CRYSTALLIZATION AND PRELIMINARY CRYSTALLOGRAPHIC STUDIES OF A CYTOKININ-SPECIFIC BINDING PROTEINS

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Cytokinin-specific binding proteins (CSBP) have been only found in legume plants so far. They appear to be low-identity (20%) homologues of pathogenesis-related class 10 (PR10) proteins. They show high affinity for natural and synthetic (urea-derived) cytokinins. We have overexpressed and purified the CSBP protein from Vigna radiata (Vr). Recombinant VrCSBP was purified from bacterial lysate by ammonium sulfate precipitation, DE52-cellulose chromatography, and two steps of FPLC on MonoQ and Superdex 75. In early crystallization experiments the protein turned out to be very sensitive to organic agents, which invariably produced amorphous precipitates. The protein could be crystallized only in the presence of cytokinins as cofactors. We have cocrystallized VrCSBP with two cytokinins, natural zeatine and synthetic N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU). The complexes have been crystallized using high-concentration organic salts (sodium citrate and sodium malonate) as precipitants. 1.65 Å diffraction data have been collected for the zeatine complex using synchrotron radiation and cryogenic conditions. The crystals are hexagonal with 3-4 molecules in the asymmetric unit. Initial attempts to solve the VrCSBP/zeatine structure by molecular replacement using a lupine PR10 model as a probe have failed, probably due to the low level of similarity and/or high number of molecules in the asymmetric unit. Current work includes derivatization for MIR approach and crystallization experiments aimed at producing suitable single crystals of other forms.
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Keywords: CYTOKININ-SPECIFIC BINDING PROTEINS, PATHOGENESIS-RELATED PROTEINS CLASS 10, CSBP-CYTOKININ COMPLEXES

CRYSTALLIZATION AND PRELIMINARY CRYSTALLOGRAPHIC STUDY OF TRANSGLUTAMINASE FROM STREPTOMYCES

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Transglutaminases (TGase, R-glutaminyl-peptide:amine γ-glutamyl-transferase, EC 2.3.2.13) are enzymes capable of catalyzing the formation of covalent ε-(γ-Glu)-Lys bond between Lys and Glu side chains of inter/intra proteins. TGases are widely distributed in animal organisms, tissues, and extracellular fluid, including liver, hair follicles, epidermis, and plasma. They were reported to involve in several biological phenomena, such as blood clotting, wound healing, keratinocyte differentiation, epidermal keratinization, and stiffening of the erythrocyte membrane. TGases have also been found in marine organisms, plants, and microorganism. Because of their abilities to catalyze the formation of ε-(γ-Glu)-lysine bonds in many proteins, microbial TGases have been used as valuable proteins with various applications in food industry, pharmaceutics, and medical treatments.
TGases from streptomyces have been successfully purified to homogeneity and characterized. These enzymes are different from those obtained from animal organisms in two main categories. TGases from streptomyces are Ca2+ independent and show activity in broader pH and temperature range. They are secreted proteins and exist in cultured broth in two forms: a 45 kDa pro-protein and a 38 kDa mature one. Though the functions of TGases from different sources are similar, the sequence of TGase from streptomyces shared low homology with those obtained from animal organisms. It is therefore important to analyze their action mode in more detail, that is, in the structural basis. Here we report the crystallization and preliminary crystallographic study of TGase from streptomyces.

Keywords: **TRANSGLUTAMINASE CRYSTALLIZATION STREPTOMYCES**

ELASTICITY OF LYSOZYME CRYSTALS BY BRILLOUIN SCATTERING

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The elastic properties are among the most important and least studied physical properties of macromolecular crystals. They are critical for understanding such phenomena as mechanical deformation, the energetics of defect formation, and the role of inter- and intramolecular compressibility. Brillouin scattering is a promising non-contact method to investigate the elastic tensor of protein crystals. Here we report the results of Brillouin scattering investigations of the second-order elastic moduli of tetragonal hen egg-white lysozyme crystals. In hydrated crystals the measured sound velocities in the (110) plane vary between 2.14(2) km/s along the [001] direction and 2.29(4) km/s along the [110] direction. The effect of dehydration is to increase the sound velocities and to decrease the velocity anisotropy in (110) from 6.3% to 2.8% between 98% and 79% relative humidity. A discontinuity in velocity and an inversion of the anisotropy is observed with increasing dehydration providing support for the existence of a structural transition below 88% relative humidity. At equilibrium hydration (98% relative humidity), the inversion of the measured sound velocities in the (110) plane yields the following: $C_{11} + C_{12} + 2C_{66} = 12.78(3)$ GPa, $C_{33} = 5.57(5)$ GPa, $C_{44} + 0.50\ C_{13} = 3.01(1)$ GPa. By combining the Brillouin quasi-longitudinal results with axial compressibilities determined from high-pressure x-ray diffraction, we obtain an adiabatic bulk modulus of 4.9(4) GPa. The elastic constants are in agreement with previously measured Young's moduli in lysozyme.

Keywords: **LYSOZYME, ELASTICITY, BRILLOUIN SCATTERING**

HUMAN COMPLEMENT PROTEIN, C9: EXPRESSION, PURIFICATION AND CRYSTALLIZATION

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Activation of the complement pathway leads to stimulation of the immune system, one of the effects being the formation of the Membrane Attack Complex (MAC). The function of the MAC is to assemble upon the surface of foreign cells, killing them by inducing lysis. The specific role of the protein C9 within the complex is to insert into the membrane and directly cause this lysis. How C9 transforms from a soluble protein to a membrane inserted protein is unknown and is of structural interest.
C9 was expressed as a secreted molecule (known as bC9) using baculovirus infected insect cells. The bC9 was purified from the conditioned media by chromatography steps similar to those used for serum C9 purification. Comparison of bC9 with serum C9 showed that they had similar properties with respect to aggregation and haemolytic activity. Notably there was a reduced molecular weight of bC9 due to decreased glycosylation, typical of insect expressed protein. Given a lack of success in crystallizing serum C9, this altered form of C9 was hypothesized to be more amenable to crystallization. Crystallization trials of bC9 showed no promising conditions until after 6 weeks, where tiny spines were observed.
Optimisation of these conditions resulted in large, thin spines. These crystals were washed, dissolved and then analyzed by silver-stain SDS-PAGE and Western blot. This confirmed that the crystals were of intact, monomeric bC9. We conclude that the reduced glycosylation aids the crystallization of C9.

Keywords: **IMMUNOLOGY COMPLEMENT PATHWAY BACULOVIRUS**