Proteins of the *Bacillus stearothermophilus* ribosome

A 5 Å structure analysis of protein S5

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The structure of protein S5 from the small subunit of the *Bacillus stearothermophilus* ribosome is described to a resolution of 5 Å. The molecular boundary is visible and shows the protein to be essentially compact although slightly elongated in one dimension.

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

1. INTRODUCTION

We have reported the crystallisation of the ribosomal protein S5 from *Bacillus stearothermophilus* [1] and here describe the X-ray analysis and structure of the protein to a resolution of 5 Å. Although the structures of other ribosomal proteins have been reported [2,3], this is the first from the small subunit.

The isolation of protein S5 from *B. stearothermophilus* has been described in [4], and it was shown to be intact and folded, by gel electrophoresis and NMR studies, respectively. The sequence determination [5] revealed an M_r of 17628 and a chain length of 166 amino acids. Furthermore, the homology with S5 from *Escherichia coli* was clearly established, the two sequences being identical at 55% of the positions. Thus at the tertiary structural level, the proteins from the two organisms are likely to be very similar. This will allow us to relate the extensive functional information obtained from the *E. coli* protein [1] to the structure of S5 from *B. stearothermophilus*.

2. MATERIALS AND METHODS

2.1. *Protein crystallisation*

Crystals were routinely grown by the sitting drop vapour-diffusion technique [6]. Conditions were ~6 mg protein/ml and 1.4 M phosphate, pH 7.3 at 20°C. These optimal conditions for growing large single crystals are slightly different from those in [1]. The space group is $P3_121$ and the cell dimensions are $a = b = 59.3$ Å and $c = 109.8$ Å.

2.2. *Data collection and analysis*

Data were collected photographically using an Enraf-Nonius Arndt-Wonacott oscillation camera. CuKα 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 collected and evaluated to a nominal resolution of 5 Å to permit rapid screening of heavy atom derivatives.

3. RESULTS AND DISCUSSION

Two derivatives, $KAu(CN)_2$ and $(NH_4)_2PtCl_4$, produced good intensity changes (see table 1), and 5 Å data were collected for each. The difference Patterson syntheses proved difficult to solve, and direct methods were employed in an attempt to locate the heavy atom positions [7]. In this technique, the structure factor differences between the derivative and the native are assumed to approximate the structure factors of the isolated heavy

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Table 1

Summary of the 5 Å X-ray data for S5

Crystal	Heavy atom reagent	Soak time	R_{sym} (%)	Δ_{iso} (%)
(1) Native protein	–	–	7.9	–
(2) $(NH_4)_2PtCl_4$ derivative	5 mM	2 days	13.2	21.3
(3) $KAu(CN)_2$ derivative	10 mM	5 days	5.4	29.4

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

atoms within the unit cell. Using these differences, the heavy atom constellation is then solved using the program MULTAN. The method worked excellently for the platinum derivative and the 2-site solution indicated by the ABSFOM figure of merit was clearly correct giving a satisfactory solution for the difference Patterson synthesis. The gold derivative, in contrast, produced several solutions with comparable ABSFOM values. However, a difference Fourier for the gold derivative using the single isomorphous + anomalous phases calculated from the platinum revealed 2 gold sites. These also explained the major peaks in the difference Patterson synthesis. The reverse cross-difference Fourier showed the original platinum sites plus a further minor site.

The results of the least-squares refinement of these heavy atom positions are shown in table 2. The gold derivative, despite having one site very close to a 2-fold rotation axis in the unit cell, gave an excellent minimum. The platinum derivative gave a poor minimum and is unlikely to be of value at higher resolution. The space group is $P3_121$, the enantiomorph having been defined from the anomalous scattering of the heavy atom derivatives. Phases based on both derivatives were calculated to 5 Å with a mean figure of merit of 0.72.

An electron density map was calculated using these phases, and fig.1 shows a 12 Å slab of the unit cell normal to the *ab* plane and extending from $Z = 0.37$–0.48. There is good contrast between protein and solvent regions and a molecular boundary is indicated in fig.1. Although this boundary is not completely unambiguous due to the close contact between symmetry related molecules, it is clear that the molecule is essentially compact and only slightly elongated with an axial ratio of about 1.5:1. Hydrodynamic studies of S5 from *E. coli* [8] have suggested that the molecule is more elongated, but the measured axial ratios of between 6:1 and 7:1 are an over-estimate. One explanation is that the hydrodynamic studies were carried out with S5 isolated in the presence of 6 M urea which is known to cause denaturation of the

Table 2

The parameters for the least-squares refinement of the heavy atom derivatives

Derivative		Occupancy	x	y	z	B	f_H	E	R_c
(1)	$(NH_4)_2PtCl_4$	0.60	0.054	0.437	0.022	15	141	88	0.554
		0.52	0.933	0.158	0.045	15			
		0.19	0.404	0.897	0.110	15			
(2)	$KAu(CN)_2$	0.53	−0.019	0.587	0.169	15	205	98	0.407
		1.03	0.713	0.399	0.102	15			

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 the RMS lack of closure error. R_c is the R factor for the centric terms during the refinement:

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

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

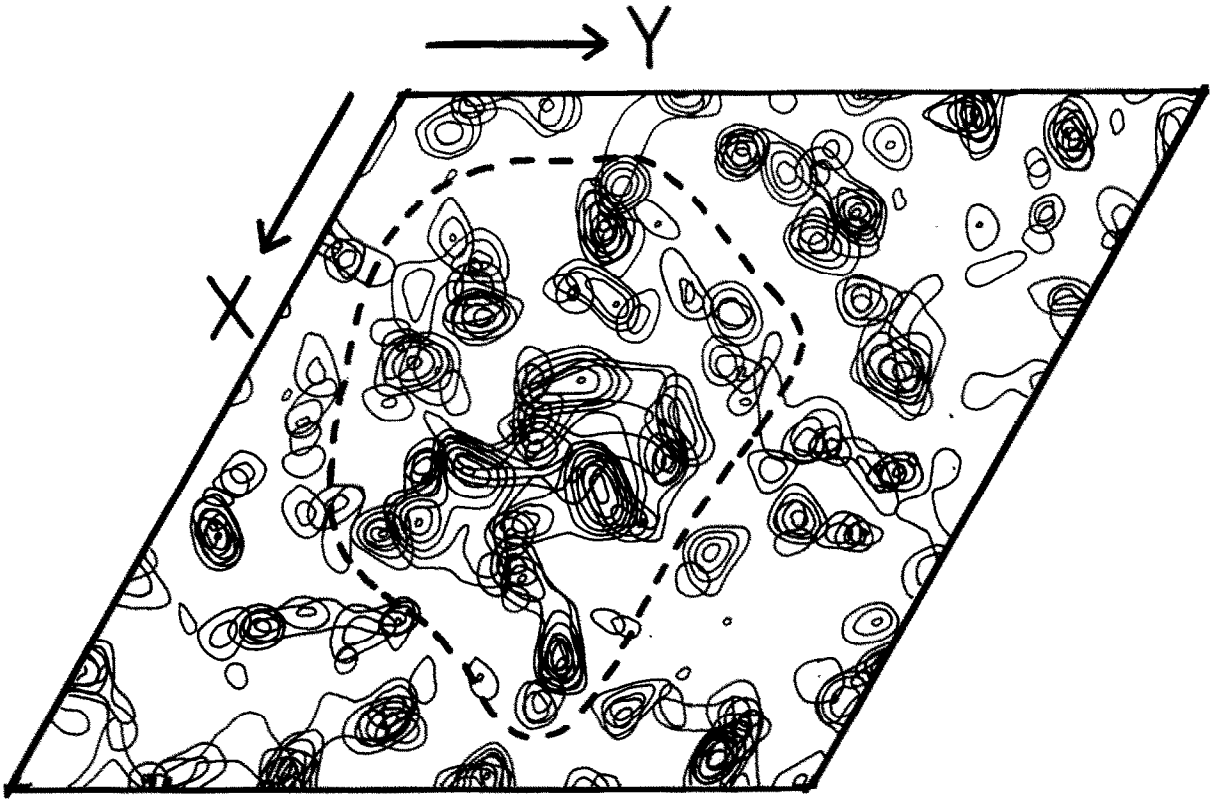

Fig.1. A superposition of 7 sections from $z = 0.37$ to 0.48 of the 5 Å electron density synthesis of protein S5. The dotted line indicates the most probable molecular boundary. A complete unit cell in the x and y directions is shown in these sections, with the origin of the cell at the top left.

protein molecule. In [9] neutron scattering data have indicated the S5 molecule to be compact.

We are searching for new derivatives, extending the present data to 3 Å, and hope to calculate a medium resolution image of the molecule in the near future.

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