Proteins of the *Bacillus stearothermophilus* ribosome

The structure of L6 at 6 Å resolution

Krzysztof Appelt, Isao Tanaka, Stephen W. White and Keith S. Wilson[+]

Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, D-1000 Berlin-Dahlem 33, Germany

Received 24 October 1983

The structure of protein L6 from the large subunit of the *Bacillus stearothermophilus* ribosome is described at a nominal resolution of 6 Å. The protein appears to be compact, with an axial ratio significantly below 2:1.

Ribosomal protein *Large subunit* *X-ray analysis*

1. INTRODUCTION

We have previously reported the crystallisation of the ribosomal protein L6 from *Bacillus stearothermophilus* [1] and now describe the X-ray analysis and structure of the protein to a nominal resolution of 6 Å. The structures of other ribosomal proteins have been reported from this group [2,3] and by authors in [4].

The isolation of protein L6 from *B. stearothermophilus* has been described elsewhere [5]. Sequence determination gave M_r values of 19168 and 18834 and chain lengths of 177 and 176 amino acids for the proteins from *B. stearothermophilus* and *E. coli* respectively [6,7]. The homology between the L6 from these two organisms is extensive, with identical residues at 48% of the positions, and the alignment required no insertions or deletions [6]. This is similar to the results reported for other ribosomal proteins [2,3] and clearly indicates very close similarity in tertiary structure. Thus we can expect to relate easily structural results on *B. stearothermophilus* to the functional studies carried out on *E. coli*.

Other structural studies of L6 have to date been restricted to NMR, CD and limited proteolysis of the protein from *E. coli* and *B. stearothermophilus* [5,8] and hydrodynamic studies of the shape of L6 from *E. coli* [9].

Little is known about the function of L6 in the eubacterial ribosome. The *E. coli* protein appears to bind to the 3′ end of 23 S RNA [10], and recently determined cross-linking between L6 and nucleotides 2473–2481 supports this hypothesis [11]. L6 has been located on the surface of the 50 S ribosomal subunit, close to proteins L11 and the L7/12-10 complex [12]. So far there is no evidence for the direct involvement of L6 in the binding of factors to the ribosome or in the activity of the peptidyl transferase center. However, L6 can affect the accuracy of translation [13]: bacteria containing mutant L6 are resistant to gentomycin (which causes misreading) and failed to translate a leaky 'amber' codon.

2. MATERIALS AND METHODS

2.1. *Protein isolation*

Extraction and purification of protein L6 has been described elsewhere [5]. The protein was kindly provided by Dr Jan Dijk.

[+] Present address: Department of Physics, University of York, York, England

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

2.2. *Protein crystallization*

Crystals are routinely grown by the hanging drop vapour-diffusion technique [14]. The protein concentration is about 8–12 mg/ml and the optimal conditions are 1.8 M phosphate, 5% dioxane (pH 7.6–8.2) at 20°C. The space group is $P6_122$ and the cell dimensions are $a = b = 72.7$ Å and $c = 124.9$ Å.

2.3. *Data collection and analysis*

Data were collected photographically using an Enraf-Nonius Arndt-Wonacott oscillation camera. CuK_α X-radiation was provided by a Seifert stationary anode operating with a fine focus tube at 40 kV and 30 mA. Native and derivative data were initially evaluated to a nominal resolution of 6 Å to permit rapid screening of potential heavy atom derivatives and calculation of a low resolution image of L6.

3. RESULTS AND DISCUSSION

Three heavy-atom reagents, $(NH_4)_2PtCl_4$, $K_2Pt(NO_2)_4$ and $K_2Pt(CN)_4$ produced good intensity changes, and 6 Å data were evaluated for each (table 1). The difference Patterson syntheses were difficult to solve and the direct methods programme MULTAN was used to locate the heavy atom positions [15]. The programme produced single site solutions for the $K_2Pt(NO_2)_4$ and $K_2Pt(CN)_4$ derivatives, which in retrospect gave a satisfactory solution of the difference Patterson syntheses. These interpretations were checked by cross-phased difference Fourier syntheses, using phases calculated from one derivative with single isomorphous + anomalous phase determination in the computation of the Fourier for the other. Similar maps showed the $K_2Pt(NO_2)_4$ derivative to be an essentially identical derivative to the $(NH_4)_2PtCl_4$, and defined the space group to be $P6_122$ rather than the enantiomorph.

The least-squares refinement of the heavy-atom parameters is summarised in table 2. Phases calculated with all 3 derivatives had a mean figure of merit of 0.77. The $K_2Pt(CN)_4$ derivative appears from photographs to give no significant intensity changes beyond about 5 Å resolution. The isomorphous differences are larger for the other two

Table 1

Summary of the 6 Å X-ray data for L6

Crystal	Concentration of heavy atom reagent (mM)	Soak time (days)	R_{sym} (%)	Δ_{iso} (%)
1. Native protein	–	–	3.46	–
2. $(NH_4)_2PtCl_4$	2	1	5.70	30.7
3. $K_2Pt(NO_2)_4$	2	2	5.20	28.2
4. $K_2Pt(CN)_4$	10	7	4.92	18.0

R_{sym} is defined as:

$$\sum_h \sum_i | I_{ih} - \bar{I} | \; / \; \sum_h \sum_i I_{ih}$$

where $\bar{I}$ is the mean intensity of i equivalent reflections with indices given by h. Δ_{iso} is the mean fractional isomorphous difference summed over all reflections

Table 2

The parameters for the least-squares refinement of the heavy atom derivatives of L6 at 6 Å resolution

Derivative	Occupancy	x	y	z	B	f_H	E	R_c
1. $(NH_4)_2PtCl_4$	0.900	0.033	0.758	0.030	15.0	193	169	67.7
2. $K_2Pt(NO_2)_4$	0.930	0.032	0.757	0.029	15.0	225	193	67.2
3. $K_2Pt(CN)_4$	0.672	0.806	0.496	0.051	15.0	160	125	64.0

The occupancies are on an arbitrary scale and the temperature (B) values have not been refined. f_H is the mean calculated heavy atom contribution, E is the r.m.s. lack of closure error. R_c is the R factor for the centric terms:

$$R = \sum_h || F_{PH} - F_P | f_H | \; / \; \sum_h | F_{PH} - F_P |$$

where F_P is the structure factor amplitude of the native protein and F_{PH} that of the heavy atom derivative

platinum reagents, but in both cases the crystals become more disordered and more susceptible to radiation damage. We are currently investigating means of improving these derivatives for use at higher resolution, and are also searching for other derivatives.

An electron density map was calculated using the phases from the platinum derivatives, and a slab of the density is shown in fig.1. There is reasonable contrast between protein and solvent regions, but the boundary between symmetry related molecules, which appear to be in close contact with one another, is not clear. Hence no molecular boundary is indicated in the figure. The most likely boundary, when the various symmetry elements (particularly the 2-fold rotation axes) within the unit cell are taken into account and also giving a location of the heavy-atom sites on the surface of the protein molecule, indicates a quite compact and globular molecule.

Therefore, from our 6 Å map, it would be premature to give definite values for the dimensions of the protein. However, even allowing for the various possibilities L6 is certainly constrained to have an axial ratio significantly below 2:1. The shape of L6 from *E. coli* has been reported from hydrodynamic studies to be a prolate elipsoid with

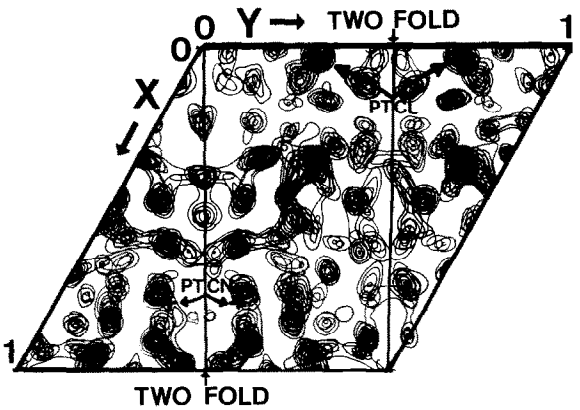

Fig.1. A superposition of 8 sections of the 6 Å electron density map of L6 in the *xy* projection. The slab of density is 15.6 Å thick and extends from $y = 0.028$ to $y = 0.153$. The heavy atom positions are indicated by the arrows and are disposed about the two 2-fold axes in the plane of the figure. 'PTCN' is the binding site for the $K_2Pt(CN)_4$ derivative, and 'PTCL' is the common binding site for the $(NH_4)_2PtCl_4$ and the $K_2Pt(NO_2)_4$ derivatives.

axial ratio 4–5:1 [9]. As for proteins L30 and S5 [2,3] the hydrodynamic studies have clearly overestimated this ratio, and our results to date give strong support to our feeling that the ribosome contains rather typical well-folded globular proteins.

ACKNOWLEDGEMENTS

We thank Professor H.-G. Wittmann for his support of this work and Howard Terry for technical assistance. The work has been in part funded by the Deutsche Forschungsgemeinschaft (SFB9).

REFERENCES

[1] Appelt, K., Dijk, J., White, S.W. and Wilson, K.S. (1983) FEBS Lett. 160, 75–77.
[2] Appelt, K., Dijk, J., White, S.W., Wilson, K.S. and Bartels, K. (1983) FEBS Lett. 160, 72–74.
[3] White, S.W., Appelt, K., Dijk, J. and Wilson, K.S. (1983) FEBS Lett. 163, 73–75.
[4] Leijonmarck, M., Eriksson, S. and Liljas, A. (1980) Nature 286, 824–826.
[5] Appelt, K. and Dijk, J. (1983) J. Biol. Chem., in press.
[6] Kimura, M., Rawlings, N. and Appelt, K. (1981) FEBS Lett. 136, 58–64.
[7] Chen, R., Arfsten, U. and Chen-Schmeisser (1977) Hoppe-Seyler's Z. Physiol. Chem. 358, 531–535.
[8] Wittmann, H.G., Littlechild, J. and Wittman-Liebold, B. (1980) in: Ribosomes: Structure, Function and Genetics (Chambliss, G. et al. eds) pp.51–88, University Park Press, Baltimore.
[9] Giri, L., Littlechild, J. and Dijk, J. (1977) FEBS Lett. 79, 238–244.
[10] Zimmermann, R.A. (1980) in: Ribosomes: Structure, Function and Genetics (Chambliss, G. et al. eds) pp.135–169, University Park Press, Baltimore.
[11] Wower, I., Wower, J., Meinke, M. and Brimacombe, R. (1981) Nucleic Acids Res. 9, 4285–4302.
[12] Stöffler-Meilicke, M., Epe, B., Steinhäuser, K.G., Woolley, P. and Stöffler, G. (1983) FEBS Lett. 162, 94–98.
[13] Kühberger, R., Piepersberg, W., Petzet, A., Buckel, P. and Böck, A. (1979) Biochemistry 18, 187–193.
[14] Davies, D.R. and Segal, D.M. (1971) Methods Enzymol. 22, 266–269.
[15] Wilson, K.S. (1978) Acta Crystallogr. B34, 1599–1608.