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Crystallization of $tRNA^{Leu}$-Synthetase from Baker's Yeast

(x-ray diffraction/3.5-Å resolution)

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ABSTRACT **$tRNA^{Leu}$-Synthetase from baker's yeast has been crystallized from ammonium sulfate solution. The crystals are of orthorhombic symmetry, falling into space group I_{222} or $I_{2_12_12_1}$ with $a = 75.5$ Å, $b = 110.7$ Å, and $c = 124.0$ Å. This unit cell contains four molecules of the native (dimeric) enzyme, with eight asymmetric units, each corresponding to the enzyme subunit. Diffraction patterns obtained for the three major projections contain reflections to spacings of less than 3.5 Å, indicating that this crystal form is amenable to structure analysis to atomic resolution.**

An aminoacyl-tRNA synthetase catalyzes the activation of one particular amino acid and its subsequent transfer to a homologous tRNA. The specificity of recognition among enzyme, amino acid, and tRNA, which is of great interest, must lie in large measure in the topography of the enzyme. $tRNA^{Leu}$-Synthetase from baker's yeast is of special interest because it recognizes a cognate tRNA ($tRNA_3^{Leu}$) not only in a native conformation, but also in a denatured one (1–3). Thus, it is inhibited by the denatured conformer of $tRNA_3^{Leu}$ (3), with which it forms a stable complex (ref. 4, and manuscript in preparation), even though it cannot catalyze its aminoacylation (3).

As an approach to understanding tRNA-synthetase recognition, we have focused on $tRNA_3^{Leu}$ and its cognate synthetase: we determined the sequence of the tRNA (5), its physical and chemical properties in native and denatured forms (2, 6, 7), and their interaction with various tRNA-specific enzymes (3). We have also purified the synthetase to homogeneity and characterized it (4, *J. Biol. Chem.*, submitted). The enzyme has a molecular weight of 120,000 and contains two presumably identical subunits. We now report the crystallization of this enzyme and some characteristics of the crystals.

RESULTS

$tRNA^{Leu}$-synthetase

The purification procedure (*J. Biol. Chem.*, submitted) was scaled up to process 50 kg of yeast, with yields of 300–400 mg of near-homogeneous enzyme. For the latter preparation, stationary phase but unripened yeast was the source instead of the logarithmic-phase cells used earlier.

Before crystallization, the purified protein was characterized. On velocity ultracentrifugation, a major component was observed with $s^0_{20,w} = 6.05$, characteristic of the native enzyme, along with a minor component (about 15%) with an $s^0_{20,w} = 8.0$, which may be an aggregate of the native enzyme. Polyacrylamide gel electrophoresis (Fig. 1*a*) revealed a major (>90%) band and some very minor ones. Of 12 other amino-acid acceptor activities checked, only that for tyrosine was present, and only in trace amounts. The specific activity of this preparation for aminoacylation of tRNA with leucine was at least 30% of the maximum value observed for enzyme purified from log-phase cells.

Crystallization

Protein (40–65 mg/ml) in 0.14 M phosphate buffer (pH 7.5) was dialyzed against a solution containing 50 mM phosphate (K^+), pH 7.5–0.2 mM EDTA–0.2 mM dithiothreitol, then clarified by centrifugation. Crystals were obtained in 10–14 days by dialysis against 50%-saturated ammonium sulfate in Sorensen's buffer (pH 4.6–6.5) at 4°. Upon transfer from the mother liquor to 60%-saturated ammonium sulfate at the same pH, the crystals remain stable for at least a month at 4°. The yield of crystalline protein was 10–30%, as estimated from the size and number of crystals obtained.

The crystals (Fig. 2) are typically soft but resilient, with well-formed faces and edges and obvious external symmetry. Typical crystals have dimensions 0.4–0.5 × 0.4–0.5 × 0.2–0.3 mm, although some are substantially larger.

Biochemical characterization of the crystalline enzyme

Polyacrylamide gel electrophoresis under nondenaturing conditions of isolated crystals washed with 60%-saturated ammonium sulfate (Fig. 1*b*) reveals a single homogeneous band of protein with the same mobility as the major component observed before crystallization (Fig. 1*a*). Crystallization, therefore, removes the minor contaminants.

The pattern of bands after treatment of the crystalline protein with 1% mercaptoethanol–1% sodium dodecylsulfate (100°, 3 min) was also examined. Under such denaturing conditions, the homogeneous enzyme before crystallization typically shows a predominant and sometimes single band corresponding to a molecular weight of 55,000–60,000, i.e., half the molecular weight of the native enzyme. However, in some preparations two additional bands of protein, corresponding to molecular weights of 76,000 and 38,000, are observed in lesser amounts. For the crystalline protein, such gels (Fig. 3*b*) show two major bands. The band of higher molecular weight (55,000–60,000) contains about twice the protein in the band of lower molecular weight (35,000–40,000). Two trace bands, corresponding to 70,000 and 80,000, are also evident. Considering that the crystalline protein shows a single band under nondenaturing conditions, and that the crystal symmetry (see

below) is consistent with identical subunits, we conclude that the asymmetric fragmentation pattern on gels run in the presence of sodium dodecyl sulfate is artifactual. In any case, the fragments observed correspond to fragments found previously for preparations of active enzyme from log-phase yeast.

Because of the need to conserve crystals, only one was washed carefully to remove mother liquor and assayed for aminoacylation of yeast tRNA. Such assays indicate leucine aminoacylation activity in a reasonable amount as compared to homogeneous enzyme before crystallization (10–100%). However, an accurate value for specific activity could not be obtained due to the small quantity of protein in the crystal and possible inhibition of the assay by the ammonium sulfate present.

The foregoing observations identify the crystalline protein as tRNA^{Leu}-synthetase.

Characterization of the crystals

Crystals were mounted in glass capillaries for x-ray diffraction analysis. Precession photographs were taken at 22–24° for about 24 hr with CuKα radiation from an Elliott rotating anode source.

Fig. 4 shows the three major projections of the tRNA^{Leu}-synthetase crystals. The unit cell is orthorhombic, with lattice dimensions $a = 75.5$ Å, $b = 110.7$ Å, and $c = 124.0$ Å. It is not possible from precession photographs to distinguish between the two possible space groups, I_{222} or $I_{2_12_12_1}$. The hk0 projection (Fig. 4*a*) shows a sharper dropoff in spot intensity in the h00 direction than in the 0k0 direction. Nevertheless, the diffraction record extends to the 3.5-Å resolution limit permitted by the precession angle in all directions of reciprocal space. The absence of a similar dropoff in the other projections (Fig. 4*b* and *c*) suggests that this is not a temperature effect, but rather characteristic of the structure.

The crystals suffer extensive deterioration in crystalline order after 24 hr of exposure to the x-ray beam. We have not established whether this is due to the treatment of the crystals before irradiation, the change of ambient temperature from

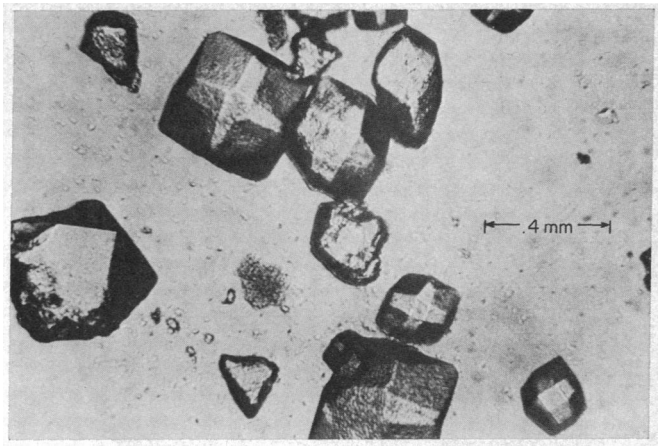

FIG. 2. Photomicrograph of single crystals of tRNA^{Leu}-synthetase.

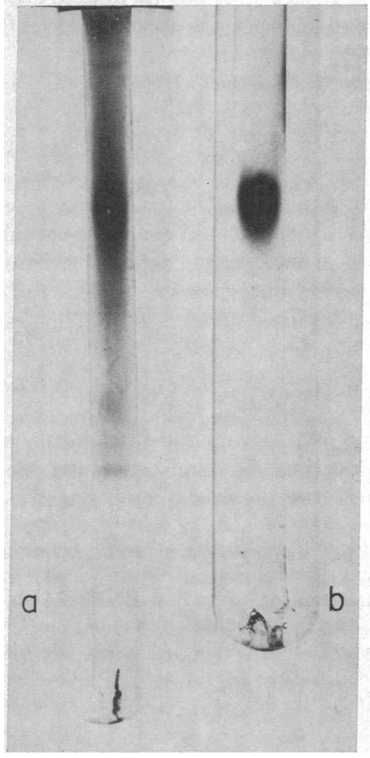

FIG. 1. Polyacrylamide gel electrophoresis of tRNA^{Leu}-synthetase: (*a*) source material for crystallization; (*b*) crystalline protein. Electrophoresis gels of 0.3-mm diameter were run at 4°, 1 mA/tube, for 4 hr. For comparison, the two gels are aligned at the starting point (*top*).

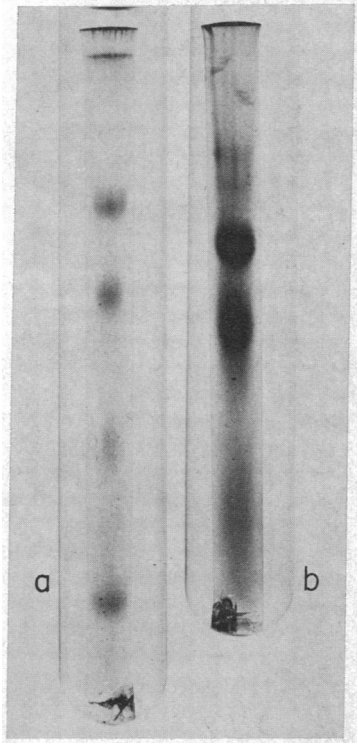

FIG. 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis: (*a*) bovine serum albumin, chymotrypsinogen, ovalbumin, and lysozyme as marker proteins; (*b*) crystalline tRNA^{Leu}-synthetase. The photographic exposure renders the faster-moving band more intense than is actually the case. Gels were run as in Fig. 1, except at 2 mA/tube, until a bromophenol blue marker band reached the bottom of the gel.

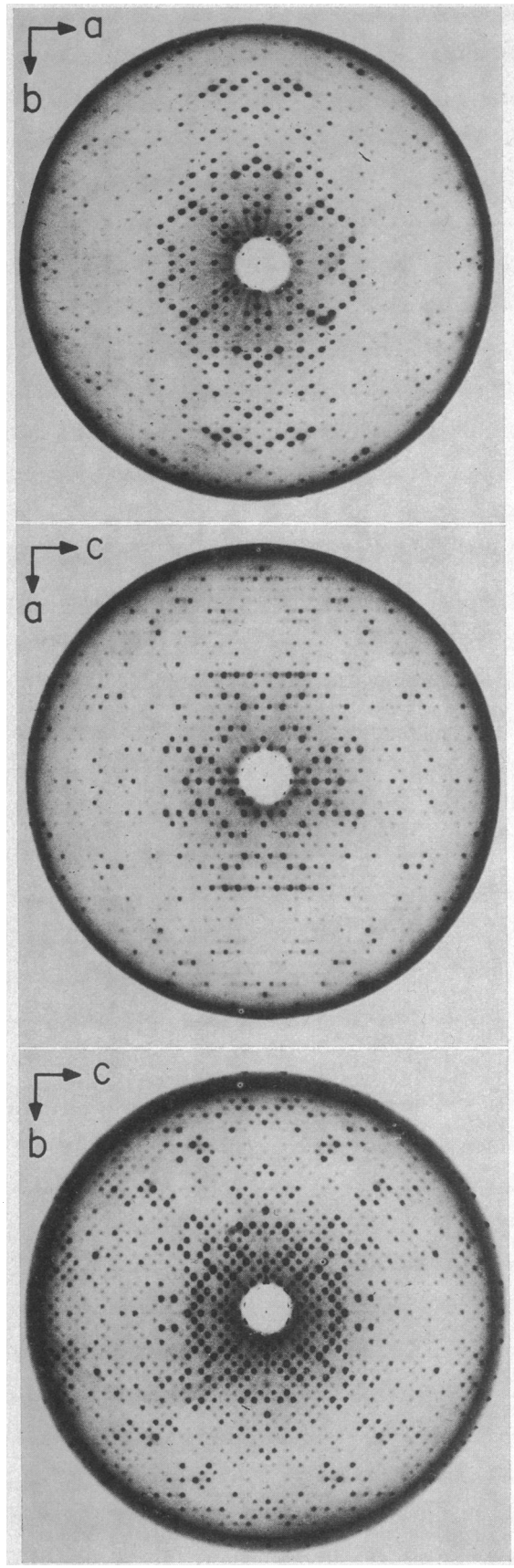

FIG. 4. Precession photographs of the three major projections of crystals of $tRNA^{Leu}$-synthetase. The precession angle was 13°, and the crystal–film distance was 75 mm. *Top:* hkO; *middle:* hOl; *bottom:* Okl.

4°C to 22–24°, radiation damage, or some combination of these factors.

When account is taken of the eight asymmetric units required by the space group, the dry volume of the protein (145,600 Å^3; $\bar{v}$ = 0.736) and the unit cell volume of 1.04 × 10^6 Å^3, it becomes apparent that the unit cell contains four molecules of native enzyme of molecular weight 120,000. On this basis, each asymmetric unit corresponds to the biochemically identified subunit of molecular weight 60,000; it follows that the subunits are identical, as presumed. The volume of 259,000 Å^3 occupied by each enzyme molecule in the crystal is close to the value of 282,000 Å^3 calculated from the hydrodynamically determined Stokes radius. Moreover, the value of V_M (8) calculated by assigning the subunit molecular weight to the asymmetric unit is 2.16 Å^3/dalton, a value that lies near the middle of the frequency distribution of V_M values found for many other protein crystals. From this V_M value, it follows that the crystals are 57% protein by volume. Independently, we calculate from the unit cell volume and the anhydrous volume of the protein that the crystals are 56% protein by volume. The crystal density, measured in a bromobenzene–xylene gradient, is 1.245 g/cm^3.

DISCUSSION

Although many aminoacyl-tRNA synthetases have been purified to homogeneity, only $tRNA^{Lys}$-synthetase from yeast has been crystallized in native form (9). These crystals have an unusually high solvent content, which probably contributes to the anisotropy and limited resolution of the x-ray diffraction patterns (10). A large enzymatically active fragment of $tRNA^{Met}$-synthetase obtained from *Escherichia coli* after tryptic digestion, but not the native enzyme, has also been crystallized in a form with favorable diffraction characteristics (11).

In the present work, native $tRNA^{Leu}$-synthetase from yeast was crystallized in a form that appears to be particularly favorable for structure analysis to atomic resolution. The unit cell is of a tractable size with no inconvenient lattice dimensions, and the symmetry of the native enzyme is incorporated into the symmetry of this unit cell. The crystals diffract to a resolution that should permit detailed structure determination. The chemistry of the enzyme appears to be attractive as well. With two identical subunits and 8–9 methionine residues in each, the sequence analysis that must accompany the structure determination is realistic, even for a polypeptide chain of 500–600 residues.

We are now attempting the preparation of heavy-atom isomorphous replacements and examining the possibility of binding substrates to the crystalline enzyme.

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