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Crystallization and Preliminary X-ray Diffraction Study of a Bacterially Produced T-cell Antigen Receptor Vα Domain

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A recombinant form of the variable domain of the α chain of a murine T-cell receptor specific for the N-terminal nonapeptide of myelin basin protein in association with the major histocompatibility complex class II I-A^u molecule has been crystallized in a form suitable for X-ray diffraction analysis. This protein was secreted into the periplasmic space of *Escherichia coli* cells and affinity-purified using a nickel chelate adsorbent. The crystals are orthorhombic, space group $P2_12_12$, with unit cell dimensions $a=97{\cdot}7$ Å, $b=79{\cdot}6$ Å, $c=30{\cdot}4$ Å and diffract to beyond 2·2 Å resolution. The ability to crystallize a T-cell receptor domain produced in bacteria strongly suggests that the periplasmic space can provide a suitable environment for the correct *in vivo* folding of this class of antigen recognition molecules.

Keywords: T-cell receptor; α chain; Vα domain; crystallization

T-cell receptors (TcRs‡) are highly diverse heterodimeric glycoproteins on the surface of T-lymphocytes responsible for antigen recognition by these immune system cells (Kronenberg *et al.*, 1986; Davis & Chien, 1993). The majority of T-lymphocytes bear TcRs composed of α and β chains, while about 10% express γδ heterodimers (Raulet, 1989). Both types of TcRs consist of variable (V) and constant (C) regions homologous to those of immunoglobulins. However, in contrast to antibodies, which recognize intact antigens, αβ TcRs recognize proteolytic fragments of antigens bound to molecules of the major histocompatibility complex (MHC). To investigate the structural basis for the corecognition of peptide and MHC by TcRs, protein engineering has been used to generate different soluble forms of these transmembrane molecules for potential use in X-ray crystallographic studies (Gascoigne *et al.*, 1987; Mariuzza & Winter, 1989; Lin *et al.*, 1990; Slanetz & Bothwell, 1991; Gregoire *et al.*, 1991; Novotny *et al.*, 1991; Ward, 1991, 1992; Engel *et al.*, 1992; Weber *et al.*, 1992; Soo Hoo *et al.*, 1992; Kurucz *et al.*, 1993). We have previously reported crystallization of the extracellular portion of the β chain, including both V and C domains, of a murine TcR specific for a hemagglutinin peptide of influenza virus in the context of the MHC class II I-E^d molecule (Boulot *et al.*, 1994). Here we describe the crystallization of the bacterially produced Vα domain of a murine TcR (named 1934.4) which recognizes the N-terminal peptide of myelin basic protein presented by I-A^u (Acha-Orbea *et al.*, 1988; Wraith *et al.*, 1989; Ward, 1992). Injection into mice of the T-cell clone from which the 1934.4 TcR was isolated has been shown to induce experimental autoimmune encephalomyelitis, a disease which serves as an animal model for multiple sclerosis in humans (Zamvil *et al.*, 1985). Determination of the three-dimensional structure of this TcR Vα domain, together with that

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‡ Abbreviations used: TcR, T-cell receptor; V, variable; C, constant; MHC, major histocompatibility complex.

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of a VβCβ chain (Boulot *et al.*, 1994), should significantly advance our understanding of antigen recognition by this class of receptors.

The 1934.4 Vα protein bearing a C-terminal His6 tag (1934.4 VαHis6) was purified from the periplasm of transformed *Escherichia coli* cells by affinity chromatography on Ni^{2+}-NTA agarose columns as described (Ward, 1992). As all attempts to crystallize this protein were unsuccessful, we suspected our failure might be due to the presence of the potentially flexible His6 sequence. We therefore eliminated this tag by limited proteolysis using carboxypeptidase A (Hochuli *et al.*, 1988). In a typical preparation, 4·8 mg of 1934.4 VαHis6 in 3·0 ml 50 mM Tris · HCl (pH 8·0) were digested with carboxypeptidase A from bovine pancreas (Boehringer) at 25 °C for seven hours at an enzyme substrate ratio of 1 : 160. Isoelectric focusing in PhastGel IEF 3-9 media (Pharmacia) was used to monitor the course of the reaction. Untreated 1934.4 VαHis6 focused as three major bands of pI 5·5 to 5·8; following digestion, the protein appeared as two major bands of pI 3·8 to 4·0, consistent with the removal of basic residues. The reaction was terminated by the addition of EDTA to a final concentration of 50 mM.

Further purification was achieved by chromatography on a Pharmacia Mono Q anion exchange column equilibrated with 20 mM bis-Tris · HCl (pH 6·5) and developed with a linear KCl gradient. The 1934.4 Vα protein eluted at between 0·20 and 0·24 M KCl. Peak fractions were dialyzed against 20 mM Tris · HCl, 25 mM NaCl (pH 7·5) and concentrated for crystallization trials. Final recovery was 1·6 mg. This material eluted as a single peak of about 25 kDa on a Superose 12 FPLC gel filtration column (Pharmacia), suggesting that the 1934.4 Vα domain exists predominantly as a homodimer in solution.

Screening for crystallization using the broad screening method of Jancarik & Kim (1991) revealed a number of conditions at which at least microcrystals could be obtained: these corresponded to solutions 6, 9, 16, 20, 30, 31 and 32. The most promising crystals grew in solution 16 (2·0 M Li_2SO_4, 0·1 M Hepes, pH 7·5), whose composition was modified to improve crystal quality. The best results were obtained at room temperature in hanging drops (McPherson, 1982) by mixing 2 μl of a solution containing 1·6 M Li_2SO_4, 0·1 M Tris · HCl (pH 8·0) with an equal volume of protein solution at 7·3 mg/ml; equilibration was against a reservoir solution of 1·9 M Li_2SO_4, 0·1 M Tris · HCl (pH 8·0). In addition, we found that inclusion of low-melting agarose (FMC BioProducts) in the hanging drops at a final concentration of 0·05% (w/v) significantly reduced the number of nucleation centers and increased crystal size, as suggested by Robert *et al.* (1992).

Crystals grew as plates with dimensions up to 0·5 mm × 0·5 mm × 0·2 mm and belong to the orthorhombic space group $P2_12_12$. The space group was determined by both precession photography and by indexing a set of three-dimensional diffraction data recorded on a Siemens area detector. The cell dimensions are $a = 97{\cdot}7$ Å, $b = 79{\cdot}6$ Å, $c = 30{\cdot}4$ Å, and the volume of the unit cell is compatible with the presence of one homodimer in the asymmetric unit with a crystal solvent content of approximately 50% (v/v). Area detector data were collected from two crystals using Cu-Kα radiation emitted from a Siemens rotating anode equipped with focusing mirror optics. Data processing using XENGEN (Howard *et al.*, 1987) resulted in 49,645 observations to 2·2 Å which merged to give 12,487 unique reflections with R_{merge} of 9·9% on intensities. Of these, 9235 had $F > 2\sigma(F)$ in the resolution range 10 Å to 2·2 Å (73·6% completeness) and 720 in the range 2·3 Å to 2·2 Å (46·7% completeness). Screening for potential heavy-atom derivatives is underway. Solution of the structure by molecular replacement will also be attempted using an immunoglobulin fold as the search model.

Several groups have reported the expression of TcR V domains in bacteria, either individually or as single chain Vα-Vβ fragments (Ward, 1991, 1992; Novotny *et al.*, 1991; Soo Hoo *et al.*, 1992; Kurucz *et al.*, 1993). Except in the case of the anti-fluorescein single chain TcR described by Novotny *et al.* (1991), however, antigen binding activity has not been demonstrated for these bacterially produced TcRs. In contrast, recombinant TcRs expressed in mammalian cells have been shown to specifically recognize their cognate peptide–MHC complexes (Weber *et al.*, 1992). Furthermore, except for the TcR fragments expressed by Ward (1992), bacterially produced TcRs have been characterized by poor solubility; again, this has not been observed for recombinant TcRs from mammalian sources. Indeed, only the V domains described by Ward (1992) could be isolated in soluble form directly from *E. coli*; all others were found in inclusion bodies or as insoluble aggregates and required *in vitro* renaturation. These difficulties raise the concern that bacterially produced TcRs may be at least partially misfolded, thereby accounting for their tendency to aggregate. Our finding that a TcR V domain produced in *E. coli* may be readily crystallized in a form suitable for high resolution X-ray diffraction analysis strongly implies that the periplasmic space into which it is secreted provides a suitable environment for correct *in vivo* folding, as it does for immunoglobulin domains (Skerra & Pluckthun, 1988; Better *et al.*, 1988). This is significant, as *E. coli* expression systems are potentially more convenient for detailed structure–function studies than mammalian ones. In addition, one may anticipate that the powerful new technologies which have been developed for the display and selection of antibody molecules on bacteriophage (Huse, 1991; Griffiths, 1993) will also prove applicable to T-cell receptors.

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