NEOCARZINOSTATIN: AN ANTITUMOR PROTEIN

A PRELIMINARY X-RAY DIFFRACTION STUDY

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SUMMARY The antitumor protein, neocarzinostatin, has been crystallized and examined by X-ray diffraction. Crystals of this globular protein are of space group $P2_12_12_1$ with cell parameters a = 27.4 Å, b = 33.9 Å and c = 102.0 Å. There is one molecule of approximately 27 Å diameter per asymmetric unit. Crystals soaked in a K_2HgI_4 solution give diffraction patterns significantly different from native crystal diffraction patterns.

Recently, several protein antibiotics have attracted interest because of their antitumor activity and relatively low toxicity. These agents are small proteins that have been isolated from the culture filtrate of various microorganisms belonging to the genus Streptomyces and include Carzinostatin (1), Mitomalcin (2), Actinoxanthin (3), Macromomycin (4), Lymphomycin (5), Actinocarcin (6) and Neocarzinostatin (7).

Neocarzinostatin (NCS) isolated from culture filtrate of Streptomyces carzinostaticus Var F41 (7,8) inhibits growth of several gram-positive organisms (7) and human leukemic cells (CCRF-CEM) in suspension culture (9). Experimental antitumor activity has been demonstrated for NCS against a number of transplantable solid tumors and leukemias in mice (7,10). In clinical trials in man, a remission rate of 50% was obtained in a group of patients with acute leukemia (11). Tumor regression was also observed in certain solid tumors, particularly metastatic gastrointestinal tumors (12,13). Mode of action experiments indicate that NCS inhibits DNA synthesis (14,15), and

also causes degradation of existing DNA (15) in Sarcina lutea. It was recently reported that NCS induces DNA breakages in intact HeLa S3 and L1210 cells in vivo (16-18) and SV 40 and calf thymus DNA in vitro (17,18).

NCS is a single-chain polypeptide with 109 amino acid residues, and is the first antibiotic protein for which the amino acid sequence has been determined (19,20). Two disulfide bonds and a large proportion of alanine, glycine, valine, serine and threonine contribute to a tightly folded conformation which is highly resistant to proteolysis and biochemical modification. Circular dichroic and infrared studies indicate the protein is composed of β-pleated sheet and little or no α-helix structures (21).

Chemical modification studies have been initiated with the intent of determining the important features of this molecule for biologic activity and hopefully of increasing its antitumor potency (21). Structure-activity correlation related to the three-dimensional structure of the molecule

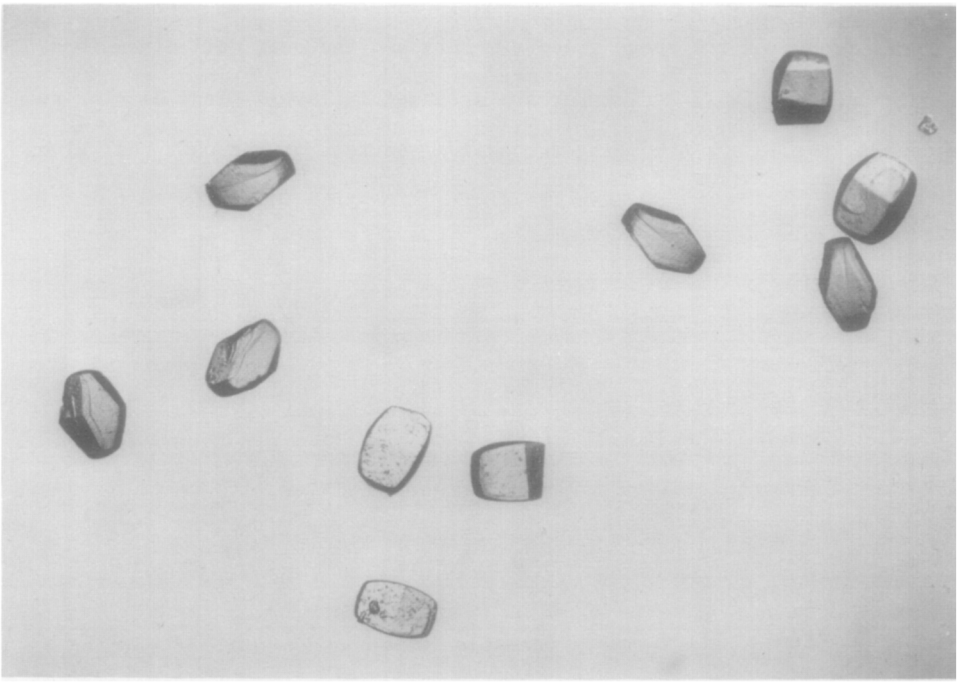

Fig. 1. Microphotograph of NCS crystals.

should provide insight into its mode of action. We report here the crystallization and preliminary X-ray diffraction analysis of crystals of NCS.

Crystals of NCS were grown from a 1% protein solution in 0.1 M sodium acetate buffer, pH 5.5, by increasing the ammonium sulfate concentration in the solution to 30% saturation (1.2 M) at 4°C. The ionic strength of the medium was increased either by addition of crystalline ammonium sulfate or by equilibrium vapor-diffusion. Crystals varying in size from 0.1 to 0.2 mm develop in 2-4 weeks. Fig. 1 shows a microphotograph of NCS crystals.

X-ray diffraction patterns reveal the crystal to be orthorhombic with a space group of $P2_12_12_1$ and cell parameters: a = 27.4 Å, b = 33.9 Å and c = 102.0 Å. Fig. 2 shows the diffraction patterns of the h0ℓ and 0kℓ zones. The unit cell volume is 94,800 Å and from the known molecular weight of 10,700 daltons, the volume per dalton, V_M, calculates to be 2.21 for one molecule per asymmetric unit. This value for V_M falls into the normal range for typical protein crystals (22).

If the molecules are assumed to be globular, a plausible arrangement can be deduced from the space group symmetry and the unit cell dimensions. Molecules of roughly 27 Å diameter are arranged in layers perpendicular to the c-axis, the layers occupying successive quarters of the unit cell along

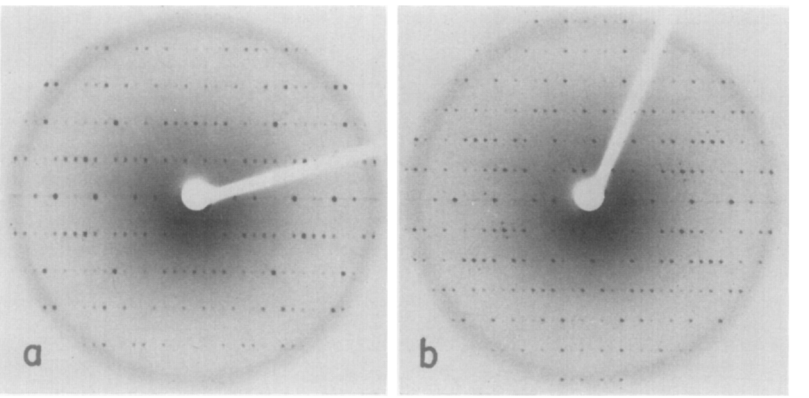

Fig. 2. X-ray diffraction patterns taken at precession angles of 8°. (a) h0ℓ zone, (b) 0kℓ zone.

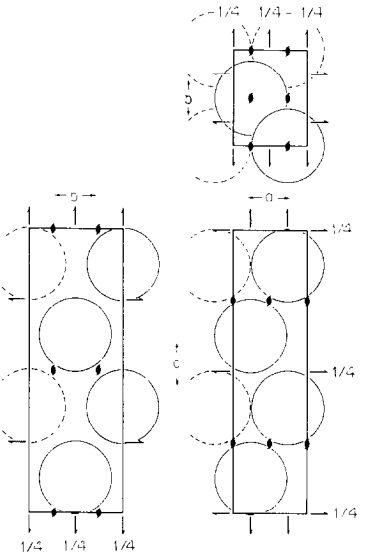

Fig. 3. Schematic diagram showing a possible arrangement of the molecules in the unit cell. The solid lined spheres depict one set of four molecules in a unit cell.

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c. Molecules in adjacent layers are related by screw axes parallel to a and b at c = 0, 1/2 and at c = 1/4, 3/4 respectively. Molecules in the third and fourth layers are related to those in the first and second by screw axes parallel to c. The exact positions of the molecules along a and b will depend on the particular shape of the molecules and the intermolecular forces acting between them. An approximate arrangement of the molecules illustrated by spheres is shown in Fig. 3.

A search for isomorphous heavy atom derivatives has been initiated and crystals soaked in 2 x 10^{-3} M K_2HgI_4 for 6 days give diffraction patterns with significant intensity changes. Anomalous scattering for both Hg and I is substantial for CuK_α radiation, suggesting the possibility of achieving a complete solution of the structure on the basis of the single isomorphous-anomalous (SIA) method (23-25). In any case, the survey for additional useful derivatives will be continued.

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