Crystallization and Preliminary X-ray Investigation of Recombinant Lactobacillus leichmannii Nucleoside Deoxyribosyltransferase*

(Received for publication, October 2, 1989)

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Crystals of recombinant bacterial nucleoside deoxyribosyltransferase have been grown from solutions of ammonium sulfate. The crystals are cubic, space group I23 or I213; the axial length is 151.1(2) Å. The crystals are stable to x-rays for at least 5 days and diffract beyond 2.8-Å resolution. It appears that the molecule, which is a hexamer, utilizes the symmetry of the space group, resulting in two or three subunits per asymmetric unit.

Nucleoside deoxyribosyltransferase (trans-N-deoxyribosylase; nucleoside:purine(pyrimidine) deoxyribosyltransferase, EC 2.4.2.6) catalyzes the direct transfer of the deoxyribosyl moiety from a purine (or pyrimidine) residue on a nucleoside to a purine (or pyrimidine) base. The enzyme has been found only in Lactobacillus species and related microorganisms that require deoxyribonucleosides for growth (1). The nucleoside deoxyribosyltransferase from Lactobacillus leichmannii appears to have low specificity for both donor and acceptor substrates (2); in contrast, two different nucleoside deoxyribosyltransferases with distinct substrate specificities have been reported for Lactobacillus helveticus (3, 4). One L. helveticus enzyme showed specificity for transfer between purine bases, while the second enzyme catalyzed the transfer of the deoxyribosyl moiety between purines or pyrimidines as well as from a purine to a pyrimidine. The molecular weight of L. leichmannii nucleoside deoxyribosyltransferase has not been reported; the reported molecular weight of L. helveticus nucleoside deoxyribosyltransferase is about 86,000 (3).

Compared to conventional chemical synthetic methods, enzymatic transglycosylation cannot simply the synthesis of certain purine and pyrimidine nucleoside analogs, but also facilitate purification of the desired nucleoside analog product. The Lactobacillus nucleoside deoxyribosyltransferases have been employed for synthesis of a variety of nucleoside analogs, including purine 2'-deoxynucleosides that have antileukemic and immunosuppressive activity (5). Although it was thought that the Lactobacillus nucleoside deoxyribosyltransferase was specific for nucleosides containing a 2'-deoxyribosyl moiety, Carson and Wasson (6) have shown recently that 2',3'-dideoxynucleosides can also serve as substrates, although the reaction proceeds at a much slower rate.

Microcrystals of nucleoside deoxyribosyltransferase obtained from L. helveticus have been reported (7), but these were not of sufficient quality to be used for x-ray studies. In this paper, we report conditions for the crystallization of recombinant L. leichmannii nucleoside deoxyribosyltransferase and the crystallographic data obtained by x-ray diffraction methods.

EXPERIMENTAL PROCEDURES

The L. leichmannii nucleoside trans-N-deoxyribosylase gene, the ntd gene, was cloned; the complete DNA sequence was determined, and the nucleoside deoxyribosyltransferase encoded by the ntd gene was expressed in Escherichia coli. E. coli cell-free extracts containing the nucleoside deoxyribosyltransferase were prepared and fractionated as described previously for Lactobacillus extracts (2, 7), except that the nucleoside deoxyribosyltransferase was purified using Mono Q column FPLC* chromatography. The purified enzyme was dialyzed against 100 mM sodium phosphate buffer, pH 6.0, and diluted to a final protein concentration of 40 mg/ml. The subunit molecular weight for the L. leichmannii nucleoside deoxyribosyltransferase expressed in E. coli was calculated from the ntd open reading frame and confirmed by electrophoresis of the purified enzyme in sodium dodecyl sulfate-polyacrylamide gels (8). The subunit composition of the holoenzyme was determined by gel filtration through a Pharmacia LKB Biotechnology Inc. Superose 12 FPLC column using cytochrome c (Mr = 12,400), carbonic anhydrase (Mr = 29,000), yeast alcohol dehydrogenase (Mr = 155,700), and β-amylase (Mr = 200,000) as molecular weight standards. Protein concentrations were measured by the method of Lowry et al. (9).

Crystals suitable for x-ray analysis were obtained by vapor diffusion equilibration of 2-μl droplets hanging from siliconized coverslips inverted on Linbro plates. The droplets consisted of 1 μl of a solution containing 40 mg of protein/ml plus 1 μl of a 5% saturated solution of ammonium sulfate in 0.05 M citrate buffer, pH 5.4. These droplets were equilibrated against 1 ml of 5% saturated solution of ammonium sulfate in 0.05 M citrate buffer, pH 5.4. After 3-4 days at room temperature, rhombic dodecahedral crystals with dimensions up to 0.3 mm on edge were obtained.

For x-ray studies, crystals were transferred to a 30% saturated solution of ammonium sulfate in 0.05 M citrate buffer, pH 5.4. Crystals were mounted in glass capillaries and were photographed on a precension camera at 22 °C using Cu Kα radiation from a Rigaku RU-300 rotating anode generator operating at 40 kV and 100 mA. A complete native data set was collected on a Nicolet X-100A area detector using the same radiation source.

RESULTS AND DISCUSSION

Crystals of L. leichmannii nucleoside deoxyribosyltransferase exhibit rhombic dodecahedral morphology. Crystals grew in the pH range 4.6-8.0, but the largest crystals grew below pH 5.8.

* The abbreviation used is: FPLC, fast protein liquid chromatography.

1 S. A. Short and C. R. Richards, unpublished data.

2 This research was supported by Grants GM-38823, CA-13148, and DE-02670 from the National Institutes of Health, Grant CH-213 from the American Cancer Society, and a grant from the National Foundation for Cancer Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
X-ray diffraction data were initially collected to 2.8-Å resolution using the area detector. Oscillation frames covered 0.25° and were measured for 5 min. Two crystals were used to measure a total of 141,326 reflections; these were merged into 11,929 unique reflections. Of the 2,677 reflections in the highest resolution range (2.8–3.0 Å), 1,083 reflections (40%) had I > 3σI. Indexing and integration of intensity data were carried out using the XENGEN processing programs. The data indexed in the cubic system with a = 151.1(2) Å and confirmed the Laue group m3. The systematic absence of hkI reflections with (h + k + l) = (2n + 1) indicates either space group I23 or I213. Subsequent x-ray precession photographs of nucleoside deoxyribosyltransferase confirmed the possible space groups and unit cell axis. The crystals are stable to x-rays at room temperature and diffract beyond 2.8-Å resolution.

Molecular weight studies performed on the purified protein reveal a molecular weight for the intact enzyme of approximately 110,000 and a subunit molecular weight of approximately 18,000. This molecular weight is significantly higher than the reported molecular weight of the L. helveticus enzyme (3). The amino acid sequence, deduced from the ntd DNA sequence, consists of 157 amino acids; the calculated molecular weight is 18,081. Therefore, the L. leichmannii nucleoside deoxyribosyltransferase is a hexamer of six identical subunits.

The rotational symmetry elements in the space group I23 or I213 that can be utilized in the quaternary structure include 2-fold and 3-fold axes. Based on a subunit molecular weight of 18,081 for the L. leichmannii nucleoside deoxyribosyltransferase expressed in E. coli, the calculated value of Vm / (11) for three subunits/crystallographic asymmetric unit is 2.65. Assuming a partial specific volume of 0.74 cm³/g, this value corresponds to a solvent volume fraction of 34%. The Vm value for two subunits/crystallographic asymmetric unit is 3.97, with a solvent volume fraction of 69%. Both of these values lie in the range reported for other protein crystals (11). Therefore, it appears that the molecule utilizes either the 2-fold or 3-fold symmetry of the space group, resulting in three or two subunits per asymmetric unit, respectively.

REFERENCES
Crystallization and preliminary X-ray investigation of recombinant Lactobacillus leichmannii nucleoside deoxyribosyltransferase.
W J Cook, S A Short and S E Ealick


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