

A new crystal form of the complex between seryl-tRNA synthetase and tRNA^{Ser} from *Thermus thermophilus* that diffracts to 2.8 Å resolution

A.D. Yaremchuk^a, M.A. Tukalo^a, I. Krikliviy^a, N. Malchenko^a, V. Biou^b, C. Berthet-Colominas^b and S. Cusack^b

^aInstitute of Molecular Biology and Genetics, Academy of Sciences of the Ukraine, 252627 Kiev, Ukraine and ^bEuropean Molecular Biology Laboratory, Grenoble Outstation, c/o ILL, 156X, 38042 Grenoble, France

Received 23 July 1992

Two distinct complexes between seryl-tRNA synthetase and tRNA^{Ser} from *Thermus thermophilus* have been crystallized using ammonium sulphate as a precipitant. Form III crystals grow from solutions containing a 1:2.5 stoichiometry of synthetase dimer to tRNA. They are of monoclinic space group C2 with unit cell dimensions $a = 211.6$ Å, $b = 126.8$ Å, $c = 197.1$ Å, $\beta = 132.4^\circ$ and diffract to about 3.5 Å. Preliminary crystallographic results show that the crystallographic asymmetric unit contains two synthetase dimers. Form IV crystals grow from solutions containing a 1:1.5 stoichiometry of synthetase dimer to tRNA. They are of orthorhombic space group P2₁2₁2₁ with unit cell dimensions $a = 124.5$ Å, $b = 128.9$ Å, $c = 121.2$ Å and diffract to 2.8 Å resolution. Preliminary crystallographic results show that these crystals contain only one tRNA molecule bound to a synthetase dimer.

Aminoacyl-tRNA synthetase; tRNA; Protein-RNA complex; Crystallization; *Thermus thermophilus*

1. INTRODUCTION

The specific recognition of tRNAs by aminoacyl-tRNA synthetases is a key step in the translation of the genetic code and numerous biochemical, genetic and structural studies have been aimed at understanding the molecular basis of the specific interactions between a synthetase and its cognate tRNA(s) [1,2]. In the last three years important progress has been made with the determination of the crystal structures of two synthetase-tRNA complexes, that of the *E. coli* glutamyl-system [3] and the yeast aspartyl-system [4]. These examples are fortuitously representatives of the two distinct classes of synthetases (class 1 and class 2) as identified by sequence analyses [5,6] and the three-dimensional structure of the seryl-tRNA synthetase [7]. Comparison of the structures of these two synthetase-tRNA complexes reveals quite different modes of interaction of the tRNA with the different catalytic domains of the two classes [4]. Nevertheless in both cases base-specific recognition occurs in the region of the tRNA acceptor stem and anticodon. Indeed it has been found by biochemical and genetic methods that for the majority of tRNAs both of these elements contribute significantly to the tRNA identity [1,8]. In *E. coli*, tRNA^{Ser}, tRNA^{Leu} and tRNA^{Ala} are exceptional in that the anticodon plays no role in recognition by the synthetase.

tRNA^{Ser}, tRNA^{Leu} and procaryotic tRNA^{Tyr} are distinguished from all other tRNAs by having a long extra (variable) arm composed of more than ten nucleotides as opposed to the normal 4 or 5 nucleotides. No crystal structure is yet available for a long variable arm tRNA. However, recent results suggest that the long variable arm of tRNA^{Ser} is a specific recognition element for seryl-tRNA synthetase [9,10].

From the five known synthetase structures and analysis of the primary sequence of all twenty synthetases it can be suggested that whereas there may well be common features within each synthetase class in the mode of binding of the acceptor stem to the catalytic domain of each class, other tRNA binding domains are much more idiosyncratic, having probably evolved at a later stage [6]. It thus remains of great interest to obtain structures of other synthetase-tRNA (and indeed other substrate) complexes to determine the common and variable features of each case.

We have been studying the seryl-tRNA synthetase in both *E. coli* [11] and the extreme thermophile *Thermus thermophilus*. Both enzymes are α_2 dimers with respectively 430 and 421 residues per subunit and a sequence identity of 35% (Tukalo et al., unpublished results). Crystal structures of the seryl-tRNA synthetase in the absence of substrates are known for both organisms at 2.5 Å resolution ([7]; Fujinaga et al., unpublished results). In addition the structure of the complex with ATP in the case of *T. thermophilus* is currently being refined (Berthet et al., unpublished results). Both enzymes have similar structures including a remarkable

Correspondence address: S. Cusack, European Molecular Biology Laboratory, Grenoble Outstation, c/o ILL, 156X, 38042 Grenoble, France. Fax: (33) (76) 20 7199.

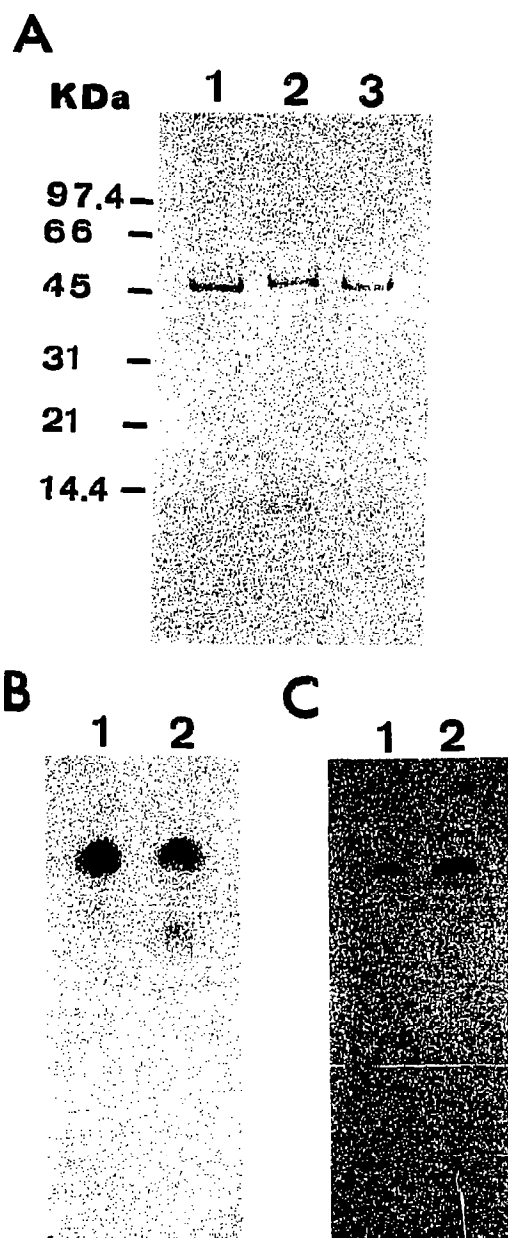


Fig. 1. SDS/polyacrylamide gel electrophoresis of dissolved form III and form IV crystals of the complex between seryl-tRNA synthetase and tRNA^{Ser} from *Thermus thermophilus*. (A) SDS/polyacrylamide gel electrophoresis of purified control seryl-tRNA synthetase (lane 1) and washed and dissolved form 3 (lane 2) and form 4 (lane 3) crystals. The gel was stained with Coomassie blue. (B) 8 M-urea/polyacrylamide gel electrophoresis of purified control tRNA^{Ser1} (lane 1) and dissolved single crystal form 3 (lane 2). The gel was stained with Stains All. (C) 8 M-urea/polyacrylamide gel electrophoresis of purified control tRNA^{Ser2} (lane 1) and dissolved single crystal form 4 (lane 2). The gel was stained with Stains All.

N-terminal antiparallel coiled-coil domain which is presumed to be important for specific binding of tRNA^{Ser}.

Recently two different tetragonal crystal forms of the complex between tRNA^{Ser1} and seryl-tRNA synthetase from *T. thermophilus* have been characterized (forms 1

and II), both with a very long unit cell *c*-axis (470 Å) and neither diffracting beyond about 6.0 Å resolution [12]. It was thus desirable to find new crystal forms diffracting to higher resolution in order to obtain yield structures in atomic detail. We report here the crystallization of two new crystal forms of the complex between tRNA^{Ser} and seryl-tRNA synthetase from *T. thermophilus*, one of which diffracts to high resolution.

2. MATERIALS AND METHODS

2.1. Preparation of tRNA

tRNA^{Ser1} and tRNA^{Ser2} from *T. thermophilus* were prepared from bulk tRNA as described [12]. Determination of the primary structure of both tRNAs is in progress.

2.2. Purification of seryl-tRNA synthetase

Seryl-tRNA synthetase from *T. thermophilus* was isolated by a modification of the previous purification method [13]. This time, the enzyme was purified to homogeneity in three chromatographic steps using chromatography on DEAE-Sephacel, hydrophobic chromatography on polyvinyl sorbent Toyopearl HW-65 and affinity chromatography on heparin-Sepharose. The crude extract after precipitation with ammonium sulphate (50% of saturation) and dialysis in buffer A (10 mM potassium phosphate at pH 7.9, 1 mM DTT, 0.1 mM diisopropyl fluorophosphate) was loaded onto the DEAE-Sephacel (Pharmacia) column, washed with buffer A and then eluted with a potassium phosphate linear gradient (concentration from 10 mM to 250 mM and pH 7.9 to 6.8). The fractions containing seryl-tRNA synthetase activity were collected and after precipitation with ammonium sulphate were loaded onto the polyvinyl sorbent Toyopearl HW-65 column (Toyo Soda). The enzyme was eluted by a reverse gradient of ammonium sulphate (50% to 10% of saturation) in buffer at pH 7.9 containing 20 mM Tris-HCl, 5 mM MgCl₂, 1 mM DTT and 0.1 mM diisopropyl fluorophosphate. The last step consisted of a chromatography on a column of heparin-Sepharose (Pharmacia).

Seryl-tRNA synthetase was eluted by increasing concentration of potassium chloride (0–250 mM) in buffer containing 20 mM Tris-HCl at pH 7.9, 5 mM MgCl₂, 1 mM DTT and 0.1 mM diisopropyl fluorophosphate. All steps of purification of seryl-tRNA synthetase were conducted at 4°C. About 12 mg of pure enzyme could be obtained from 1 kg of cells. The enzyme was stored at 4°C after precipitation with ammonium sulphate.

2.3. Crystallization

A search for new crystallization conditions was undertaken using two isoacceptors, tRNA^{Ser1} and tRNA^{Ser2}, and the seryl-tRNA synthetase isolated by the modified purification method described above. Crystallization was by the hanging drop vapour diffusion method, using ammonium sulphate or sodium citrate as precipitants. It has recently been shown that upon complex formation *T. thermophilus* tRNA^{Ser} becomes susceptible to cleavage by a high concentration of Mg²⁺ ions (Tukalo et al., unpublished results; see also [14]). Therefore the concentration of magnesium chloride was not increased above 2.5 mM. In the following crystallization trials the concentration of both tRNA^{Ser} and seryl-tRNA synthetase was also decreased in order to have fewer points of nucleation and hence single crystals.

2.4. Crystallography

Diffraction data were collected on a rotating anode source with graphite monochromator and a FAST detector (CNRS, Grenoble) or double-focussing mirrors and Mar-Research image-plate system (EMBL, Grenoble) and on synchrotron beamline W32 at LURE [15] using a Mar-Research image-plate detector. In the latter case the wavelength was 0.986 Å. Raw data were processed either using MADNES and the Kabsch profile fitting programs PROCOR (FAST data)

or the MOSFLM package for image-plate data [16]. Subsequent data analysis was performed with the CCP4 program package (Daresbury Laboratory, Warrington, WA4 4AD, UK). In the case of the monoclinic crystals (form III), data were collected on the FAST system for two putative heavy-atom derivatives obtained by soaking the crystals in 10^{-3} M dysprosium chloride for three days or 10^{-3} M uranyl acetate (pH 6.8) for one week.

3. RESULTS

Table I contains details of the four characterised crystal forms of the complex between seryl-tRNA synthetase and tRNA^{Ser} from *T. thermophilus*.

3.1. Form III crystals

Form III crystals grow in hanging drops containing 25 mM Tris-maleate/NaOH (pH 7.2), 2.5 mM MgCl₂, 20% saturated (v/v) ammonium sulphate, 1 mM NaN₃ and a synthetase-tRNA stoichiometry of 1:2.5 with either tRNA^{Ser1} or tRNA^{Ser2} (see Table I). The crystals are of a characteristic, but irregular shape which is very difficult to relate to the unit cell axes. They grow to a maximum size of about $0.45 \times 0.2 \times 0.15$ mm³ over a period of several weeks. The presence of tRNA and enzyme in the crystals was checked by gel electrophoresis (Fig. 1A and B).

Table I
Characteristics of the four crystal forms of the *Thermus thermophilus* seryl-tRNA synthetase-tRNA^{Ser} complex

	Form I Tetragonal	Form II Tetragonal	Form III Monoclinic	Form IV Orthorhombic
Protein:tRNA stoichiometry	1:2.4 (tRNA ^{Ser1,2})	1:2.4 (tRNA ^{Ser1,2})	1:2.5 (tRNA ^{Ser1,2})	1:1.5 (tRNA ^{Ser2})
Precipitating agent	Ammonium sulphate	Ammonium sulphate	Ammonium sulphate	Ammonium sulphate
Starting concentration (v/v sat.)	14%	14%	20%	20%
Reservoir concentration	30%	30%	32%	32%
Initial macromolecular concentration				
Protein (mg/ml)	11.2	11.2	5.6	5.6
tRNA (mg/ml)	8.3	8.3	4.2	2.6
Temperature (°C)	20	20	20	20
Magnesium chloride (mM)	15	15	2.5	2.5
Tris-maleate/NaOH buffer (mM)	50	50	25	25
pH	7.6	7.6	7.2	7.2
Sodium azide (mM)	1	1	1	1
Spermine (mM)	0.3	8	—	—
Space-group	P4 ₁ 2 ₁ 2 ₁ ^a	P4 ₁ 2 ₁ 2 ₁ ^b	C2	P2 ₁ 2 ₁ 2 ₁
Cell dimensions (Å)	$a = 127$ $b = 127$ $c = 467$	$a = 101$ $b = 101$ $c = 471$	$a = 211.6$ $b = 126.8, \beta = 132.4^\circ$ $c = 197.1$	$a = 124.5$ $b = 128.9$ $c = 121.2$
Volume of asymmetric unit (Å ³)	941,530	600,584	976,306	486,256
Contents of asymmetric unit	Synthetase dimer 2 tRNA ^c	Synthetase dimer 2 tRNA ^c	2 Synthetase dimers 4 tRNA ^c	Synthetase dimer 1 tRNA
Molecular weight (Da) ^d	148,000	148,000	296,000	124,600
Solvent content (% volume) ^e	82%	72%	66%	71%
V_m (Å ³ /Da)	6.1	3.9	3.2	3.9
Diffraction limit	6.0 Å	5–6 Å	3.5 Å	2.8 Å

^a Determined by molecular replacement solution (unpublished results).

^b Exact tetragonal space-group unknown.

^c tRNA content assumed.

^d Molecular weight of seryl-tRNA synthetase subunit = 47,806 Da. Molecular weight of tRNA^{Ser} assumed to be 29,000 Da.

^e Assuming partial specific volume of protein and tRNA are respectively 0.73 and 0.53 cm³/g.

The space-group of the crystals was determined to be monoclinic C2 by means of the MADNES auto-indexing routine of the FAST system and confirmed by subsequent data analysis. The cell dimensions are $a = 211.6$ Å, $b = 126.8$ Å, $c = 197.1$ Å, $\beta = 132.4^\circ$ and the crystals diffract to about 3.5 Å on the beamline W32 at LURE. Even using synchrotron radiation of wavelength 0.986 Å, the high resolution diffraction is short-lived, a maximum of 30° of data being obtained from a single crystal. On the other hand, reasonable data at 4.5–5.5 Å resolution could be obtained from one or two crystals using the FAST system and a rotating anode source. An advantage of the FAST system was the ability to orientate the crystals in order to optimise data collection by rotation about an axis perpendicular to the 2-fold axis.

Preliminary results have been obtained with a native dataset measured on the FAST system using 2 crystals giving 19,255 unique reflections to 4.5 Å resolution (R -merge 0.12 (0.20 in highest resolution shell), completeness 83%, average redundancy 2.7). The refined 2.5 Å structure of seryl-tRNA synthetase from *T. thermophilus* (Fujinaga et al., unpublished results) was used as a search model for molecular replacement. Since the helical arms of the molecule are known to have a variable orientation they were excluded from the model. The highest peak in the cross-rotation function (6.8σ) gave a solution to the translation function, using BRUTE [17], with a peak height of 6.5σ and a correlation on intensities of 0.37 (resolution range 6–12 Å). Using this position for one synthetase dimer, phases were derived from the protein model (lacking arms) and a 6.0 Å map was calculated using coefficients obtained with the program SIGMAA [18]. The map confirmed the position of the protein and gave a clear indication of the altered orientation of the helical arms, but no clear indication of any tRNA position.

To investigate further it was decided to measure some derivative data to provide additional phase information. Two putative derivatives were prepared by soaking crystals in either dysprosium chloride or uranium acetate (see Methods), both of which had been used successfully as derivatives of the seryl-tRNA synthetase from *E. coli* [7]. Data were collected on the FAST system. Fourier difference maps were calculated using coefficients $(F_{\text{der}} - F_{\text{nat}})\phi_{\text{calc}}$ where F_{der} and F_{nat} are respectively the measured derivative and native structure factor amplitudes and ϕ_{calc} are the phases derived from the model described above containing a single synthetase dimer. The maps showed highly significant peaks corresponding to four dysprosium atoms and three uranium atoms. Two of the dysprosium peaks were adjacent to Glu-345 in the active site of each subunit of the already located synthetase dimer, thus corresponding exactly to the dysprosium site found previously in the *E. coli* enzyme (Glu-355). One of the uranium sites is adjacent to Glu-227 in one of the subunits of the already located synthetase dimer (this site was not occupied in the

E. coli enzyme, although the glutamic acid is conserved). The other two dysprosium sites and uranium sites showed exactly the same relative configuration (however, with both Glu-204 sites occupied by uranium) and thus could be used to place a second synthetase dimer in the asymmetric unit. It was subsequently confirmed that the orientation of this second synthetase molecule corresponded to the second highest peak in the rotation function (6σ), for which no convincing translation function solution had previously been found. A new SIGMAA map calculated at 6 Å resolution with model phases including two synthetase dimers now revealed convincing density for one tRNA molecule and possible but noisy density for three other tRNA molecules. It was confirmed that the packing of two complete synthetase dimers with a total of four tRNA molecules was satisfactory. The structure determination is still in progress and will probably require better derivative data for completion.

3.2. Form IV crystals

The best crystals (form IV) were obtained using ammonium sulphate as a precipitating agent. 10 µl hanging drops containing 25 mM Tris-maleate/NaOH buffer at pH 7.2, 2.5 mM MgCl₂, 20% saturated (v/v) ammonium sulphate, 1 mM NaN₃, 2.6 mg/ml tRNA^{Ser}2 and 5.6 mg/ml seryl-tRNA synthetase (stoichiometric ratio of 1.5 tRNA molecules to one enzyme dimer), were equilibrated at room temperature against 32% saturated ammonium sulphate. Crystals appeared within 2–3 weeks and grew very slowly reaching a maximum size of 0.40 × 0.18 × 0.16 mm³ in 2–3 months. Before examination with X-rays, the crystals were stabilized by increasing slowly the concentration of ammonium sulphate in the reservoir to a final value of 37%. Analysis of washed and dissolved crystals by gel electrophoresis, ultraviolet absorbance and enzymatic assay indicates that form IV crystals contain both tRNA and enzyme (Fig. 1A and C).

Initially, only two crystals of reasonable size were available. One was examined using the FAST system and the space-group determined to be orthorhombic by the MADNES auto-indexing routine. This was confirmed in subsequent data processing. The unit cell axes are $a = 124.5$ Å, $b = 128.9$ Å and $c = 121.2$ Å. The crystals are rectangular prisms with the b -axis parallel to the long axis of the prism and the long faces being perpendicular to [101] and [10-1]. The second crystal (size 0.24 × 0.1 × 0.1 mm³) was measured on wiggler beamline W32 at LURE with a wavelength of 0.986 Å and a crystal-image plate distance of 245 mm at a temperature of 2°C. A complete dataset at 2.9 Å resolution was obtained on the one crystal by integrating 62 one-degree oscillation images (typical exposure time 8 min). 41,675 unique reflections were obtained from 79,091 measured reflections (average redundancy of 1.9) with an R -merge of 6.4% (17.2% highest resolution bin) and

completeness of 95%. The exact space-group was ambiguous from an examination of the systematic absences of this data.

The refined 2.5 Å structure of seryl-tRNA synthetase from *T. thermophilus* (Fujinaga et al., unpublished results) was used as a search model for molecular replacement as described above. A cross-rotation function yielded a single unambiguous solution for the dimer orientation with a peak of 10.2σ. This orientation was used in a translation search using BRUTE [17] and an unambiguous solution found with space-group P2₁2₁2₁ with a correlation of 0.714 (resolution range 4–8 Å). Crystal packing was verified to be satisfactory with this model of the protein position although it was immediately apparent that one of the dimer active sites was blocked by crystal contacts. Using phases derived from the protein model a 3.0 Å map was calculated using coefficients derived from SIGMAA [18]. This immediately revealed excellent density for about 50% of a single tRNA molecule bound to the synthetase dimer. This is consistent with the fact that the second tRNA site on the synthetase dimer is blocked by crystal contacts. All crystal contacts are mediated by protein which perhaps explains the high diffraction quality and relative radiation insensitivity of the crystals compared to the other crystal forms of the complex. Model building and refinement of this structure is in progress, with the aid of an atomic model of tRNA^{Ser} kindly provided by Dr. E. Westhof (IBMC, Strasbourg).

4. DISCUSSION

Four crystal forms of the *Thermus thermophilus* seryl-tRNA synthetase-tRNA^{Ser} complex have been characterised. The two most promising crystal forms have been shown to contain complexes with one tRNA per synthetase dimer in the case of form IV and probably two tRNAs per synthetase dimer in the case of form III. In the best diffracting crystals (form IV) the tRNA plays no role in crystal contacts which may be an important factor in crystal quality and stability to X-rays. It remains to be seen whether the unexpected crystallisation of a dimeric synthetase with a single tRNA is of particular biological significance as might be the case if the affinities for tRNA of the two sites differ.

The good quality of the orthorhombic crystals should permit the determination of a high-resolution structure of the seryl-tRNA synthetase-tRNA^{Ser} complex and

thus provide a good basis for a deeper understanding of the mechanism of synthetase-tRNA recognition. In particular it will be possible to understand how the long helical arm of the seryl-tRNA synthetase interacts with the tRNA and how the long variable arm of the tRNA interacts with the synthetase. In addition for the first time the tertiary structure of a long-variable arm tRNA will be determined.

Acknowledgements: We thank Roger Fourme, Jean-Pierre Benoit and colleagues for excellent help and support on beamline W32 at LURE and C. Cohen-Addad and colleagues for giving access to the FAST system at the CNRS, Grenoble. A.D.Y. received a short-term EMBO fellowship for part of the duration of this work. Both A.D.Y. and M.A.T. received visiting scientists' fellowships from EMBL for some of this work. We also express gratitude to Marianne Grünberg-Manago for her support.

REFERENCES

- [1] Schimmel, P. (1987) *Annu. Rev. Biochem.* 56, 125–158.
- [2] Schulman, L.D. (1991) *Progress in Nucleic Acid Research and Molecular Biology* 41, 23–87.
- [3] Rould, M.A., Perona, J.J., Soll, D. and Steitz, T.A. (1989) *Science* 246, 1135–1142.
- [4] Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J.C. and Moras, D. (1991) *Science* 252, 1682–1689.
- [5] Eriani, G., Delarue, M., Poch, O., Gangloff, J. and Moras, D. (1990) *Nature* 347, 203–206.
- [6] Cusack, S., Härtlein, M. and Leberman, R. (1991) *Nucleic Acids Res.* 19, 3489–3498.
- [7] Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N. and Leberman, R. (1990) *Nature* 347, 249–255.
- [8] Shimizu, M., Asahara, K., Tamura, T. and Himeno, H. (1992) *J. Mol. Evol.* (in press).
- [9] Himeno, H., Haegawa, T., Ueda, T., Watanabe, K. and Shimizu, M. (1990) *Nucleic Acids Res.* 18, 6815–6819.
- [10] Normanly, J., Ollick, T. and Abelson, J. (1992) *Proc. Natl. Acad. Sci. USA* 89, 5680–5684.
- [11] Lebermann, R., Härtlein, M. and Cusack, S. (1991) *Biochem. Biophys. Acta* 1089, 287–298.
- [12] Yaremchuk, A.D., Tukalo, M.A., Krikliiviy, I.A., Melnik, V.N., Berthet-Colominas, C., Cusack, S. and Leberman, R. (1992) *J. Mol. Biol.* 224, 519–522.
- [13] Yaremchuk, A.D., Tukalo, M.A., Konovalenko, A.V., Egorova, S.P. and Matsuka, G.Kh. (1989) *Biopol. Kletka (Ukraine)* 5, 83–86.
- [14] Beresten, S., Jahn, M. and Soll, D. (1992) *Nucleic Acids Res.* 20, 1523–1530.
- [15] Fourme, R. et al. (1992) *Rev. Sci. Instr.* 63, 982–987.
- [16] Leslie, A.G.W. (1992) *Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography*, No. 26 (April 1992) Daresbury Laboratory, Warrington WA4 4AD, UK.
- [17] Fujinaga, M. and Read, R.J. (1987) *J. Appl. Crystallogr.* 20, 517–521.