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Crystal Structure of Bovine Antithrombin III

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Abstract

The crystal structure of bovine antithrombin III has been solved by molecular replacement, using α_1-antitrypsin (30% sequence homology with human antithrombin III) as a model. The protein crystallizes with two molecules in the asymmetric unit. The use of different resolution ranges was essential in determining the true orientations of the two molecules, because these were the orientations that appeared most consistently in the rotation function, albeit with different scores and slightly different values. Accuracy and correctness of the orientations of the two independent molecules was crucial for the success of the translation function. Stripping off surface atoms from the trial model resulted in a better signal-to-noise ratio in the Crowther–Blow translation function, which turned out to be very discriminative, even though the translational search was performed with less than 30% of the asymmetric unit. In this way, the orientation and position of each of the two independent molecules was separately determined; the crystal packing of the corresponding dimer showed no bad contacts. Phases calculated with this model allowed the determination of heavy-atom sites in a derivative which had previously resisted interpretation. The current R factor after energy minimization and a 1 ps molecular-dynamics simulation is 32% at 3·6 Å resolution.

Introduction

Antithrombin III (M_r = 56 600) is a plasma antiserine protease involved in the coagulation process. Its physiological role is thought to be the inhibition of thrombin and/or factor Xa; factor Xa, together with factor Va and Ca^{2+} ions, helps cleave prothrombin into thrombin, which then cleaves fibrinogen into fibrin (Furie & Furie, 1988). Fibrin then polymerizes in molecular nets which, together with activated platelets, repair blood vessel damage around a wound. The rate of inhibition by antithrombin III (ATIII) is modulated by a polysaccharide, heparin (Rosenberg & Damus, 1973). The binding site of heparin in ATIII has been mapped by chemical affinity labeling techniques (Blackburn, Smith & Sibley, 1983) and genetic studies (Chang & Tran, 1986), and is located in the N-terminal domain of the protein. Furthermore, a pentasaccharide that presents the same activity as heparin for the inactivation of factor Xa by antithrombin has been chemically synthesized (Choay, Petitou, Lormeau, Sinay, Casu & Gatti, 1983; Sinay *et al.*, 1984). However, specific contacts at the molecular level between heparin and ATIII are unknown and this prevents any rational design of chemically synthesized analogs of heparin. In order to make such drug-design experiments and molecular modeling possible, the three-dimensional structure of ATIII has to be known. At a later stage, the complex between the pentasaccharide and ATIII will also have to be solved.

In this article, we report the crystal structure determination of bovine ATIII. Our strategy was to pursue isomorphous replacement and molecular replacement methods separately. The latter method proved to be successful and helped interpret some results from the first. Because of some difficulties encountered in the application of the molecular replacement approach, we feel that a detailed description of our results is useful in further assessing the importance of the different parameters.

Our main conclusions are that (i) different resolution ranges have to be tried in the rotational search to identify the correct peaks; furthermore, even if a maximum seems clear, the actual angles of the rotation can, in some cases, be sufficiently inaccurate – even if a very fine grid is used – to make the translation search fail; (ii) a model containing only 30% of the asymmetric unit is enough to provide a good signal for the rotation and the translation functions; and (iii) 50% of the asymmetric unit is enough to phase a derivative. A possible way around the required accuracy and correctness of the orientation angles, especially when different conditions (*e.g.* resolution ranges) give slightly different positions for the maxima, is to run several translation searches, using orientations systematically varied around the maxima of the rotation function, with a step size of 3°.

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Experimental

Data collection and processing

The crystals were obtained by the microdialysis method, using ammonium sulfate as a precipitating agent. Gel electrophoresis and N-terminal sequencing of the crystals suggest that the asymmetric unit contains two molecules that are cleaved at the serine protease binding site (Samama, Delarue, Mourey, Choay & Moras, 1989).

The space group is $P4_32_12$, with cell constants 91·4, 91·4 and 383·1 Å. Data were collected by oscillation photography (Winkler, Schutt & Harrison, 1979) using synchrotron radiation at either LURE (Orsay, France) or CHESS (Cornell, Ithaca, NY, USA). The typical crystal size was 0·3 × 0·3 × 0·3 mm. The oscillation angle was 2° for each photograph and the usual crystal-to-film distance was 100 mm; the final native data set had about four observations for each reflection and the cusp region was also included. The crystals diffract up to a resolution limit of 3·0 Å.

A partial data set (68% complete at 3·5 Å) was also collected for crystals soaked for 3 days in 2m*M* $PtCl_4^{2-}$, which provided a heavy-atom derivative. This platinum derivative is only isomorphous with native crystals to 4·5 Å resolution. Another partial data set (only 40% complete at 6 Å resolution) obtained from a crystal soaked for 5 days in 16m*M* $PtCl_4^{2-}$ was also collected.

Films were scanned on a Joyce–Loebl optical rotating densitometer and processed with the program *OSC* (Rossmann, 1979). The *CCP*4 package was then used for scaling, merging and post-refinement. The final *R* factor on intensities for the native data set used to solve the structure was 8·6% for 23 500 independent reflections (19 000 reflections at 3·5 Å resolution, *i.e.* 90% complete).

Rotation function

The 3·0 Å refined structure of α_1-antitrypsin (α_1-AT; Loebermann, Tokuoka, Deisenhofer & Huber, 1984; Protein Data Bank reference 5API) was used as a model. The accessibility of every atom was calculated with the program *ACCESS* (Richmond & Richards, 1978) and atoms with an accessibility greater than 10 Å^2 were removed. This left about two thirds of the initial model (model 5API–TIDY–TOT in Table 1). In addition, residues 355 to 393 (α_1-AT is cleaved in the crystal at position 358; residue 393 is the C-terminal end of the protein) were then removed or retained for different tests (5API–CUT or 5API–TOT in Tables 1 and 2), because the junction between these two domains is likely to be different in native α_1-AT (Loebermann *et al.*, 1984).

The model was placed in a *P*1 cell with dimensions 150 × 150 × 150 Å. A smaller cell with an edge of 120 Å was also tried. Structure factors were calculated to 3·5 Å resolution using the atomic temperature factors of α_1-AT. The fast rotation function (Crowther, 1972) as implemented in the *CCP*4 package (Dodson, 1985) was employed; this version allows expansion with 60 Bessel coefficients. The step size was 2·5°; for normal (*F*) structure factors, an overall negative temperature factor of 20 Å^2 was applied to sharpen the peaks; the origin cutoff was 5 Å. As the molecular dimensions of the model were 40 × 40 × 60 Å, four different outer radii of integration were tried, from 30 to 45 Å.

Different resolution ranges were used for normal (*F*) or normalized (*E*) structure factors: 15–4, 10–6, 10–4, 10–3·5, 6–4 Å and 20–6, 20–5, 20–4, 20–3·5 Å, respectively.

Translation function

Possible orientations found by the rotation function were applied to the model. The center of mass of this model (one molecule) was placed at the origin of a *P*1 cell that had the same dimensions as that containing the unknown structure. Partial structure factors were than calculated for each symmetry-related molecule and then merged.

The Crowther–Blow translation function (Crowther & Blow, 1967) was used, as implemented by Tickle (1985) in the *CCP*4 package. This program computes the Fourier coefficients of a three-dimensional map (see Harada, Lifchitz, Berthou & Jolles, 1981), which is then sorted according to the value of the translation function. Normalized structure factors between 20 and 4 Å resolution or between 20 and 3·5 Å resolution were used. The program was run for each of the two possible enantiomeric space groups. Self vectors were omitted. The search was performed with every possible peak of the rotation function. In addition, at least nine slightly different orientations were tried around each of the possible solutions, using a step size of 3° for each Eulerian angle.

The *R*-search program of *CCP*4 was used to check the solutions for each molecule in a limited box of 21 × 21 × 21 points, with a grid of 0·5 Å and data between 10 and 7 Å. Similarly, the *BRUTE* program (Fujinaga & Read, 1987), which allows variation of both the rotational and translational parameters, was also used to check the different solutions in a limited six-dimensional search.

Initial rigid-body refinement

The two molecules of the asymmetric unit were put together and allowed to move independently as two different rigid bodies. The *CORELS* program

Table 1. *Rotation-function results*

The height of the signal (in parentheses) is the number of standard deviations of the map above the mean. Unless otherwise stated, the Crowther rotation function was run with an external radius of 40 Å, a resolution range of 4–10 Å and a cell edge of 150 Å, using the model 5API–TOT.

	Rot1				Rot2				Rot3			
$R = 45$ Å	337·5	35·0	65·0	(4·1)	142·5	67·5	12·5	(3·3)	125·0	77·5	32·5	(3·6)
					132·5	62·5	7·5	(3·3)				
$R = 40$ Å	337·5	35·0	65·0	(4·1)	142·5	67·5	10·0	(3·2)	125·0	77·5	32·5	(3·6)
					132·5	62·5	7·5	(3·2)				
$R = 35$ Å	337·5	35·0	65·0	(4·0)	142·5	67·5	7·5	(3·1)	125·0	77·5	35·0	(3·5)
					132·5	62·5	7·5	(3·0)				
$R = 30$ Å	337·5	35·0	65·0	(3·7)	142·5	67·5	7·5	(3·0)	125·0	77·5	32·5	(3·4)
					135·0	62·5	7·5	(2·9)				
Cell edge = 120 Å	337·5	35·0	65·0	(4·0)	132·5	62·5	7·5	(3·3)	125·0	77·50	32·5	(3·6)
5API–TIDY–TOT (see Fig. 1)	337·5	35·0	65·0	(4·1)	135·0	62·5	5·0	(2·8)	122·5	80·0	35·0	(3·2)
					142·5	67·5	10·0	(3·5)				
F 15–4 Å	337·5	35·0	65·0	(3·3)	140·0	72·5	5·0	(2·8)				
F 6–4 Å	335·0	35·0	67·5	(2·3)	142·5	67·5	12·5	(2·9)				
					132·5	62·5	5·0	(2·6)				
					145·0	72·5	2·5	(2·8)				
F 10–6 Å	335·0	32·5	67·5	(4·8)	Background				120·0	77·5	35·0	(4·1)
F 10–3·5 Å	337·5	35·0	65·0	(3·9)	142·5	67·5	10·0	(3·2)	125·0	77·5	32·5	(3·4)
					132·5	62·5	7·5	(3·1)				
E 20–6 Å	335·0	32·5	67·5	(3·4)	Background							
E 20–5 Å	332·5	32·5	70·0	(3·6)	Background							
E 20–4 Å	332·5	32·5	67·5	(3·3)	142·5	67·5	10·0	(3·4)	120·0	80·0	17·5	(3·2)
E 20–3·5 Å	332·5	32·5	70·0	(3·1)	135·0	65·0	0·0	(3·8)				
Final solution	335·0	34·0	65·0		131·5	61·5	10·5					

(Sussman, Holbrook, Church & Kim, 1977) was used with an initial resolution range of 10–9 Å (320 reflections), extended to 10–8 Å (900 reflections) and to 10–7 Å (1677 reflections).

Phasing the derivative

Phases derived from this crude model were used to calculate a Fourier difference map of the platinum derivative. Occupancies of the heavy-atom sites were refined against centrosymmetric reflections and then fixed to phase all reflections. Definitions of the relevant statistics are described by Dodson (1976).

Results

Rotation function

The results are summarized in Table 1, with, in each case, the values of the Eulerian angles (α, β, γ) of the two solutions (Rot1 and Rot2) that turned out to be correct, as well as their height. Fig. 1 shows a plot of the value of the rotation function for the maximum of each β-section, in the resolution range 10–4 Å, using *F* or *E* values and 5API–TIDY–TOT as the model. From this figure, it can be seen that two major peaks dominate at sections $\beta = 35°$ and $\beta = 70°$. The first one (Rot1), appeared to be correct while the second one (Rot2) had Eulerian angles quite different from the final answer (see below). Almost all the maxima of the β-sections around the first peak (Rot1) correspond to rotations very similar to Rot1: they just build up the peak. However, on closer inspection, there is a shoulder on the Rot2 peak around $\beta = 62·5°$ and also another, smaller peak around section $\beta = 80°$, where the angles, although close to those of Rot2, are sufficiently different for it to be a different peak. Indeed, the three sections $\beta = 77·5$, 80·0 and 82·5° build up another peak that will be referred to as Rot3 because it also appeared consistently in different conditions (see Table 1).

The variability of the actual values of the Eulerian angles of Rot1, Rot2 and Rot3 in different conditions was found to be significant (that is to say,

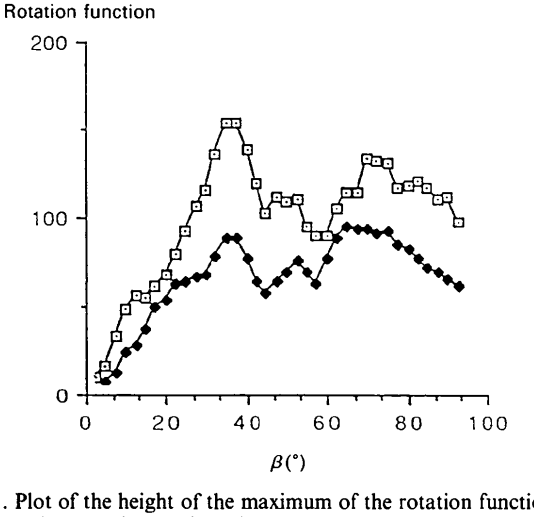

Fig. 1. Plot of the height of the maximum of the rotation function for each β-section, using the 5API–TIDY–TOT model, an integration radius of 40 Å, a cell edge of 150 Å, data in the 10–4 Å resolution range, with either normalized (□) or normal (◆) structure factors.

enough to make the translation function fail) for all of these orientations and most pronounced for Rot2: in some cases the peak corresponding to Rot2 was split. In a broader sense, Rot3 can be considered to be produced by some degeneracy of Rot2; the axes of these two different rotations are separated by an angle of 20°.

As a general trend, using F or E values (Tickle, 1985), lower resolution (10–6 Å, 20–6 Å) favors Rot1 and makes Rot2 and Rot3 weaker or even disappear in the background. On the other hand, higher resolution makes Rot2 more apparent and even, in some cases, first in the list.

The low-resolution terms are not important when using E values (20–4 Å gave the same results as 10–4 Å) but omitting too much data is not recommended (7–4 Å data gave inconsistent results). When using F values, a low-resolution cutoff of 15 Å instead of 10 Å made Rot2 and Rot3 coalesce into a single peak that was clearly wrong.

The external radii, which were chosen in accordance with the literature (Lifchitz, 1983), did not prove to be very important, although $R = 30$ Å gave lower signal-to-noise ratios.

When compared with results obtained with the complete model, the use of a model in which surface atoms were removed did not improve the signal. The main problem of these rotational searches was the degeneracy of Rot2.

Translation search

The Crowther–Blow translation function was run for each one of the previously selected orientations (Rot1, Rot2, Rot3). The various parameters were not thoroughly varied since all published results agree on the fact that E values should be used with as broad a resolution range as possible (Cygler & Anderson, 1988*a*,*b*; Tickle, 1985).

With the orientation found for Rot1 in the resolution range 10–4 Å, the search turned out to be almost immediately successful. This allowed us to answer some basic questions about the space group, the best model to be used and the required accuracy of the angles for a successful translational search. Relevant results concerning these questions are summarized in Table 2. It is clear from the height of the signal obtained for the two possible space groups $P4_12_12$ and $P4_32_12$ that the latter should be chosen. It is also clear that a correct orientation, within 5°, is an absolute requirement for obtaining a detectable signal in the translation function. This has been stressed by others for different translational searches, for example, R searches (Evans, 1985). As for the model used in the T search, the one in which all the surface atoms had been removed was found to be superior. Removal of the C-terminal part of the molecule after the cleavage site did not improve the signal of the translation function. A limited R-factor search around the position determined by the translation function essentially confirmed that we had correctly identified the location of the first molecule of the asymmetric unit. The R factor for a 10–8 Å resolution range was 50·0%. The translation to be applied to the rotated coordinates of α_1-AT is $t_x = 73{\cdot}45$, $t_y = 34{\cdot}0$, $t_z = 174{\cdot}5$ Å.

Table 2. *Results of the Crowther–Blow translation function, using E values between* 20 *and* 4 Å *resolution*

The space group is $P4_32_12$, unless otherwise stated.

Model	Signal*
Rot1	
5API–TIDY–CUT ($P4_12_12$)	$2{\cdot}0\sigma$
5API–TIDY–CUT ($P4_32_12$)	$6{\cdot}0\sigma$
5API–TIDY–TOT	10σ
5API–TOT	$4{\cdot}5\sigma$
5API–CUT	$3{\cdot}0\sigma$
5API–TIDY–CUT (rotation off by 5°)	$0{\cdot}5\sigma$
Rot2	
5API–TIDY–TOT	$4{\cdot}5\sigma$
5API–TIDY–CUT	$2{\cdot}0\sigma$

* The number of standard deviations (σ) between the highest and the first spurious peak.

We then set out to localize the second molecule, using Rot2 and Rot3 as possible orientations. Only one of them (Rot2), after an extensive search around each of these orientations, gave an acceptable signal of $4{\cdot}5\sigma$ between the major peak and the first spurious peak. This is less than that obtained with the first molecule (ten standard deviations). The R factor obtained with the second molecule ($R = 52{\cdot}0\%$) is also worse than that of the first molecule ($R = 50{\cdot}0\%$). The two molecules were then put together in the asymmetric unit and the question of the four different possible origins in the Patterson function (x, y, z; $x + \frac{1}{2}, y + \frac{1}{2}, z$; $x + \frac{1}{2}, y + \frac{1}{2}, z + \frac{1}{2}$; $x, y, z + \frac{1}{2}$) was addressed simply by calculating an R factor for each origin; only one origin gave an R factor (48%) below that obtained when using only one monomer. These results were essentially confirmed by the use of the *BRUTE* program (Fujinaga & Read, 1987). Although a complete search of the asymmetric unit, with orientations systematically varied with a step size of 3° around the peak of the rotation function, would have been computationally prohibitive in our case, it is clear that the signal would have been unmistakable. The results (Table 3) also confirm that correlation coefficients are more sensitive than R factors. The number of standard deviations between the highest and the next spurious peak, for each molecule separately, is always greater than three. Table 3 also confirms that data between 4 and 5 Å resolution are very important; this may result from the fact that these data generate inter-β-strand distances (very abundant in α_1-AT) in the Patterson function.

Table 3. *Correlation coefficients (%) given by the BRUTE program for the translational search*

The correlation coefficient is defined by: $\Sigma(x-\langle x\rangle)(y-\langle y\rangle)/[\Sigma(x-\langle x\rangle)^2]^{1/2}[\Sigma(y-\langle y\rangle)^2]^{1/2}$ where $x = F^2_{obs}$ and $y = F^2_{calc}$.

Resolution range	Model	Max.	Mean	σ
10–6 Å	Dimer	49	30	6·0
10–6 Å	Rot1	36	19	4·7
10–6 Å	Rot2	28	18	3·0
5–4 Å	Dimer	21·8	7·6	3·0
5–4 Å	Rot1	17·0	5·0	2·5
5–4 Å	Rot2	12·5	4·9	2·5
5–4·5 Å	Dimer	29·1	10·8	3·8
4·5–4 Å	Dimer	18·6	6·3	2·8

Assessing the validity of the model

At this stage, a rigid-body refinement was undertaken with the program *CORELS* (Sussman *et al.*, 1977). This led to a drop of the *R* factor to 46% in the 10–7 Å resolution range (1677 reflections). The crystal packing showed no bad contacts between crystallographically related and/or noncrystallographically related molecules (Fig. 2).

Phases derived from this partially refined model were used to calculate a Fourier difference map of the platinum derivative. This revealed three peaks (Table 4), which were found to satisfy the Patterson function. The positions of these sites in the model were graphically displayed and were found to be located near cysteine residues on the surface of the protein (see Fig. 3). Two of the sites are topologically equivalent, meaning that they are related by the noncrystallographic symmetry. The position of the noncrystallographic equivalent of the third site was calculated and found to give low scores in the Patterson function. On the other hand, this site was found to give a high score in an automatic search of the Patterson function of the 16m*M* $PtCl_4^{2-}$ derivative, using the *HASSP* program (Terwilliger, Kim & Eisenberg, 1987). This means that this site is accessible, but that, possibly for kinetic reasons, we do not see it in the 2m*M* $PtCl_4^{2-}$ derivative. Retrospectively, the fact that we had not been able to interpret this derivative, either by automatic search methods or by direct methods, might be explained by the rather special positions of the heavy atoms (*i.e.* $x \simeq y$ or $x + y \simeq \frac{1}{2}$), as well as by the high symmetry of the Patterson function.

B factors for the heavy-atom sites were estimated from their refined occupancies with centrosymmetric reflections in different resolution ranges (12–8, 8–6 and 7–5 Å). Phasing with the three sites deduced from the Fourier difference map gave the following statistics for data between 20 and 5 Å resolution:

(*a*) An *R*-factor of 55%, a correlation coefficient of 0·51 and a gradient of the plot between the calculated and observed structure factors of centrosymmetric reflections of 0·41.

(*b*) A figure of merit of 0·31 and a phasing power of 1·63 for all reflections.

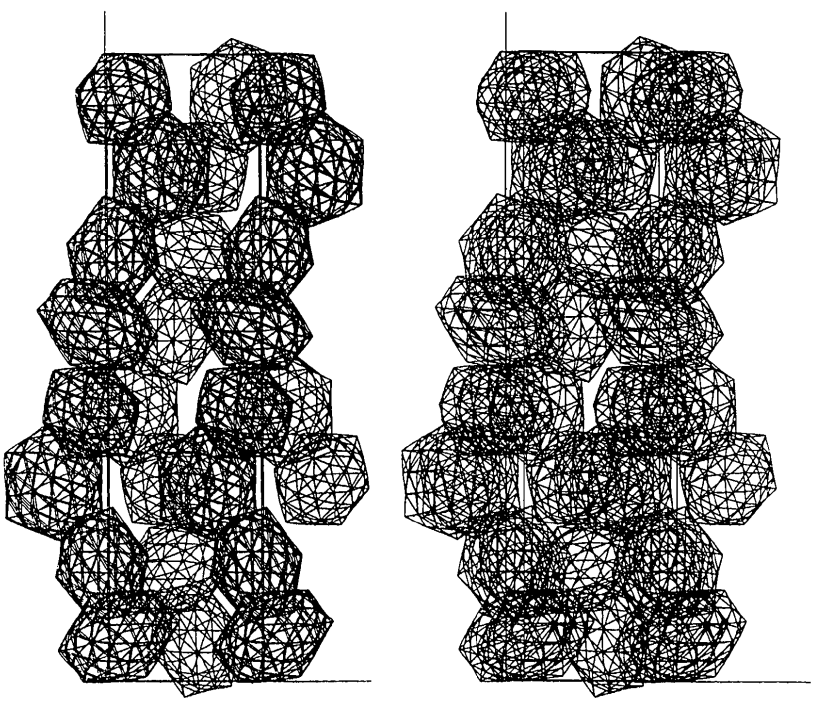

Fig. 2. Stereoview of the crystal packing, using an ideal representation of the molecule by an ellipsoid (program *PACKING*, M. Bergdoll, unpublished). The *c* axis is vertical and the *a* axis is horizontal.

Table 4. *Parameters of the different heavy-atom sites of the* $PtCl_4^{2-}$ *derivative*

Site	Dimer*	Rot1†	x	y	z	Occ.	B
Site 1	$5{\cdot}9\sigma$	$6{\cdot}5\sigma$	0·383	0·375	0·341	2·3	90·0
Site 1′	$5{\cdot}8\sigma$	$5{\cdot}4\sigma$	0·308	0·285	0·270	2·3	60·0
Site 2	$6{\cdot}5\sigma$	$5{\cdot}6\sigma$	0·396	0·103	0·212	3·4	100·0
Pseudo site 2′	Background		0·172	0·555	0·273		

* Difference Fourier map phased with the dimer; the first spurious peak is at $4{\cdot}0\sigma$; the height of the signal is given as the number of standard deviations above the mean value of the map.

† Difference Fourier map phased with only Rot1 as a model; the first spurious peak is at $4{\cdot}15\sigma$.

A dramatic improvement of these parameters was obtained on solvent flattening and noncrystallographic symmetry averaging. This allowed us to obtain a 5 Å resolution map that is essentially free of any bias introduced by the model, which was found to fit in this electron density map (data not shown).

Refinement at higher resolution is currently being carried out with the program *GROMOS* (van Gunsteren & Berendsen, 1987). Energy minimization followed by a 1 ps molecular-dynamics simulation led to an R factor of 32% at 3·6 Å resolution. Details on these calculations and further refinement will be reported elsewhere.

Discussion

Correctness and accuracy of the Eulerian angles is required for a successful translational search. Using a fine grid in the rotation function is not enough to achieve this accuracy. Instead, it is necessary to try many different resolution ranges to obtain an idea about which peaks appear most consistently (see Dijkstra, van Hess, Kalk, Brandenburg, Hol & Drenth, 1982). However, no general rule stating the relative importance of high- and low-resolution data can be derived in our case, since this appears to be different for the orientations of each of the two independent molecules of the asymmetric unit. For each possible orientation, it is then necessary to carry out a manual rigid-body refinement, which is essentially the same as that performed in the *BRUTE* program (Fujinaga & Read, 1987), except that the translation function is used instead of an R-factor search. The answer given by the Crowther–Blow translation function requires only one resolution range to be used and is essentially unambiguous, with only one major peak when the orientation is correct. The result is thus much more clear cut than the R-search procedure and less computationally intensive. This allowed us to overcome the degeneracy of the Eulerian angles of Rot2, observed for different resolution ranges in the rotation function. This method has also been successful for the structure determination of an Fab–cyclosporin complex (D. Altschuh, in preparation).

The use of a model in which surface atoms had been removed did not really facilitate the interpretation of the rotation function but definitely helped in obtaining a very high signal-to-noise ratio in the translation function, even though the translational search was performed with less than 30% of the asymmetric unit. This extends observations made by other authors on the question of the required com-

Fig. 3. Stereoview (Cα carbon representation) of the dimer of the asymmetric unit, showing the positions of the heavy-atom sites (dotted spheres) on the surface of the protein. The two monomers are related by a noncrystallographic symmetry operation ($\kappa = 123°$, $t_u = 42{\cdot}2$ Å) around a noncrystallographic axis defined by a point M ($x_M = 96{\cdot}6$, $y_M = 38{\cdot}1$, $z_M = 279{\cdot}3$ Å) and a unitary vector u (spherical coordinates $\varphi = 138°$, $\theta = 55°$).

pleteness of the model (Cygler & Anderson, 1988*a*,*b*). For the rotation function, they found that Cα carbons (12% of the asymmetric unit) were enough to obtain the correct orientations. However, at least 50% of the asymmetric unit was required for the Crowther–Blow translation function (Cygler & Anderson, 1988*b*). Another example of a successful application of the molecular replacement method with an incomplete model is the recent resolution of the complex between the trp repressor and its cognate DNA sequence (Otwinovski *et al.*, 1988).

Suprisingly, half of the asymmetric unit is enough to produce phases of sufficient quality to give an interpretable Fourier difference map for the platinum derivative. Such a possibility has also been previously noted by Evans (1985).

The two molecules in the asymmetric unit shown in Fig. 3 are related by a screw axis which is not a twofold one. A similar situation has been found for hexokinase (Steitz, Fletterick, Anderson & Anderson, 1976) and collagenase (Arnoux, 1985). It should be mentioned that antithrombin III is functionally active as a monomer in solution.

The peak corresponding to the noncrystallographic symmetry in the self-rotation function is significant only when E values between 20 and 4 Å resolution or F values between 10 and 4 Å resolution are used; the best outer radius for the integration is 35 or 40 Å, in which case the height of the peak is 12% of the crystallographic ones.

As judged from the height of the translation function, one molecule of the asymmetric unit is clearly more similar to cleaved α_1-AT than the other, suggesting that the two monomers are not strictly equivalent. However, a definite answer to this question will have to await further refinement.

The fact that the molecules making up the asymmetric unit are cleaved in the crystal (Samama *et al.*, 1989) came as a surprise, in a situation that is reminiscent of that described by R. Huber and colleagues (Loebermann *et al.*, 1984). Carrell (Carrell, 1982) suggested that the cleaved molecule may be more stable than the native one. Nevertheless, the N-terminus domain of antithrombin is intact in our crystals; this domain, which includes the heparin binding site, contains more residues than that of α_1-AT. We expect our map will allow us to describe this domain in molecular detail.

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