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Production, crystallization and preliminary X-ray analysis of the human integrin α_1 I domain

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Integrin $\alpha_1\beta_1$ is one of the main collagen receptors in many cell types. A fast large-scale production, purification and crystallization method for the integrin α_1 I domain is reported here. The α_1 I domain was crystallized using the vapour-diffusion method with a reservoir solution containing a mixture of PEG 4000, sodium acetate, glycerol and Tris–HCl buffer. The crystals beong to the *C*2 space group, with unit-cell parameters a = 74.5, b = 81.9, c = 37.3 Å, $\alpha = \gamma = 90.0$, $\beta = 90.8^\circ$. The crystals diffract to 2.0 Å and a 94.2% complete data set to 2.2 Å has been collected from a single crystal with an $R_{\rm merge}$ of 5.8%.

1. Introduction

Integrins are cell-adhesion receptors on the exterior surface of plasma membranes which mediate cell–cell and cell–matrix interactions. Integrins are often inactive, but they can be activated by intracellular modification of their cytoplasmic domains. Activation leads to highly regulated cell adhesion which transduces signals into cells. These signals regulate cell growth, differentiation and phenotype. Through their adhesive function, integrins have many roles in various physiological and pathological processes such as platelet aggregation, inflammation, immune function, wound healing, tumour metastasis, tissue migration during embryogenesis, viral infections and other diseases (Hynes, 1992; Pigott & Power, 1993).

Integrins are $\alpha\beta$ heterodimers (Hynes, 1992). Eight different β subunits (Pigott & Power, 1993) and 17 different α subunits have been identified (Camper *et al.*, 1998, and references therein). These subunits can form over 20 different integrins. Integrin $\alpha_1\beta_1$, which we are studying, is a collagen and laminin receptor in many cell types (Hynes, 1992). In addition, it is up-regulated in some inflammatory diseases of the human intestine (MacDonald *et al.*, 1990) and may have some role in wound contraction (Racine-Samson *et al.*, 1997). The α_1 subunit, like the α_2, α_{10}, $\alpha_{\rm L}$, $\alpha_{\rm M}$, $\alpha_{\rm X}$, $\alpha_{\rm E}$ and $\alpha_{\rm D}$ subunits, has an I ('inserted') domain near its N-terminus. This ~200-residue sequence has sequence similarity with von Willebrand factor, cartilage matrix protein, some collagen types and complement proteins (Dickeson & Santoro, 1998). This domain has a metal-ion dependent adhesion site (MIDAS) which plays a major role in ligand binding (Kamata *et al.*, 1994; Kern *et al.*, 1994; Tuckwell *et al.*, 1995). The structure of three different I domains, with and without bound metal ion, have been published: $\alpha_{\rm M}$ (Baldwin *et al.*, 1998; Lee, Bankston *et al.*, 1995; Lee, Rieu *et al.*, 1995; Li *et al.*, 1998), $\alpha_{\rm L}$ (Qu & Leahy, 1995, 1996) and α_2 (Emsley *et al.*, 1997), but no ligand complexes with I domains have yet been reported.

We are presently studying the details of the interactions of integrin α_1 and α_2 I domains with toxins, peptides derived from them, collagen and other molecules using site-directed mutagenesis, binding studies, molecular modelling and X-ray crystallography (Ivaska *et al.*, 1999). The crystal structure determination is essential for detailed study of the ligand binding. By comparing the structures of these two integrin I domains, we hope to establish the basis for their different collagen and peptide-inhibitor binding specificities. We have now crystallized the α_1 integrin I domain and report here the first crystal form of the α_1 integrin I domain. These crystals diffract to 2.0 Å and have proved to be suitable for X-ray structure determination.

2. Materials and methods

2.1. Production of recombinant integrin α_1 I domain

Recombinant integrin α_1 I domain was produced according to the protocol for the production of α_2 I domain (Ivaska *et al.*, 1999). The DNA encoding the α_1 I domain was inserted into the pGEX-4T-3 glutathione S-transferase (GST) expression vector using the *Bam*HI and *Sal*I restriction enzymes. The recombinant α_1 I domain has the integrin amino acids from residues 123 to 338. In addition, the recombinant α_1 I domain contains two amino acids at the N-terminus (GS) and ten amino acids at the C-terminus

(VDSSGRIVTD) from the fusion vector. The GST-r α_1 I fusion protein was cleaved with thrombin and purified using the bulk glutathione Sepharose 4B purification system, with only minor changes to the manufacturer's instructions (Pharmacia, Sweden). After purification, the recombinant integrin α_1 I domain was diluted threefold to decrease the salt concentration, and concentrated to 15 mg ml^{-1} in a Centricon 3 concentrator (Amicon Inc., USA) and a Nanosep 3K microconcentrator (Pall Filtron Corporation, USA). The protein concentration was determined using the Bradford microassay (Bio-RAD Laboratories, USA; Bradford, 1976; Sedmak & Grossberg, 1977) and the protein purity was checked by native and SDS–PAGE using PhastSystem (Pharmacia, Sweden) 8–25% gradient gels, which were stained with Coomassie Brilliant Blue.

2.2. Crystallization and preliminary X-ray analysis

The sparse-matrix sampling method was used for the initial screening of crystallization conditions (Jancarik & Kim, 1991). Crystals were grown using the hanging-drop vapour-diffusion method by mixing 2 µl protein and 2 µl reservoir solution at room temperature. One of the initial screening conditions was further modified: the best crystals grew with a reservoir solution containing 16–20% PEG 4000, 0.2 *M* sodium acetate, 20% glycerol and 0.1 *M* Tris–HCl pH 8.5. The crystals grew to their final 0.95 × 0.1 × 0.1 mm size in one week (Fig. 1). We first collected a room-temperature data set from one crystal and noticed that the crystal was twinned. The twinned crystal was, therefore, split prior to data collection in order to obtain an untwinned crystal. This crystal was directly mounted from the drop using a cryoloop and flash-cooled in a low-temperature nitrogen-gas stream. A data set was collected from this crystal using an R-AXIS IIC image plate mounted on a Rigaku RU200B rotating-anode X-ray source using Cu $K\alpha$ radiation (50 kV, 180 mA). Data were reduced with *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997). The solvent content of the crystals and the Matthews coefficient (Matthews, 1968) were calculated with *CNS* (Brünger *et al.*, 1997).

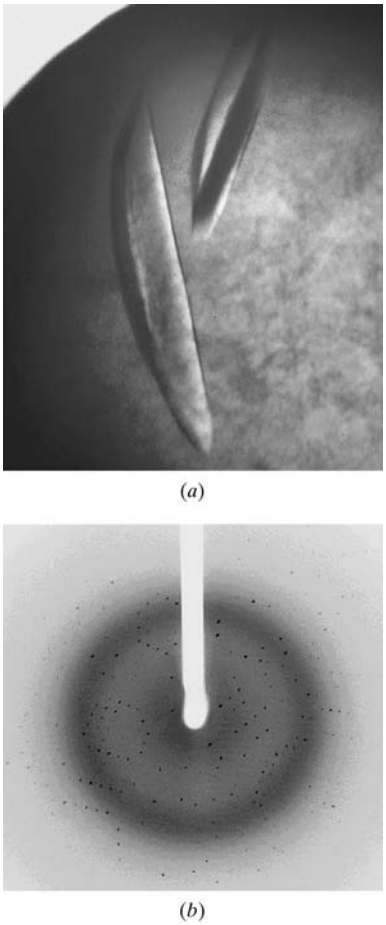

Figure 1
(*a*) Integrin α_1 I domain crystals. (*b*) Diffraction pattern of an oscillation image of integrin α_1 I domain.

3. Results and discussion

With the GST-fusion protein system we could produce 3.5–4.0 mg recombinant α_1 I domain per litre of culture medium. The fusion protein was purified with the standard GST-fusion protein purification protocol, and the α_1 I domain was cleaved with thrombin from GST. After this initial purification, we could still observe traces of other proteins on SDS–PAGE gels. Integrin α_M and α_2 I domains have also been produced as GST-fusion proteins (Baldwin *et al.*, 1998; Emsley *et al.*, 1997; Lee, Bankston *et al.*, 1995; Lee, Rieu *et al.*, 1995; Li *et al.*, 1998). Before crystallization, Lee and coworkers (Lee, Bankston *et al.*, 1995; Lee, Rieu *et al.*, 1995) extensively purified the α_M I domain using Ni^{2+}-affinity column chromatography followed by dialysis, cation-exchange column chromatography, gel filtration and desalting column chromatography; Li *et al.* (1998) used cation-exchange column chromatography, gel filtration and desalting column chromatography, and Baldwin *et al.* (1998) used cation-exchange chromatography and dialysis. Emsley *et al.* (1997) purified the α_2 I domain using Ni^{2+}-affinity column chromatography prior to crystallization. However, we managed to crystallize the α_1 I domain without any further purification steps.

The integrin α_1 I domain crystals have unit-cell parameters a = 74.5, b = 81.9, c = 37.3 Å, α = 90.0, β = 90.8, γ = 90.0° and belong to space group $C2$. Assuming one monomer (22 kDa) per asymmetric unit, the Matthews coefficient (volume-to-mass ratio, V_m) is 2.6, giving a solvent content of 51.0% (assuming a molecular density of 1.30 g cm^{-3}) and 52.9% (assuming a partial specific volume of 0.74 cm^3 g^{-1}). These values are in the range typical for proteins (Matthews, 1968). There must, therefore, only be one monomer in the asymmetric unit, as with two monomers per asymmetric unit the solvent content would be abnormally low.

The crystals diffract to 2.0 Å resolution and proved to be suitable for three-dimensional structure determination. We have collected 36079 raw measurements from the frozen crystals. The final merged data consisted of 10851 unique reflections and was 94.2% complete to 2.2 Å with an $R_{\rm merge}$ of 5.8%. The completeness of the highest resolution shell, 2.20–2.28 Å, was 74.0%. The structure of the integrin α_1 I domain will be solved with molecular replacement using the structure of the integrin α_2 I domain (Emsley *et al.*, 1997) as a search model. This should be a reasonable approach, as the sequence identity of the α_2 I domain and α_1 I domain is 52.3%. The structure determination from this data is currently under way. This structure will be the first integrin α_1 I domain structure and the first step towards our main goal: integrin α_1 I domain–ligand complex structures.

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