TWO ISOMORPHOUS HEAVY-ATOM DERIVATIVES OF CRYSTALLINE METHIONYL-tRNA SYNTHETASE FROM *ESCHERICHIA COLI*

Claude MONTEILHET, Charles ZELWER and Jean-Loup RISLER
Centre de Génétique Moléculaire du C.N.R.S. 91190 Gif-sur-Yvette, France

Received 9 July 1974

1. Introduction

Aminoacyl-tRNA synthetases play a key role in the biosynthesis of proteins by their ability to specifically recognize an amino acid and its corresponding tRNA. An understanding of the basis of such specific protein–nucleic acid recognition requires the knowledge of the structure of the molecules involved. This goal is probably not very remote now since a high resolution model of yeast phenylalanine tRNA has been obtained recently [1], while four aminoacyl-tRNA synthetases have been obtained in a crystalline form [2–6] and are the object of crystallographic studies.

In this paper we report on the preparation of two heavy-atom derivatives of crystalline methionyl-tRNA synthetase from *E.coli*. The crystallized protein, a single polypeptide chain of molecular weight 65 000 [7,8], is a fully active fragment obtained by proteolysis of the native enzyme [4,7].

The great similarities between the catalytic properties of native methionyl-tRNA synthetase and its tryptic fragment, as evidenced by a number of recent experiments [9–12], indicate that the latter is a fully representative model of the native enzyme. Crystals are monoclinic, space-group $P2_1$, and contain one molecule per asymmetric unit [4].

2. Materials and methods

Native methionyl-tRNA synthetase from *E.coli* K12 and its tryptic fragment were prepared as previously described [7,13]. The fragment was crystallized by dialysis of a 0.5% protein solution against 1.3 M ammonium citrate buffered at pH 7.0 by 40 mM phosphate [4]. Crystals were grown and stored in the cold.

2.1. *Preparation of heavy-atom derivatives*

Heavy-atom derivatives were first searched by the conventional soaking method [14] in the presence of the mother liquor containing ammonium citrate. These first attempts failed, probably because the heavy-metal ions were chelated by citrate and therefore not accessible to the protein. Crystals previously grown in the presence of citrate were thus transferred into 3.2 M ammonium sulfate (pH 7.0), with but minor changes in the diffraction pattern and unit cell parameters (table 1). In this mother liquor, uranyl fluoride $Na_3UO_2F_5$ and platinum cyanide $K_2Pt(CN)_4$ produced noticeable intensity changes with little effect on the unit cell parameters (table 1). Both the uranyl and platinum salts were subsequently used at a concentration of 5 mM with a diffusion time of 5–7 days.

2.2. *Data collection and processing*

All X-ray experiments were performed in the cold room (1°C). Reflections were recorded photographically with Enraf-Nonius precession cameras and graphite-monochromatized CuKα radiation from a Philips fine focus X-ray tube. X-ray diffraction data were collected at 3.9 Å resolution for both the native enzyme and the two derivatives, using 21 sets of photographs in each case; each set consisted of two films.

An optronics rotating drum scanner connected to a 8K Varian 620-L-100 computer (Service de Microdensitométrie du C.N.R.S. Orsay) was used to measure

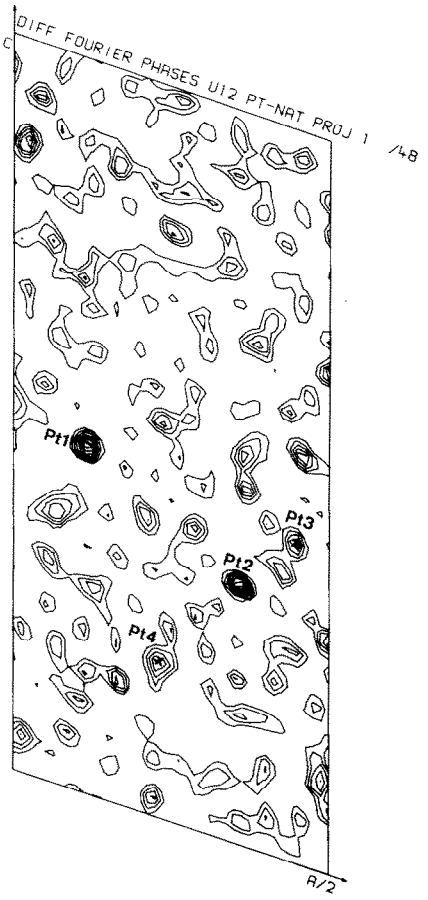

Fig. 1. Difference in electron density between the platinum derivative and the native enzyme, projected down the *b* axis. This difference Fourier map was calculated using only centric (hol) data and signs from the uranyl derivative alone. Platinum sites Pt1 and Pt2 are readily apparent, sites Pt3 and Pt4 (indicated by crosses) were later localized using full three-dimensional data.

Both derivatives (sites U12 + U3 and Pt1 + Pt2) were then used to calculate difference Fourier maps from which sites Pt3 and Pt4, and a further uranyl site (U4), were located. All the x and z coordinates thus obtained were consistent with the centric data.

The two derivatives were then refined by alternate cycles of phasing and least-squares refinement [19]. The final coordinates, relative occupancies A and gaussian form factor coefficient B of the heavy-atom sites are shown in table 2. A summary of the refinement statistics is given in fig. 2. The overall figure of merit at 3.9 Å resolution is 63%.

3. Conclusion

It appears from the data shown in fig. 2 that both the uranyl and platinum derivatives are satisfactory. In particular, the root mean square lack of closure E is significantly smaller than the calculated mean heavy-atom scattering F_c, which is an indication that the derivatives effectively participate to the phasing [20]. Furthermore, since E is smaller than F_c at 3.9 Å resolution, the two derivatives will probably be useful at higher resolution. The electron density map is presently being calculated, and it is expected that a low resolution model will soon emerge.

Acknowledgements

We are indebted to Dr V. Luzzati, in whose laboratory this work was carried out, for his continuous interest and encouragement, and to Dr J. P. Waller for

Table 2
Final heavy-atom parameters at 3.9 Å resolution

Derivative	Site	X[a]	Y[a]	Z[a]	A[b]	B[c]
	U12	0.9344	0.3599	0.8369	44.0	18.1
UO_2F_5	U3	0.9587	0.3816	0.8902	13.4	14.8
	U4	0.9062	0.5134	0.8241	5.6	15.0
	Pt1	0.1154	0.0065	0.4711	49.5	22.4
	Pt2	0.3523	0.3292	0.3529	27.4	7.9
$Pt(CN)_4$	Pt3	0.5577	0.2890	0.5647	32.7	39.8
	Pt4	0.2284	0.3867	0.2095	27.8	22.0

a – Fractional unit cell.
b – Arbitrary unit.
c – 'Temperature factor' in $Å^2$, including heavy-atom form factor.

Table 1
Unit cell parameters of methionyl-tRNA synthetase crystals in various conditions

Derivative	Supernatant (pH 7.0)	a(Å)	b(Å)	c(Å)	β(degrees)
Native[a]	Ammonium citrate 1.6 M	78.5	46.4	87.9	109.1
Native[b]	Ammonium sulphate 3.2 M	78.2	46.0	88.1	108.5
$Na_3UO_2F_5$[b]	Ammonium sulphate	78.2	46.0	87.8	108.6
$Pt(CN)_4K_2$[b]	Ammonium sulphate 3.2 M	78.0	46.3	88.0	108.6

a – Measured manually with a Nikon Shadowgraph apparatus.
b – Values given by the rotating drum densitometer.

the integrated intensities. The program written for this purpose in assembler language by one of us (C.Z.) was adapted from a FORTRAN program given to us by Drs B. W. Matthews and C. E. Klopfenstein [15]. As judged from the measurements statistics the films were generally of good quality, with reliability factors R_{sym} and R_{sca} [16] in the range 5–7% and 4–6% respectively.

Each plane of each derivative was scaled to the corresponding native plane by means of a Wilson plot (all the data processing was carried out with computer programs kindly provided by Dr D. M. Blow). The planes of native data were then brought to a common scale by using common reflections [17] and the interlayer scaling factors thus determined were applied to the derivatives. A total of 6840 reflections were measured for each derivative, which corresponds to 4610 independant reflections out of the 5218 included between (15 Å)$^{-1}$ and (3.9 Å)$^{-1}$.

2.3. *Location of heavy-atoms*

The difference Patterson synthesis for the uranyl derivative, calculated at 2.9 Å resolution using the centric (hOl) data, was first interpreted in terms of two sites (called U1 and U2); these sites were very close to each other (less than 2.5 Å) and could not be refined independently. They were therefore considered as a single site (called U12) and refined as such. A self difference Fourier map calculated with signs from U12 revealed a third site (U3).

Sites U12 and U3 were then used to calculate the difference Fourier in projection for the platinum derivative. Two sites (Pt1 and Pt2, see fig. 1) appeared clearly.

The three-dimensional difference Patterson function for the platinum derivative showed the two cross-vectors between Pt1 and Pt2 at the expected U and W coordinates and at levels V and $(1/2 - V)$. The origin along the b axis being set by assigning $y = 0$ for Pt1, the choice between the two possible sets of coordinates is arbitrary in the absence of anomalous dispersion data.

The $\triangle F^2$ synthesis for the uranyl derivative confirmed the location of sites U1 and U2 (Harker section) but no cross-vector could be found. It was therefore assumed that the y coordinates of the two sites were approximately equal. A difference Fourier map, calculated with phases from the platinum derivative (sites Pt1 and Pt2) gave the y coordinates of sites U12 and U3 (ghosts peaks due to a pseudo-symmetry center at $y = 1/4$ were much smaller and easily eliminated). The relative positions of the uranyl and platinum sites thus determined were then confirmed by the correlation function [18] calculated with $(F_{Pt} - F_U)^2$ coefficients.

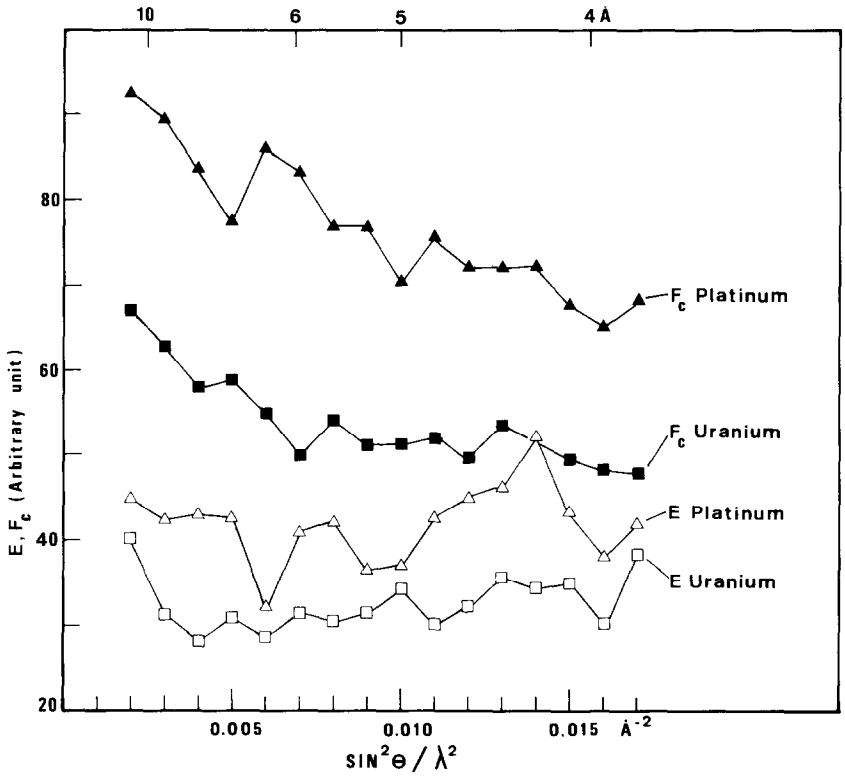

Fig. 2. Refinement statistics for the two heavy-atom derivatives. F_c is the mean heavy-atom scattering and E the root mean square lack of closure. The reliability R factor ($R = \sum \left| |F_{pH}^{obs}| - |F_{pH}^{calc}| \right| / \sum |F_{pH}^{obs}| - |F_p^{obs}|$) is 0.51 for the uranyl derivative and 0.46 for the platinum derivative.

stimulating discussions and gifts of enzyme preparations. We also thank Dr D. M. Blow for his help during the first intensity measurements and for his generous gift of computer programs. We acknowledge the skilful technical assistance of C. Pontillon. One of us (J-L. R.) was granted a short term EMBO fellowship during his stay in Cambridge (M.R.C. Laboratory of Molecular Biology). This work has been supported in part by a grant from the Délégation Générale à la Recherche Scientifique et Technique.

References

[1] Suddath, F. L., Quigley, G. J., Mc Pherson, A., Sneden, D., Kim, J. J., Kim, S. H. and Rich, A. (1974) Nature 248, 20–24.
[2] Rymo, L., Lagerkvist, U. and Wonacott, A. (1970) J. Biol. Chem. 245, 4308–4316.
[3] Lagerkvist, U., Rymo, L., Lindqvist, O. and Anderson, E. (1972) J. Biol. Chem. 247, 3897–3899.
[4] Waller, J. P., Risler, J. L., Monteilhet, C. and Zelwer, C. (1971) FEBS Letters 16, 186–188.
[5] Chirikjian, J. G., Wright, H. T. and Fresco, J. R. (1972) Proc. Natl. Acad. Sci. U.S. 69, 1638–1641.
[6] Reid, B. R., Koch, G. L. E., Boulanger, Y., Hartley, B. S. and Blow, D. M. (1973) J. Mol. Biol. 80, 199–201.
[7] Cassio, D. and Waller, J. P. (1971) Eur. J. Biochem. 20, 283–300.
[8] Koch, G. L. E. and Bruton, C. J. (1974) FEBS Letters 40, 180–182.
[9] Blanquet, S., Fayat, G. and Waller, J. P. (1974) Eur. J. Biochem. 44, 343–351.
[10] Lawrence, F., Blanquet, S., Poiret, M., Robert-Gero, M. and Waller, J. P. (1973) Eur. J. Biochem. 36, 234–243.
[11] Blanquet, S., Iwatsubo, M. and Waller, J. P. (1973) Eur. J. Biochem. 36, 213–226.
[12] Blanquet, S., Fayat, G., Waller, J. P. and Iwatsubo, M. (1972) Eur. J. Biochem. 24, 461–469.
[13] Cassio, D. and Waller, J. P. (1971) FEBS Letters 12, 309–312.

[14] Blake, C. C. F. (1968) in: Advances in Protein Chemistry (Anfinsen, C. B., Anson, M. L., Edsall, J. T. and Richards, F. M., eds.), Vol. 18, pp 59–120, Academic Press, New York and London.
[15] Matthews, B. W., Klopfenstein, C. E. and Colman, P. M. (1972) J. Phys. E. Sci. Instrum. 5, 353–359.
[16] Colman, P. M., Jansonius, J. N. and Matthews, B. W. (1972) J. Mol. Biol. 70, 701–724.
[17] Hamilton, W. C., Rollett, J. S. and Sparks, R. A. (1965) Acta Cryst. 18, 129–130.
[18] Rossmann, M. G. (1960) Acta Cryst. 13, 221–226.
[19] Blow, D. M. and Matthews, B. W. (1973) Acta Cryst. A29, 56–62.
[20] Matthews, B. W. (1970) in: Crystallographic Computing (Ahmed, F. R., Hall, S. R. and Huber, C. P., eds.), pp. 146–159, Munksgaard, Copenhagen.