer on a DNA template at elevated temperature, resulting in a high yield of desired product with less non-specific amplification products. In addition, the intermediate duplex DNA of the PCR reaction is denatured during each cycle at 93~95℃ for the next primer annealing step. It should be noted that DNA polymerase for the reaction is required to be stable at high temperature range of 93~95℃ (Saiki *et al.*, 1985; Saiki *et al.*, 1988; Holland *et al.*, 1991). Mesophilic DNA polymerase such as *E. coli* DNA polymerase I is inactivated at 73~95℃, whereas thermostable Taq DNA polymerase is stable at the high temperature range. One can add Taq DNA polymerase only once at the beginning of the PCR reaction for many cycles due to its thermostability. Not only is Taq DNA polymerase highly valuable in commercial PCR application, but it is also important in studying DNA replication. Taq DNA polymerase is apparently homologous to *E. coli* DNA polymerase I, which has long been used for DNA replication study (Kornberg and Baker, 1992; Lawyer *et al.*, 1993; Joyce and Steitz, 1994). Like *E. coli* DNA polymerase I, Taq DNA polymerase has a domain at its amino terminus (residues 1 to 290) that has 5'-3'nuclease activity and a domain at its C-terminus that catalyzes the polymerase reaction. Unlike *E. coli* DNA polymerase I, the intervening domain of Taq DNA polymerase has lost editing activity of 3'-5'exonuclease. While the structure of the Klenow fragment from *E. coli* DNA polymerase I, which includes polymerase and 3'-5'exonu-

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clease domains, has been known for 10 years, that of intact *E. coli* DNA polymerase I has proven more elusive because the crystallization of intact *E. coli* DNA polymerase I could not be accomplished (Ollis *et al.*, 1985; Derbyshire *et al.*, 1988; Beese *et al.*, 1993). The crystal structure of Taq DNA polymerase could be useful as a substitute for DNA replication study since thermostable enzymes generally tend to crystallize easier and diffract better than mesophilic enzymes (Delrarue *et al.*, 1990). The crystals of intact Taq DNA polymerase diffract to 2.4 Å resolution at −165℃ when synchrotron radiation is sufficient for the study.

A diffraction pattern breaks down the crystal structure into discrete sine waves in a Fourier series. The original shape of an object in the form of electron density may be represented as the sum of those sine waves with varying amplitudes and phases in three dimensions. The original object can be reconstructed by recombining those individual sine waves. However, when the X-ray diffraction pattern is recorded, the intensities of diffraction can be measured, but not their relative phases, so it is impossible to recombine them properly. This is the phase problem in X-ray crystallography. The phase problem can be overcome by several methods, such as multiple isomorphous replacement (MIR), molecular replacement (MR), multiple wavelength anomalous dispersion and so on. The MIR is generally preferred to other methods. The MR method is most preferred since, with some computations, it can provide the structure with phases quickly, compared to the greater tedium of other methods. If there is a known crystal structure which is very similar to a target structure, phases can be deduced from the known structure by MR, which generally consists of rotation and translation functions (Fitzgerald, 1988; Brunger, 1992). The MIR, another popular method, largely depends on a cumbersome process of obtaining protein crystals into which heavy atoms such as mercury and platinum have been soaked at certain concentrations. These heavy-atom-soaked crystals diffract differently from native crystals, and heavy-atom-soaked data were collected under conditions identical to those of native crystals. Native and heavy atom derivative data sets are compared by difference Patterson analysis to obtain proper phase information (Glusker and Trueblood, 1985; McRee, 1993).

Initially, the MR was employed to obtain phases for Taq DNA polymerase using the crystal structure of the polymerase domain of the Klenow fragment as a search model. It was suggested that the polymerase domain of the Klenow fragment was very homologous to that of Taq DNA polymerase. The correspondence between Taq DNA polymerase and the Klenow fragment in their polymerase domains is about 75% (Lawyer *et al.*, 1989). Therefore, the MR was a good initiation point to obtain phases for Taq DNA polymerase. The phase determination of the crystal structure of Taq DNA polymerase was eventually accomplished by combining both MIR and MR, and its structure determination is well described in the previous papers (Kim *et al.*, 1995; Kim, 1995), while this report will describe an attempt to determine phases to solve the crystal structure of Taq DNA polymerase only by the MR.

Materials and Methods

Purification of Taq DNA polymerase

Taq DNA polymerase was purified by the method of Engelke *et al.* (1990) with some modifications. *E. coli* MV1184 cells carrying the expression vector, pDS2 were grown at 37℃ in 2X LB medium containing 100 μg/ml ampicillin (Kwon *et al.*, 1991; Kim, 1995). When the absorbance of the culture at 600 nm reached 0.5, it was induced by the addition of isopropyl-1-thio-beta-galactopyranoside to a final concentration of 0.4 mM and allowed to grow for an additional 15 h at 37℃. Eighty grams of cells were harvested by centrifugation from 10 liter culture. The remaining procedure are well described by Kim (1995). The final fraction of the purified Taq DNA polymerase was concentrated to 10 mg/ml concentration and then crystallization was set up by the hanging drop method.

Crystallization and X-ray diffraction data collecton

Crystals of Taq DNA polymerase were grown at 22℃ in hanging drops containing 3 μl protein solution (10 mg/ml Taq DNA polymerase in 18 mM Tris-HCl, pH 8.2, 0.09 mM EDTA, 0.9 mM dithiothreitol, 90 mM KCl, 9% (v/v) glycerol and 0.7% (w/v) β-octyl glucoside) and 3 μl resorvoir solution (15% (w/v) polyethylene glycol 8000, 60 mM ammonium sulfate, 2 mM dithiothreitol, 0.2% (w/v) sodium azide and 100 mM sodum citrate, pH 5.5). It took 5~7 days for crystals to grow up to 0.1×0.1×2~3 mm, which is a usable size for data collection (Eom *et al.*, 1995). Crystals were then transferred to the first stabilizing solution (40 mM sodium citrate, pH 5.5, 10% (v/v) glycerol, 100 mM KCl, 0.4% (w/v) β-octyl glucoside and 31% (w/v) polyethylene glycol 8000) for 36 h. The crystals were then flash-frozen at −165℃ (Watenpaugh, 1991). Native data was collected for the frozen crystals using the RAXIS-IIc image plate detector system mounted on a Rigaku-200 rotating anode. Data was processed using DENZO (the program written by Z. Otwinowski) and SCALEPACK (Z. Otwinowski).

Molecular replacement

The MERLOT, an integrated program package for

MR (Fitzgerald, 1988; Stout and Jensen, 1989), was used to find a solution by means of rotation search. The reflections of native data between 10 to 4 Å resolution were used for the search. For the calculation of the rotation function, only the non-hydrogen atoms of the protein were included in a search model structure. Initially, the search model was positioned in a P1 unit cell with a=b=c=150 Å. The structure factors from this hypothetical unit cell were calculated to be between 10.0 and 4.0 Å resolution (Crowther, 1972). The rotation search was carried out using the CROSUM (a rotation function program of the MERLOT), which is Crowther's rotation function, with a Patterson vector radius of 40 Å. In Eulerian space, sampling reciprocal space ranged from $\alpha=0$ to 360°, $\beta=0$ to 120°, and $\gamma=0$ to 180° (α, β and γ are Euler angles) in steps of 5.0°, 1.67° and 1.0°, respectively. The Patterson correlation (PC) refinement was then carried out between 10.0 and 4.0 Å resolution for the top 56 peaks from the rotation search using the PC-refinement routine of the X-PLOR program package (Brunger, 1992). The PC-refinements of the top 56 peaks from the rotation search were carried out with the search model oriented according to the orientations of the 56 selected peaks in Euler angles. The peaks which showed a higher root mean square deviation (r.m.s) were selected and subjected to the translation function routine of the X-PLOR package (Brunger, 1992). Translation functions were calulated using data between 10.0 to 4.0 Å resolution with a 2 r.m.s. cutoff above the mean. The grid sizes of the translation searches are x=1/43.0, y=1/43.0 and z=1/66.0 at fractional coordinates of x, y and z. The grid ranges are 0 to 1 for x, 0 to 1 for y and 0 to 1 for z.

Results

Selection of a search model

E. coli DNA polymerase I consists of polymerase, 3′-5′exonuclease and 5′-3′exonuclease domains. Taq DNA polymerase also contains polymerase and 5′-3′ exonuclease domains but has only a smaller 3′-5′ exonuclease domain with no detectable activity compared to *E. coli* polymerase I. Taq DNA polymerase shows a considerable amino acid sequence similarity to *E. coli* DNA polymerase I. One possible sequence alignment yields 38% identity between Taq DNA polymerase and E. coli DNA polymerase I. Especially the polymerase domains, which correspond to the amino acid positions 520 to 928 in *E. coli* DNA polymerase I, and 424 to 832 in Taq DNA polymerase, show extraordinary similarity in amino acid sequences. The polymerase domain of Taq DNA polymerase shows 55% identity and 77% homology in amino acid sequences to *E. coli* DNA polymerase I (Lawyer *et al.*, 1989). Since such significant similarity of amino acid sequences are observed in those two polymerase domains, it was conceivable to attempt MR using the polymerase domain of *E. coli* DNA polymerase I as a search model. The structure of the intact *E. coli* DNA polymerase I is not known but that of the Klenow fragment, which consists of polymerase and 3′-5′exonuclease domains, was solved and refined to 2.5 Å resolution (Beese *et al.*, 1993). Therefore only the polymerase domain of the Klenow fragment was chosen as a search model for the MR, specially residues 520 to 928 of the Klenow fragment, which correspond to the polymerase domain. However, the residues 573 to 623 of the model sequence were deleted because they are disordered in the structure of the Klenow fragment. Instead of using the structure of the polymerase domain of the Klenow fragment directly, it was presumed that it would be beneficial to build the model structure of the polymerase domain of Taq DNA polymerase, based on the structure of the Klenow fragment. The model structure was built based on the PDB file coordinates of the Klenow fragment. Since the two polymerase domains show very significant homology, it was straight forward to align amino acids in the polymerase domain of *E. coli* polymerase I to corresponding ones of Taq DNA polymerase. All amino acid residues in the PDB file-coordinate of the Klenow fragment were substituted for those in Taq DNA polymerase so that the model of the polymerase domain of Taq DNA polymerase could be built based on the structure of the Klenow fragment. Consequently, the basic PDB file coordinates of polyalanine for the model of the polymerase domain of Taq DNA polymerase are from the structure of the Klenow fragment but those of the side-chains are registered with those of the Taq DNA polymerase sequence using the program O (Lawyer *et al.*, 1989; Jones and Kjeldgaard, 1994). Sequentially, the built model of the polymerase domain of Taq DNA polymerase was subject to the positional refinement routine of the X-PLOR package (Brunger, 1992) so that the substituted side-chains of amino acids in the model of Taq DNA polymerase were positioned at possible energy minimum states. The refined model of the polymerase domain of Taq DNA polymerase was then ready to use for MR. The final model contains 358 amino acids with all side-chains out of the total 832 residues of intact Taq DNA polymerase, which is equivalent to a scattering mass of 43%.

Native X-ray data collection

The rod crystals grown at 18% (w/v) polyethylene

Table 1. Collection of orientations from the cross rotation function program, CROSUM

Peak index	Rotataion function values in r.m.s.	α	β	γ
1	5.3	68.63	66.08	104.23
2	4.5	110.00	43.00	85.00
3	4.3	231.79	96.85	11.64
4	4.3	35.00	69.00	118.33
5	4.2	35.00	68.00	0.00
6	4.0	291.16	136.93	97.80

α, β, and γ are Euler angles in the Merlot.

Table 2. Collection of the 4 highest peak orientations as shown in Fig. 1 after the PC-refinement for the top 56 selected peaks of the CROSUM rotation search

Peak index	Rotation function values from the CROSUM in r.m.s.	θ_1	θ_2	θ_3
1	5.3	189.53	65.37	343.86
16	3.7	10.18	114.77	316.81
27	5.4	189.58	65.40	343.96
56	3.6	9.57	114.56	316.02

*Peaks No. 1 and 27, and 16 and 56 were at the same orientations respectively. The orientations of peak No. 1 and 16 were used for translation searches.

*θ_1, θ_2 and θ_3 are the coordinate angles defined in the X-PLOR and α, β, γ are defined at the MERLOT. The relationship between two conventions are $\theta_1=\alpha+120°$, $\theta_2=\beta$, and $\theta_3=\gamma+240°$.

*Symmetry operators for the $p3_121$ space groups:

(x, y, z)
(−y, x−y, 2/3+z)
(−x+y, −x, 1/3+z)
(y, x, −z)
(x−y, −y, 1/3−z)
(−x, −x+y, 2/3−z)

*The PC-refinement consists of 15 steps of rigid body energy minimization and additional 20 steps with the three separate subdomains; group=((residues 424~448) or (residues 560 to 832)), group=(residues 449~476), group=(528 to 559).

glycol 8000 in sodium citrate buffer (final pH 6.0) was used for a native data collection at cryogenic temperature. The crystals belong to space group $P3_121$ or $P3_221$ and have unit cell dimensions of a=b=108.0, c=171.2 Å, α=β=90° and γ=120°. Data was collected at an RAXIS-IIc image plate detector. The data collection procedure at −165℃ has been well described by Kim (1995). The native data used for the MR diffracted to 3.2 Å resolution. R_{merge} was 8.0% and the data was completed to 95% to 3.2 Å resolution bin. The crystal diffracted to 2.4 Å resolution using a synchrotron radiation source, and so this data set was used for the final structure determination (Kim *et al.*, 1995) but the

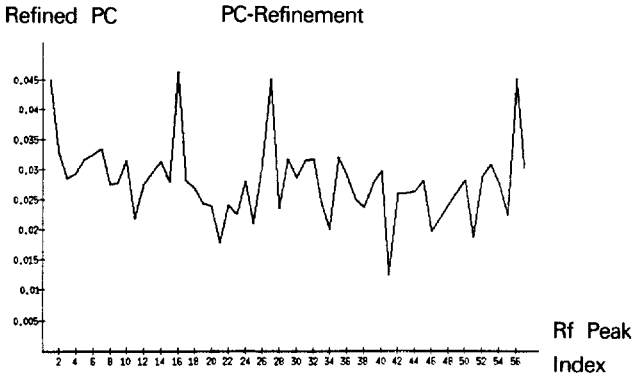

Fig. 1. The PC-refinement for the selected top 56 peaks from the CROSUM search. The PC-function values after the PC-refinement are shown. The PC-function are described by Brunger (1992).

3.2 Å data set collected at the RAXIS-IIc image plate detector was used for the MR.

Rotation search

The previously built model of the polymerase domain of Taq DNA polymerase based on the structure of the Klenow frament was used as a search model for rotational search. The program CROSUM resulted in presumably a solution peak with 5.4 r.m.s for the rotation search at α=68.63°, β=66.08° and γ=104.23°. The collection of an additional top six peaks with their Euler angles are listed in Table 1. Next, the PC-refinement of the X-PLOR program was carried out for the selected peaks from the CROSUM. The top 56 peaks, including the highest peak, were collected and subject to the PC-refinement. Since the convention of Euler angles in the MERLOT is different from that of the X-PLOR, those Euler angles were converted to the ones of the X-PLOR (Fitzgerald, 1988, Brunger, 1992; see Table 2). The PC-refinement was then performed between 10.0 and 4.0 Å resolution on the orientations produced by the cross rotation search of the CROSUM. This PC-refinement consists of 15 steps of rigid body energy minimization for the orientation of the molecule followed by the molecule being divided into three individual domains; namely, residues 424 to 448 and 560 to 832, residues 449 to 476, and residues 528 to 559. Sequentially, 20 steps of rigid body energy minimization for the three domains were carried out (Brunger, 1992). During the PC-refinement, the Patterson correlation values were calculated and ploted as shown in Fig. 1. Four outstanding peaks were selected as listed in Table 2 but the last two of them are identical to the first two, respectively. The first peak in Table 2 had the same orientation as the highest peak from the CROSUM search. The PC-refined orientation turned out to be $\theta_1=189.53°$, $\theta_2=65.37°$, and

Table 3. Collection of orientations from the translation function search

Peak index	Translation function value in r.m.s.	Fractional coordinates in $P3_121$ unit cell		
		x	y	z
1	7.35	0.250	0.600	0.985
2	3.53	0.233	0.600	0.985
3	3.46	0.250	0.600	1.000
4	3.11	0.250	0.533	0.985
5	2.67	0.250	0.533	1.000
6	2.54	0.250	0.600	0.917

$\theta_3=343.86°$ for the highest peak orientation of the CROSUM search where the CROSUM search showed the orientation at $\alpha=68.63°$, $\beta=66.08°$, and $\gamma=104.23°$, which can be converted into $\theta_1=188.63°$, $\theta_2=66.08°$, and $\theta_3=344.23°$ in the X-PLOR coordinate system. Apparently, the PC-refinement optimized the peak orientation from the CROSUM rotation search. It turned out that the PC-refinement of the peak orientation facilitated the next translation search.

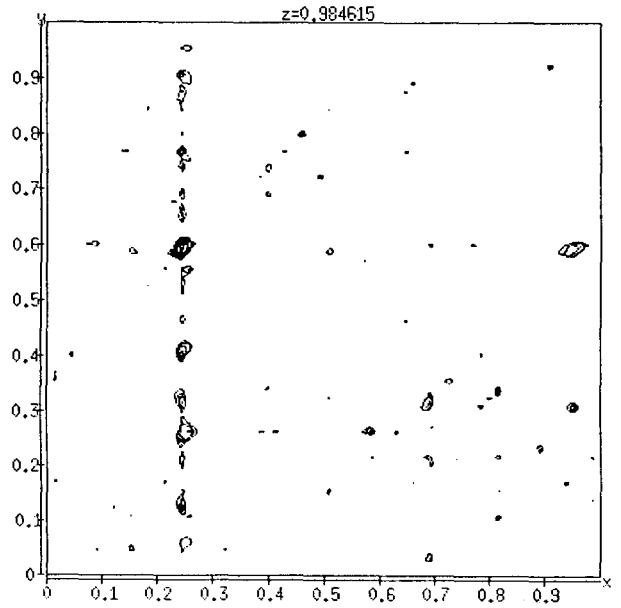

Fig. 2. Contour plot of the translation function at 10 ^{1} Å resolution (using the space group of $P3_121$). The highest peak is at 7.4 r.m.s above the mean and yield the fractional translation vector at x=0.250, y=0.600, and z=0.985.

Translational search

The orientations of peak No. 1 and No. 2 as shown in Table 2, where θ_1, θ_2 and θ_3 were subject to translational search using the translation function routine of the X-PLOR package (Brunger, 1992). At this stage, the translation searches were carried out for those two orientations, assuming the correct space group either P3121 or P3221. Therefore the total four translational searches were carried out for the two orientations with two possible space groups at 8~4 Å resolution. The four translation searches resulted in the solution for the translation search using the peak No. 1 orientation and assuming the space group as $P3_121$. Several peak heights, including the highest solution peak of the translation search, are listed in Table 3. The solution peak is located in the orientation of x=0.250, y=0.600, z=0.985 or 0.485 (either one in this space group) as shown in Fig. 2, showing a maximum peak of 7.35 r.m.s over the mean. The next highest peak showed 3.53 r.m.s, which is a a 3.82 r.m.s difference compared to the highest solution peak. This assured that this orientation and space group was correctly chosen. The other three translational searches resulted in all mediocre peaks under less than 5 r.m.s values, and all their peak heights were very closely located to the next ones. There were no big differences between the No. 1 and 2 peak heights, supporting that the No. 1 peak orientation and the $P3_121$ space group as the right solution for the translation search.

The model structure of the polymerase domain of Taq DNA polymerase was correctly positioned according to the orientataion from the rotation and translation searches. The model was then subject to the rigid body energy minimization of the X-PLOR program (Brunger, 1992). The crystallographic R-factor after the rigid body refinement was 0.485 for the data between 6 to 3.2 Å resolution.

Discussion

The highest peak value in the translation search results in an oustanding 7.35 r.m.s above the mean, and there was a 3.82 r.m.s gap to the second highest peak, which indicates that the translation search was correct.

Initially, the space group was supposed to be either $P3_121$ or $P3_221$ because the correct one could not be determined by diffraction symmetry. The translation searches assured that the correct space group should be $P3_121$.

The polymerase domain of Taq DNA polymerase was located by MR, and the Cα backbone structure is shown in a trigonal unit cell in Fig. 3. The search model was rotated according to the rotation angles from the rotation search and translated by the angles from the translational search using the X-PLOR program. The picture shows that only the polymerase domain is located in the trigonal unit cell, with a disordered loop region (residues 477 to 528) of the 'thumb' subdomain deleted. The phases were calculated based on the PDB coodinate of the search model after it was properly positioned according to the orientation of the MR solution. They were then applied on calculat-

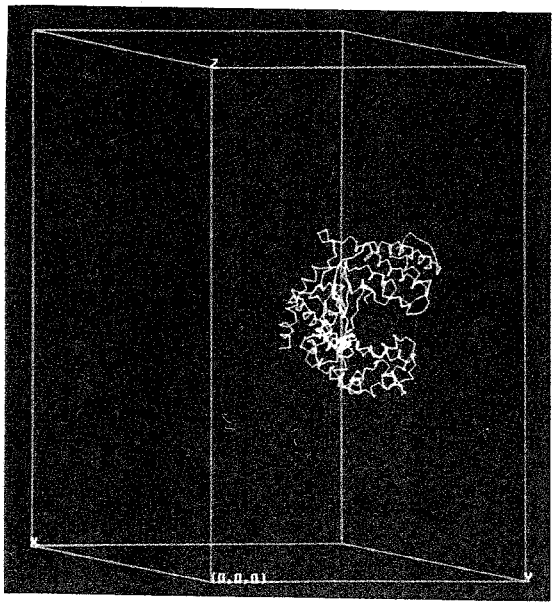

Fig. 3. The orientation of polymerase domain of Taq DNA polymerase according to the rotation and the translation searches. The polymerase domain is in Cα backbone. The structure of the whole Taq DNA polymerase was solved by the MR and MIR and superimposed on the model of polymerase domain from the MR. They were superimposed with 1 Å r.m.s in Cα backbone (Kim *et al.*, 1995). The molecule is located in a trigonal $P3_121$ unit cell. The z coordinate is 0.485 (or 0.985 in the space group).

ing the electron density map of the whole Taq DNA polymerase. The polymerase domain could be defined in the electron density map because there exists a corresponding domain in the search model, but there was not much useful phasing information in the other two domains of 3′-5′exonuclease and 5′-3′exonuclease. The phases of the whole Taq DNA polymerase were useful in defining the polymerase domain but it was not helpful to describe the shape of the other domains. A phase combination using the X-PLOR and the SIGMA programs (Read, 1986) was attempted to extend the phase information to the two domains but it did not improve the phases much. Since the scattering mass of the search model was equal to 43% of the whole Taq DNA polymerase, the search model could not be extended to provide reasonable phasing information for the whole Taq DNA polymerase. Therefore, the structure of the whole Taq DNA polymerase was determined by combining both MR and MIR. It is well described how the structure of the whole Taq DNA polymerase was determined by combining the MIR and MR methods (Kim *et al.*, 1995; Kim, 1995).

In summary, phasing infromation from MR was useful in the MIR phasing experiment. The calculated phases based on the search model was good enough to identify the heavy atom positions of the MIR phasing experiment. In practice, it was not easy to determine the positions of the heavy atoms in the $P3_121$ unit cell only from the difference Patterson analysis because the difference Patterson peak in the Harker sections was not outstanding in this high symmetry space group. So, the heavy atom positions for the MIR experiment were determined by difference Fourier analysis using the phases deduced by MR. In addition, since the structure of the polymerase domain of Taq DNA polymerase was determined by MR, it was a straightforward procedure to build the structure of the polymerase domain in determining the whole Taq DNA polymerase. The structure of the polymerase domain from the MR solution was well superimposed on that of the 2.4 Å refined whole Taq DNA polymerase in Cα backbone with 1 Å r.m.s deviation (Kim *et al.*, 1995).

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