A new conformation of the integrin-binding fragment of human VCAM-1 crystallizes in a highly hydrated packing arrangement

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1. Introduction

Vascular cell adhesion molecule 1 (VCAM-1/CDM8; Osborn et al., 1989; INCAM-110; Rice et al., 1990) is a cell-surface receptor molecule that represents an integrin-binding subgroup of the immunoglobulin superfamily (IgSF; Wang et al., 1995). VCAM-1 preferentially binds the integrin heterodimer of the very late antigen (VLA-4) β1 family (Wang & Springer, 1998) containing α4 (CD49d) and β1 (CD29) subunits (Vonderheide & Springer, 1992). The VCAM-1–integrin interaction mediates intercellular adhesion of leukocytes to the blood-vessel wall (Osborn et al., 1989) and also regulates trans-endothelial emigration at inflammatory sites (Wang & Springer, 1998). VCAM-1 may also act as a host receptor for viruses and parasites (Huber, 1994), as well as having important implications in tumour formation and metastasis (Rice et al., 1990).

The extracellular region of VCAM-1 typically consists of seven contiguous arranged IgSF domains (VCAM-7D; Chothia & Jones, 1997) that adopt an extended rod-like structure (Osborn et al., 1994). Ligand-binding surfaces specific to VLA-4 integrin are exposed on homologous IgSF domains 1 and 4 of VCAM-7D (Wang & Springer, 1998). X-ray crystal structures are available for a functional ligand-binding fragment of domains 1 and 2 of VCAM-1 (Jones et al., 1995; Wang et al., 1995, 1996) and ligand-binding domains of related immune-cell adhesion molecules of the subfamily, including ICAM-1 (Casasnovas et al., 1998; Bella et al., 1998), ICAM-2 (Casasnovas et al., 1997), MAdCAM-1 (Tan et al., 1998), CD2 (Jones et al., 1992; Bodian et al., 1994) and CD4 (Brady et al., 1993; Ryu et al., 1994). Here, we present a novel crystal form of VCAM-1 that adopts a different conformation and exists in an exceptionally highly hydrated packing arrangement.
2. Materials and methods

2.1. Crystallization

Purified protein was a gift from Dr P. Lake. Crystals were grown at room temperature using the hanging-drop method and could be obtained using either polyethylene glycol or ammonium sulfate as the precipitant. Large hexagonal crystals used for data collection were obtained from 10 μl hanging drops. These drops comprised 5 μl of protein solution (10 mg ml⁻¹ in 50 mM HEPES buffer pH 7.5) added to a 5 μl drop of well solution [50 mM Tris buffer pH 7.1, 42%(v/v) saturated ammonium sulfate].

2.2. Structure determination

A summary of the crystallographic data is given in Table 1. Crystals of dimensions 1.0 × 1.0 × 0.8 mm were mounted in thin glass Lindemann tubes. X-ray data were collected at room temperature using a MAR image plate and processed using DENZO (Otwinowski & Minor, 1993). The structure was solved by molecular replacement using the program AMoRe (Navaza, 1994), with one domain of the VCAM structure as the model (Jones et al., 1995). Positional and B-factor refinement were performed using X-PLOR Version 3.1 (Brünger, 1993); water molecules were placed on appropriate peaks in subsequent difference Fourier maps.

2.3. Density measurements

Aqueous stock solutions of Ficoll 400 (Pharmacia) were made up at 60, 50, 40, 30, 20 and 10%(w/v). Aliquots of approximately 40 μl were layered into a glass tube with an internal diameter of 2 mm. The gradient was calibrated using 3.1. X-ray structure and solvent channels

Statistics for the refined structure are given in Table 1. The packing diagram for this form of VCAM (Fig. 1) shows a spectacularly large solvent channel with $V_m = 3.44 \text{ Å}^3 \text{Da}^{-1}$ and over 80% of the crystal being occupied by solvent. In order to show that lack of electron density in the crystal channels was indeed arising from solvent and not disordered VCAM, density measurements of the crystal using the Ficoll method were carried out.

The single crystal of VCAM which had been used for X-ray intensity measurements was introduced onto the top of the Ficoll gradient tube and within 30 s sank to a point corresponding to a crystal density ($d$) of 1.12 g cm⁻³. After centrifugation for 5 min at 4000g the crystal sank to a point corresponding to 1.135 g cm⁻³. The crystal continued to drop in the gradient tube until stabilizing after about 60 min with an apparent density of 1.17 g cm⁻³. This increase of crystal density with time has been commonly observed (Dragovich et al., 1996; Borgstahl et al., 1996) and is attributed to the incorporation of Ficoll into the lattice. The number of molecules per unit cell ($n$) can be calculated from (Westbrook, 1985)

\[
 n = (NV / Mv_p)(\rho_s - \rho_v) / ([1/v_p - \rho_s]),
\]

Figure 1
A highly hydrated packing arrangement in VCAM-1 shown in projection along $c$. The unit cell is shown.

Table 1
Crystallographic data.

<table>
<thead>
<tr>
<th>Unit-cell parameters (Å³)</th>
<th>$a = b = 152.94$, $c = 45.98$, $\alpha = \beta = 90$, $\gamma = 120$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>$P_{3}1$</td>
</tr>
<tr>
<td>$Z$ (No. of molecules in the unit cell)</td>
<td>6</td>
</tr>
<tr>
<td>$d_{	ext{calc}}$ (Å)</td>
<td>3.0 (3.05–3.0)</td>
</tr>
<tr>
<td>Total No. of observed reflections†</td>
<td>183518 (5762)</td>
</tr>
<tr>
<td>No. of unique reflections†</td>
<td>12530 (622)</td>
</tr>
<tr>
<td>Merging $R$ factor† (%)</td>
<td>11.5 (59.6)</td>
</tr>
<tr>
<td>Multiplicity†</td>
<td>9.4 (9.5)</td>
</tr>
<tr>
<td>Completeness† (%)</td>
<td>99.9 (100)</td>
</tr>
<tr>
<td>$% &gt; I/\sigma(I)$†</td>
<td>74.8 (55.4)</td>
</tr>
<tr>
<td>Final $R_{	ext{free}}$ (%)</td>
<td>20.8</td>
</tr>
<tr>
<td>Final $R_{	ext{free}}$ (%)</td>
<td>23.8</td>
</tr>
<tr>
<td>R.m.s.d.</td>
<td>0.006</td>
</tr>
<tr>
<td>Bonds (Å)</td>
<td>1.31</td>
</tr>
<tr>
<td>Angles (°)</td>
<td>1.2</td>
</tr>
<tr>
<td>Ramachandran plot</td>
<td></td>
</tr>
<tr>
<td>Most favoured region (%)</td>
<td>82.7</td>
</tr>
<tr>
<td>Allowed (%)</td>
<td>16.2</td>
</tr>
<tr>
<td>Disallowed (%)</td>
<td>1.2</td>
</tr>
<tr>
<td>$B$ values (Å²)</td>
<td></td>
</tr>
<tr>
<td>Average $B$ value for protein (s.d.)</td>
<td>45.03 (20.81)</td>
</tr>
<tr>
<td>Average $B$ value for main chain (s.d.)</td>
<td>42.00 (18.60)</td>
</tr>
<tr>
<td>Average $B$ value for side chain (s.d.)</td>
<td>48.20 (22.47)</td>
</tr>
<tr>
<td>Average $B$ value for the 50 waters (s.d.)</td>
<td>61.58 (19.56)</td>
</tr>
</tbody>
</table>

† Values in parentheses are for the highest resolution shell of data. ‡ $R$ factors were calculated using all data in the resolution range 24.0–3.00 Å.
where \( V \) is the volume of the unit cell (930 600 Å\(^3\)) and \( M \) is the molecular weight (22 491 Da); \( \rho_c \) is the measured crystal density and \( \rho_s \) is the density of the total solvent compartment. The protein partial specific volume (\( \rho_p \)) was taken as 0.737 cm\(^3\) g\(^{-1}\) (Bode & Schirmer, 1985). The initial concentration of the hanging drop was 20% saturated ammonium sulfate (\( \rho_s = 1.117 \) g cm\(^{-3}\)). Protein crystals are modelled as a lattice of solvated protein surrounded by channels of free solvent (Westbrook, 1985). The calculation of \( n \) is very sensitive to the difference between \( \rho_c \) and \( \rho_s \). With an initial measured crystal density of 1.12 g cm\(^{-3}\), \( n \) is calculated to be 11.5, 6.0 and 0.3 for \( \rho_s \) values of 1.0 (water), 1.07 and 1.117 g cm\(^{-3}\) (20% saturated ammonium sulfate), respectively. The value of \( n = 6 \), required for space group \( P3_121 \), is consistent with the large solvent channels being partially filled with ammonium sulfate solution. This is supported by the crystallographic refinement, which gives no trace of connected electron density in the solvent channels.

### 3.2. Interdomain flexibility

IgSF domains of VCAM-1 and homologous members of the Ig subfamily are comprised of two antiparallel \( \beta \)-pleated sheets packed face-to-face (Jones, 1993). The common core structure is stabilized by interconnecting loops and disulphide bridges (Lesk & Chothia, 1982). A connecting linker peptide (residues 90–95) mediates the intramolecular dimerization of VCAM-1 Ig domains, with the last \( \beta \)-sheet of domain 1 joining to the first \( \beta \)-sheet of domain 2 (Jones, 1993) (Fig. 2). The linker peptide mediates interdomain flexibility at the hydro-
phobic interface of domains 1 and 2. The average B factor of 42.0 Å² (s.d. 18.6 Å²) for main-chain atoms in this structure solved at room temperature is rather high (Table 1) and only 50 solvent molecules were located. Despite this, the linker region (residues 88–96) between the two domains is rather well defined and has an average main-chain B factor of 36.4 Å² (s.d. 4.51 Å²) and slightly more mobile side chains with an average B factor of 40.4 Å² (s.d. 12.1 Å²). All ϕ and ψ angles of this linker are found to lie within the allowed regions of the Ramachandran plot.

The analysis of dihedral angles measured from all available structural results (Table 2) suggests that interdomain flexibility is not uniquely determined by the ϕ, ψ angles of any one linker residue but is rather determined by changes in the conformation of some or all of the residues in the sequence Ile88–Glu96. In particular, Ile88, Tyr89, Ser90, Pro92 and Glu96 play a major role. The ϕ angle of Ser90 of −128.5° for the new VCAM-1 form varies considerably from the narrow range of values obtained for the other crystal forms (−144.3 to −149.6°). The ϕ and ψ rotation angles of Pro92 of −66.7° and −45.5°, respectively, both deviate from values obtained for the other four monomers (Table 2). Previous analyses attributed interdomain flexibility to changes in the conformation of the pivot residue Tyr89 (Wang et al., 1995) or Ile88, Pro92 and Glu96 (Jones et al., 1995).

A least-squares fit of main-chain atoms of domain 2 (residues 96–196) provides an optimized structural alignment for each of the five crystallographically independent VCAM-1 structures (Table 3; Fig. 3). The ‘tilt’ angle between the domains (Table 2) is calculated as the angle between the two lines defining the principal axes for each of the domains (Bork et al., 1996). The tilt angle is essentially two-dimensional and, in association with the ‘skew’ angle, defines the orientation of the domains. The tilt and skew values obtained for the VCAM structure presented here (19.4°, 77.7°) lie between the tilt (7.3–39.9°) and skew (55.3–87.8°) angles measured for the other four monomers. The VCAM structure reported here adopts a unique conformation, as evident from the tilt and skew angles which demonstrate projection of domain 1 with respect to domain 2. This orientation provides an intermediate VCAM conformation that facilitates interaction of domain 1 with both the flexible C E and FG loops of domain 2.

The torsion angles of the linker residues may in part be governed by the interdomain contacts. Indeed, the least-squares fit of the domains of each of the five structures shows that there are large differences in the conformations of the longer FG and C E loops (Table 3). The inherently mobile C E and FG loops located at the hydrophobic interface of Ig domains 1 and 2 are likely to change conformation in order to accommodate any changes in the relative positions of domains 1 and 2 (Chothia & Jones, 1997).

Table 3
Domain 2 pairwise superposition of VCAM structures.

<table>
<thead>
<tr>
<th>Template</th>
<th>Superimposed molecule</th>
<th>R.m.s. deviation (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM (ij9)</td>
<td>1vca (A)</td>
<td>0.394</td>
</tr>
<tr>
<td>VCAM (ij9)</td>
<td>1vca (B)</td>
<td>0.443</td>
</tr>
<tr>
<td>VCAM (ij9)</td>
<td>1vsc (A)</td>
<td>0.862</td>
</tr>
<tr>
<td>VCAM (ij9)</td>
<td>1vsc (B)</td>
<td>0.858</td>
</tr>
</tbody>
</table>

Mutagenesis experiments revealed the general integrin-binding motif of VCAM-1 as the sequence Q38IDSPL located on the loop connecting β-strands C1 and D1 of the first domain of VCAM-1 (Fig. 2; Newham et al., 1997). Asp40 is a key integrin-binding residue located at the tip of the CD loop. It provides a negative charge that may complete a divalent cation-binding site in the counter-receptor molecule (Jones et al., 1995). Mutagenesis studies have implicated the sequence G64NEH exposed on the EF loop in α4 integrin binding to domain 1 of VCAM-1 (Osborn et al., 1994). The EF loop of domain 1 lies in close proximity to the CD loop to form a general integrin-presenting surface (Wang et al., 1996) positioned near the interface of domains 1 and 2 (Wang et al., 1995).

Mutational analysis provides the first evidence for the existence of C E loop–integrin interactions (Newham et al., 1997) that support findings of VCAM-1 interdomain contacts. Residues of the C E loop (Asp143, Ser148) and E β-strand (Glu150, Lys152 and Glu155) create a negatively charged surface required for specific binding to α4β7 integrin. Sequence analysis suggests equivalent negatively charged residues are a common requirement for α4β7 binding to VCAM-1 and MAdCAM-1 (Newham et al., 1997). Specific integrin binding of α4β1 to VCAM-1 maps to residues K(79)LEK that reside in the G β-strand of domain 1 (Kilger et al., 1997) (Fig. 2).

The structural data presented here provides further insight into the mechanism of adhesive interactions in the Ig subfamily of integrin-binding proteins. The conformation of our X-ray crystal structure demonstrates interdomain motion stabilized by the extensive loops of domain 2.

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References


Taylor et al. • VCAM-1 integrin-binding fragment 1583