Proteins of the \textit{Bacillus stearothermophilus} Ribosome

CRystallization of Proteins L30 and S5

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Proteins L30 and S5 from the 50 S and 30 S subunits, respectively, of the \textit{Bacillus stearothermophilus} ribosome have been crystallized. L30 crystals are tetragonal and the space group is P4\(_2\)2\(_1\)2 (or P4\(_2\)2\(_1\)2) with cell dimensions \(a = b = 46.3 \, \text{Å}\) and \(c = 61.4 \, \text{Å}\). S5 crystals are trigonal with the space group P3\(_2\)1 (or P3\(_2\)1) and cell dimensions \(a = b = 59.3 \, \text{Å}\) and \(c = 109.8 \, \text{Å}\). In both cases, there appears to be a single molecule in the asymmetric unit.

The purpose of this study is to describe crystals of the ribosomal proteins L30 and S5 from \textit{Bacillus stearothermophilus}. Both are of sufficient quality to allow high resolution analyses of their structures. This work follows from our preliminary report of crystallographic studies of ribosomal proteins (1).

L30 and S5 from \textit{B. stearothermophilus} were originally designated L34 (1) and S6 (2), respectively, according to their positions on two-dimensional gels. However, recent amino acid sequence information from \textit{B. stearothermophilus} ribosomal proteins has made it possible to redesignate the proteins according to their \textit{Escherichia coli} homologues. Thus, the proteins L30 and S5 described here are homologous to L30 and S5 from \textit{E. coli}. The sequences of all four proteins have been determined (4–6).

Protein L30 either from \textit{B. stearothermophilus} or \textit{E. coli}, when prepared under mild conditions, has a well ordered globular structure and is one of the smallest ribosomal proteins. The \textit{B. stearothermophilus} protein has a molecular weight of 7053 and contains 62 amino acids but lacks cysteine, tyrosine, tryptophan, or phenylalanine (3). Little is known of its globular structure and is one of the smallest ribosomal proteins from \textit{E. coli}.

Protein L30 consists of a sequence that has been found to be homologous to the \textit{E. coli} protein (6). The ribosomes lacking L30 have been obtained from erythromycin-dependent revertant mutants of \textit{E. coli} (6), and the mutants have growth rates roughly comparable to the wild type. This suggests that L30 is not an essential ribosomal protein. On the other hand, an indication of the functional significance of L30 is the reduced rate of poly(Phe) synthesis observed when prepared under mild conditions, has a well ordered globular structure and is one of the smallest ribosomal proteins. The \textit{B. stearothermophilus} protein has a molecular weight of 7053 and contains 62 amino acids but lacks cysteine, tyrosine, tryptophan, or phenylalanine (3). Little is known of its globular structure and is one of the smallest ribosomal proteins from \textit{E. coli}.

The molecular weight of S5 from \textit{B. stearothermophilus} is 17,628 and it contains 166 amino acids. There is much evidence from \textit{E. coli} that the molecule has an essential function in protein biosynthesis although this cannot be precisely defined. Affinity immune electron microscopy (9) and photoaffinity labeling with analogues of mRNA (10) and puromycin (11) demonstrate that it is situated close to the decoding region of the 30 S subunit. Furthermore, antibiologically induced mutants of \textit{E. coli} have been isolated which contain specifically altered S5 proteins (5). The mode of action of these antibiotics (12) together with other evidence (13, 14) suggest a role for S5 in the translocation process.

EXPERIMENTAL PROCEDURES

Protein Extraction—The procedure is essentially the same as described previously and is briefly summarized below. The major difference is that 70 S "tight couples" (15) are used as starting material instead of 50 S subunits in order to maximize the yield of proteins currently being studied by x-ray diffraction.

The ribosomes were washed for 12 h with a solution of 1 M NaCl in HEPES* buffer, pH 7.0, in the presence of Mg\(^2+\)*, and the extract was fractionated on a carboxymethyl (CM)-Sepharose CL-6B column using an NaCl gradient (0.07–0.7 M). The process was repeated after a second wash with 2 M NaCl. L30 was obtained from both extracts and eluted from the CM-Sepharose column at around 0.25 M NaCl. S5 appeared only in the first extraction and eluted at approximately 0.35 M NaCl. Both proteins were contaminated with other ribosomal proteins and were further purified by gel filtration on a Sephadex G-50 (superfine) column.

Crystallization—Crystals of L30 and S5 were grown by the "hang-drop" vapor diffusion technique (16). The conditions were determined from a series of experiments using a variety of precipitants and buffers. The materials and methods used have been fully described in a previous publication (1).

Gel Electrophoresis—To confirm that the crystals contain intact protein and not proteolytic fragments, the crystalline material was analyzed by sodium dodecyl sulfate-polyacrylamide electrophoresis. Several large crystals were washed twice in stabilizing solution (see "Results") and dissolved in 30 µl of distilled water followed by 60 µl of sample buffer and 10 µl of mercaptoethanol. A 20-µl aliquot of the mixture was analyzed on a 15% sodium dodecyl sulfate gel (17) together with 2 µg of noncrystalline material. The results clearly showed that the crystals contain pure, intact protein.

X-ray Diffraction—Diffraction patterns were recorded on a Nonius precession camera with a crystal to film distance of 75 mm and a 0.5-mm collimator. CuK\(_\alpha\) x-rays were produced from a Seifert stationary anode operating with a fine focus tube at 55 kV and 35 mA.

RESULTS

L30—Crystals of L30 grow reproducibly in 2–4 weeks from 3.85 M \((\text{NH}_4)_2\text{SO}_4\), between pH 7.6 and 8.8, using as buffer HEPES or Tris between pH 7.6 and 8.4 and Bicine for the pH range 8.2–8.8. Crystals grow optimally at pH 8.4 in 0.1 M Tris-Cl buffer, 3.85 M \((\text{NH}_4)_2\text{SO}_4\), and at protein concentrations of 4–10 mg/ml. The crystals grow as tetragonal bipyr- amids up to 0.5 mm in each dimension (Fig. 1a). Unfortunately, such large single crystals grow very seldom, and it is often necessary to seed smaller crystals into fresh protein solution.

The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Bicine, N,N-bis(2-hydroxyethyl)glycine.
The crystals are well ordered and diffract to at least 2.5-Å resolution. Fig. 2a shows a precession photograph of the \(\text{hk}0\) major zone of the native protein. The 4-mm symmetry of the \(\text{hk}0\) and \(\text{hkl}\) zones shows the crystals to be tetragonal. Systematic absences for the \(\text{h}0\text{0}\) reflections \((h \neq 2n)\) and \(\text{00l}\) reflections \((l \neq 4n)\) clearly identify the space group as \(P4_2\) or its enantiomorph \(P4_222\). The cell dimensions are \(a = b = 46.3\) Å and \(c = 61.4\) Å, giving a unit cell volume of \(V = 131,623\) Å\(^3\). Taking the known molecular weight of L30 (7053), the presence of 1 molecule/asymmetric unit gives a value of \(V_m\) (volume of the asymmetric unit/molecular weight) of 2.33 Å\(^3\)/dalton. This value lies close to the mean value, 2.4 Å\(^3\)/dalton, found for a range of protein crystals (18).

\text{S5}—Crystals of S5 grow in 4-6 weeks from 1.2 M phosphate (NaH\(_2\)PO\(_4\)/K\(_2\)HPO\(_4\) mixture) between pH 7.2 and 8.2 and at a protein concentration of 5-10 mg/ml. Large single crystals grow optimally between pH 7.2 and 7.6, and showers of smaller ones tend to occur at higher pH. The small crystals are useful as seeds when transferred to a protein solution in the optimal conditions. The crystals have a hexagonal morphology and grow up to 0.7 mm in width and 0.5 mm in thickness (Fig. 1b). Fully grown crystals can be conveniently stored in a stabilizing solution of 1.5 M NaH\(_2\)PO\(_4\)/K\(_2\)HPO\(_4\) at pH 7.4.

The crystals diffract strongly to a resolution better than 3.0 Å and survive continuous irradiation at room temperature for approximately 3 days. Fig. 2b shows a precession photograph of the \(\text{hk}0\) zone (into the hexagonal face of the crystal). The former indicates either a trigonal or a hexagonal space group, but the upper level zones, \(\text{hkn}\), clearly show 3-fold symmetry coincident with the \(c\) axis. Systematic absences for the \(\text{00l}\) reflections \((l \neq 3n)\) identify the space group as \(P3_1\) or its enantiomorph \(P3_2\). The cell dimensions are \(a = b = 59.3\) Å and \(c = 109.8\) Å, and the unit cell volume \(V = 334,382\) Å\(^3\). Taking the known molecular weight of S5 (17,628) and assuming 1 molecule in the asymmetric unit, the packing density in the crystal is 3.2 Å\(^3\)/dalton. This implies a relatively loosely packed unit cell but is nevertheless within the range for typical protein crystals (18).

**DISCUSSION**

The relationship of the present L30 crystals to those reported in a preliminary report (1) (when the protein was labeled BL34) is not obvious. The morphology is strikingly similar to that of the original tetragonal crystals. However, the unit cell dimensions obtained, from the admittedly very small crystals with maximum dimension 0.2 mm, were previously \(a = 44\) Å and \(c = 129\) Å; that is, a closely similar value for \(a\), but with the \(c\) axis more than doubled in length. Given the very poor diffraction originally observed, we have not been able to establish any relationship between the old and new tetragonal crystals. Clearly the present crystals are more suitable for crystallographic analysis.

S5 is the first small subunit ribosomal protein to be successfully crystallized. The crystallization of L30 and several other large subunit proteins demonstrates that they behave as normal globular proteins when purified under nondenaturing conditions (1). It may now be anticipated that the same will be true for at least some of the small subunit proteins purified by the same technique.

**FIG. 1.** Crystals of L30 (a) and S5 (b) grown by the hanging-drop vapor diffusion technique (17). The bar indicates a length of 0.5 mm.
Fig. 2. Precession photographs of L30 and S5. a, 15° precession photograph of the native L30 protein (hk0 zone); b, 12° precession photograph of the native S5 protein (hk0 zone). Both photographs were recorded in 12 h using CuKα, nickel-filtered radiation from a Seifert stationary anode source (35 kV and 35 mA) with an Enraf-Nonius precession camera. The crystal to film distance was 75 mm.

Diffraction data from L30 and S5 native crystals have been collected using an Arndt-Wonacott oscillation camera to a resolution of 2.5 and 3.5 Å, respectively. Also, potential heavy atom derivatives have been found for each, and data collection is in progress.

Finally, it should be noted that two independent preparations of S5 show differences in their amino acid sequences (3). One preparation has been completely sequenced, and the second only partially. The crystals discussed in this paper were produced from the latter preparation. However, crystals with an identical morphology have recently been obtained from the preparation for which the entire sequence is available, and these are currently being investigated for any differences that may be attributable to the altered amino acid sequence.

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REFERENCES
1. Appelt, K., Dijk, J., Reinhardt, R., Sanhuesa, S., White, S. W., Wilson, K. S. & Yonath, A. (1981) J. Biol. Chem. 256, 11787–11790
2. Isono, S. & Isono, K. (1975) Eur. J. Biochem. 50, 483–488
3. Kimura, M. (1983) J. Biol. Chem., in press
4. Ritter, E. & Wittmann-Liebold, B. (1975) FEBS Lett. 60, 153–155
5. Wittmann-Liebold, B. & Greuer, B. (1978) FEBS Lett. 95, 91–98
6. Dabbs, E. R. (1979) J. Bacteriol. 140, 734–737
7. Coblentz, J. A. & Nomura, M. (1976) J. Biol. Chem. 251, 209–221
8. Aron, P. E. & Fahnstock, S. R. (1981) J. Biol. Chem. 256, 10105–10110
9. Keren-Zar, M., Boublik, M. & Ofengand, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1054–1058
10. Towbin, H. & Elson, D. (1978) FEBS Lett. 90, 203–206
11. Nierhaus, K. H. & Wittmann, H. G. (1980) Naturwissenschaften 67, 234–250
12. Thomas, G., Sweeney, R., Chang, C. & Noller, H. F. (1975) J. Mol. Biol. 95, 91–102
13. Marsh, R. C. & Parmenter, A. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 151–155
14. Noll, M. & Noll, H. (1974) J. Mol. Biol. 99, 477–494
15. Davies, D. R. & Segal, D. M. (1971) Methods Enzymol. 22, 266–269
16. Matthews, B. W. (1968) J. Mol. Biol. 33, 491–497
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