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Crystallization and Preliminary Characterization of Three Crystal Forms of Human Recombinant Transforming Growth Factor-α

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Three crystal forms of human recombinant TGF-α have been grown from solutions containing 2-methyl-2,4-pentanediol. One of the forms belongs to the orthorhombic space group $C222_1$ and the other two belong to the monoclinic space group C2. Two of the crystal forms diffract to approximately 2.3Å Bragg spacings. X-ray diffraction data has been collected for all three forms. These data appear to be suitable for crystal structure determination, using either heavy atom isomorphous replacement methods or molecular replacement, for phase determination.

Transforming growth factor-α (TGF-α) is a 5.7 kilodalton protein that affects growth and differentiation of a variety of cells of ectodermal and mesodermal origin (1). Patterns of expression observed for this mitogen during embryonic stages of development suggest a role for it in driving proliferation of particular cell populations (2,3). TGF-α has also been detected in several types of adult normal cells including those of skin keratinocytes (4), macrophages (5), gastric mucosa (6), brain (7), pituitary gland (8) and mammary epithilia (9).

TGF-α was first isolated from retrovirus transformed mouse fibroblasts (10) and has since been found to be prevalent in a variety of oncogene-transformed (11,12) and tumor-derived (13) human and rodent cell lines. Transfection of the growth factor gene into certain cell lines results in their transformation (14,15,16) and its overexpression in transgenic mice is correlated with a high incidence of neoplasias (17,18,19). Moreover, TGF-α has been found to play a role in neovascularization (20), digestion of extracellular matrices (21) and maintenance of a hypercalcemic state (22). These observations suggest a link between TGF-α expression and the generation or maintenance of neoplasias.

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TGF-α is a member of a family of structurally related growth factors which also includes epidermal growth factor (23), vaccinia virus growth factor (24), amphiregulin (25) and a heparin binding epidermal growth factor-related protein (26). The proliferative effects of all of these growth factors are mediated by binding to the epidermal growth factor receptor, a 170 kilodalton transmembrane protein with tyrosine kinase activity (23). Protein domains containing a structural motif similar to that of this growth factor family (i.e. $X_nCX_7CX_{2-3}GXCX_{10-13}CXCX_{3-4}X_9XRCX_4LX_n$) have been identified in a variety of seemingly unrelated classes of proteins (27). These classes include serine proteases involved in blood coagulation, intercellular adhesion molecules, lipoprotein receptors and Drosophila homeogene products. An understanding of the structures of TGF-α and the related growth factors is therefore of interest in order to elucidate structure-function relations of the growth factors themselves as well for the insights it may lend into the structure of the related domains found in some of the other proteins. Extensive NMR studies of TGF-α (28,29) and epidermal growth factor (30,31) as well as the EGF domains of human factors IX and X (32,33) have been carried out by several groups. A preliminary characterization of EGF crystals has also been reported (34).

We are interested in pursuing structural studies of TGF-α by crystallographic methods. Comparison of the x-ray crystallographically obtained coordinate sets to those obtained by NMR is of interest from both biophysical and methodological standpoints. Here we describe the crystallization of human recombinant TGF-α and the preliminary characterization of the crystals.

Methods

Human recombinant TGF-α was purified as described by Winkler et al. (35). The protein was precipitated from the water/acetonitrile solution by addition of ammonium sulfate to 55% saturation. The precipitated protein was then resuspended in Tris-HCl buffer, pH 7.5. Crystals were grown by vapor phase equilibration of 10 microliter sitting droplets of the protein solution containing 40% 2-methyl-2,4-pentanediol against a reservoir containing 50% 2-methyl-2,4-pentanediol.

Precession photographs were taken with a Huber precession camera mounted on a multiwire generator at a crystal to film distance of 60mm. Diffraction data were collected on a Seimens electronic detector mounted on a Rigaku RU-200 rotating anode generator operating at 50 kV and 100 mA. The detector was mounted 10 cm from the crystal, with a carriage angle of 10 degrees. Data was processed using the XENGEN package (36).

Results and Discussion

Three crystal forms of TGF-α were obtained under the conditions described above. The morphologies of the three forms were similar. Crystals usually grew as

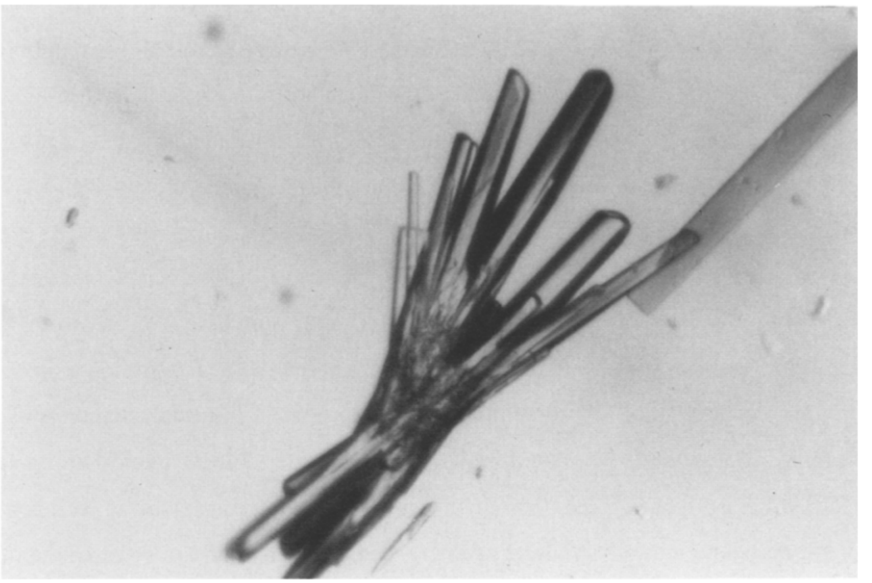

Figure 1. TGF-α crystals of the orthorhombic form. Typical dimensions of individual crystals were 0.3mm x 0.1mm x 0.05mm.

plates with typical dimensions of 0.3mm x 0.1mm x 0.05mm (Fig.1). Space groups and unit cell dimensions were determined by inspection of precession photographs (Fig. 2) and corroborated, subsequent to refinement of diffraction data using XENGEN (36), by

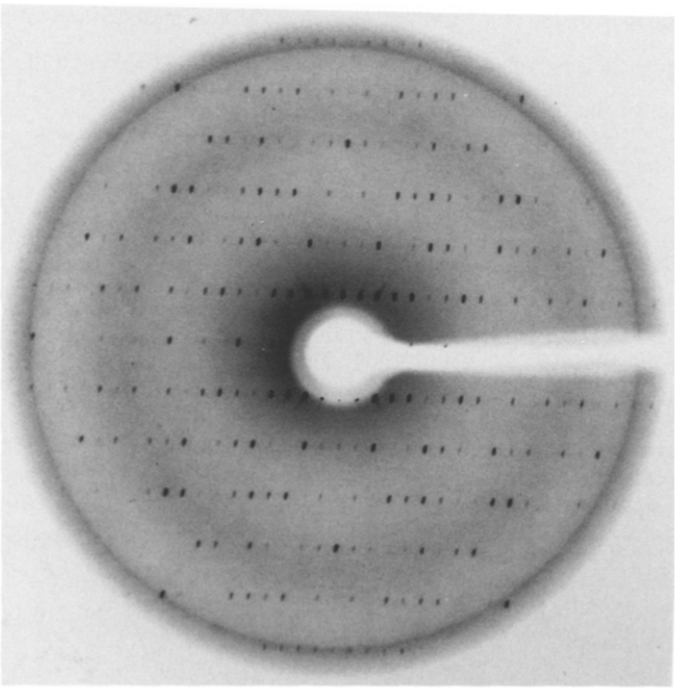

Figure 2. Precession photograph of orthorhombic form of TGF-α crystal. The Okl zone is shown. Crystal to film distance is 60mm and precession angle, μ is 15°.

Table 1. *Crystallographic Parameters of Three Crystal Forms of TGF-α*

	form 1	**form 2**	**form 3**
space group	C2	$C222_1$	C2
unit cell dimensions			
a (Å)	42.3	49.7	37.1
b (Å)	39.5	37.4	39.8
c (Å)	29.9	55.5	59.0
β (degrees)	101.8		92.6
molecules/unit cell	4	8	8
molecules/asymmetric unit	1	1	2
Å^3/dalton (Matthew's constant)	2.1	2.3	1.9

calculation of the reduced cell for the observed lattice using the method of Andrews (37). Crystallographic parameters of the three crystal forms are listed in Table 1. The orthorhombic form (form I) was the most prevalent. Matthew's constants are consistent with one molecule per assymetric unit for forms 1 and 2 and two molecules per asymmetric unit for form 3. The larger crystals diffract to approximately 2.3Å Bragg spacings. Data was collected to 2.35Å for forms 1 and 2 and to 3.0Å for form 3. The data are suitable for structure determination. Toward that end, we are currently pursuing phase determination using the method of multiple isomorphous replacement as well as that of molecular replacement. For the latter method, the NMR derived model of TGF-α is being used as the starting model (28). We expect that structure determinations of the multiple crystal forms will be informative in terms of the effects crystal contacts may have on the TGF-α crystal structure. This type of information is particularly important in this case, of a small protein molecule with considerable conformational flexibility (29,30).

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