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Jel44 monoclonal Fab fragment specific for HPr of the phosphoenolpyruvate:sugar phosphotransferase system of *Escherichia coli* and the complex of Jel44 Fab fragment with HPr: preparation, crystallization and preliminary crystallographic analysis

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Jel44 is a mouse monoclonal antibody specific for the histidine-containing phosphocarrier protein (HPr), a component of a sugar-transport system in *Escherichia coli*. Because Jel44 binding to HPr is dependent upon ionic strength and the enthalpic and entropic contributions do not vary over the temperature range 277–310 K, the complex is of great interest. A single crystal of the Jel44 Fab fragment was obtained and diffracted X-rays to a maximum resolution of 4.6 Å on an in-house X-ray source. The crystal belongs to space group $P2_1$, with unit-cell parameters a = 68.6, b = 67.7, c = 105.5 Å, β = 96°. Although crystals of the complex of Jel44 Fab fragment with HPr could not be fully characterized owing to suspected crystal twinning, it was encouraging that they diffracted X-rays to 2.5 Å on an in-house X-ray source. It is thus foreseen that improvement of crystal quality will allow the complete solution of this novel structure.

1. Introduction

The interaction between antibodies and protein antigens is a significant event in the immune response and with the availability of monoclonal antibodies a number of protein antigen–Fab fragment complex structures have been described by X-ray diffraction (Davies & Cohen, 1996). These structures provide a better understanding of the molecular events in antibody–antigen recognition. The structure of the complex between histidine-containing phosphocarrier protein (HPr) and the Fab fragment of Jel42, an antibody specific for HPr from the *Escherichia coli* phosphoenolpyruvate:sugar phosphotransferase system, has been obtained (Delbaere *et al.*, 1989; Prasad *et al.*, 1998).

Jel42, Jel44 and Jel323 are three monoclonal antibodies specific for HPr and their epitopes on HPr and binding constants for the antibody–HPr and Fab fragment–HPr complexes have been described (Sharma *et al.*, 1991; Smallshaw *et al.*, 1998). Jel44 binding to HPr exhibited two unusual aspects: binding was dependent upon ionic strength and the enthalpic and entropic contributions did not vary over the temperature range 277–310 K. Because of these observations, crystallographic analysis of the Fab fragment of Jel44 and of the Jel44 Fab fragment–HPr complex was initiated, as reported here.

2. Jel44 Fab fragment preparation

Jel44 IgG mouse monoclonal antibody was produced in gram quantities from the ascites fluid of pristine-primed Balb/c mice and purified by Sephacryl S-200 chromatography (Mosmann *et al.*, 1979). Papain digestion led to the destruction of the light chain; therefore, Fab fragments were instead produced using trypsin digestion. Jel44 IgG was incubated at room temperature for 12 h with 3% trypsin, 10 m*M* 2-mercaptoethanol, 0.1% Triton X100 in 10 m*M* Tris–HCl buffer pH 8.0. The incubation mixture was dialyzed against two changes of 10 m*M* Tris–HCl buffer pH 8.0 and the Fab fragment was purified on a G-100 Sephadex column (1 × 30 cm) equilibrated with 10 m*M* Tris–HCl buffer pH 8.0. Fractions containing the Fab fragment, detected by SDS–PAGE (Laemmli, 1970), were loaded onto a Q-Sepharose High Performance column (1.5 × 20 cm) equilibrated with 10 m*M* Tris–HCl buffer pH 8.0 and eluted using a 0–1 *M* NaCl gradient. SDS–PAGE was used to detect fractions with homogeneous Fab fragments. Isoelectric focusing gels pH 7–9 (Sharma *et al.*, 1993) showed that the Jel44 Fab fragment had three major forms. Multiple forms are commonly found with Fab fragments. Each of the components was separated on a C 10/20 chromatofocusing column (bed height 15 cm) using a combination of Pharmacia Polybuffers

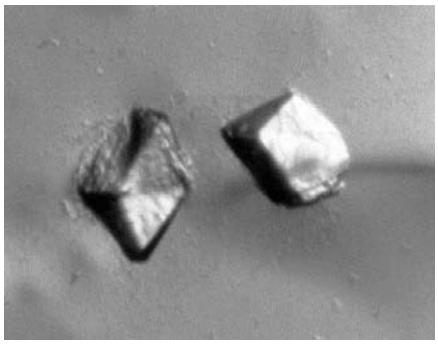

Figure 1
Two crystals of the Jel44 Fab fragment from *E. coli*. See text for details.

74 and 96 [7.5 µmol (pH unit)$^{-1}$ ml^{-1}] with Polybuffer exchanger PBE 94 within a pH interval of 6–8.5.

3. Crystallization

All three forms of the Jel44 Fab fragment were used in crystallization trials; the most basic form yielded crystals. Crystallization was carried out using the hanging-drop method at 290 K. A drop consisting of equal volumes of protein solution (9–10 mg ml^{-1} Fab fragment in 10 m*M* Tris–HCl pH 7.5) and a reservoir solution (16% polyethylene glycol 8000 in 50 m*M* phosphate buffer pH 8.0 with 0.1 *M* NaCl) was equilibrated against the reservoir solution. Single crystals (Fig. 1) with approximate dimensions of 0.2 × 0.2 × 0.15 mm grew within eight months and showed bright birefringence under cross-polarized light. The colourless crystals exhibited the forms {001}, {110} and {010}.

For the Jel44 Fab fragment–HPr complex, the crystallization drop consisted of equal volumes of Fab fragment (9 mg ml^{-1}) and wild-type HPr (7 mg ml^{-1}) in 25% saturated ammonium sulfate and 25 m*M* sodium citrate buffer pH 5.0. HPr was obtained as described previously (Anderson *et al.*, 1991). The drop was equilibrated against a reservoir containing 60% saturated ammonium sulfate and 50 m*M* of the same buffer. Colourless crystals of the Jel44 Fab fragment–HPr complex grew to dimensions of approximately 0.2 × 0.2 × 0.2 mm.

4. X-ray analysis

Crystals were mounted in a thin-walled glass capillary along with a small amount of mother liquor for X-ray analysis; both ends of the capillary were sealed with epoxy glue. Diffraction data were collected at 288 K on a FAST area detector using Cu $K\alpha$ radiation generated by a Nonius FR571 rotating-anode generator and were processed with the *MADNES* software package (Messerschmidt & Pflugrath, 1987). The Jel44 Fab fragment crystal diffracted X-rays to a maximum resolution of 4.6 Å. Characterization of the crystal indicated it to belong to the monoclinic space group $P2_1$, with unit-cell parameters $a = 68.6$, $b = 67.7$, $c = 105.5$ Å, $\beta = 96^\circ$. 47 out of 50 reflections were initially indexed. After calculation of the unit cell, an effective mosaic spread of 0.77 was observed over all reflections. Assuming two molecules in the asymmetric unit, $Z = 4$ gives a V_M value of approximately 2.5 Å^3 Da^{-1} and a solvent content of 50%. These values lie within the expected range for protein crystals (Matthews, 1968).

Crystals of the Jel44 Fab fragment complexed with HPr diffracted X-rays to a maximum resolution of 2.5 Å on the same in-house system. They were not characterized further owing to suspected crystal twinning.

Improvement of the crystallization conditions is expected to improve the X-ray diffraction quality. The solution of the structure using the molecular-replacement method is anticipated once good-quality data of higher resolution are obtained. The determination of the structures of both the free and complexed forms of Jel44 Fab fragment will determine whether any significant conformational changes are caused by the interaction with HPr. Moreover, structural comparisons with other complexes (Davies & Cohen, 1996; Prasad *et al.*, 1998) will be made. Valuable information concerning antibody–antigen interactions should be gained, in addition to insights into the design of molecules with novel recognition properties.

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