A T Cell Receptor Vα Domain Expressed in Bacteria: Does It Dimerize in Solution?

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Summary

To evaluate the potential for dimerization through a particular T cell receptor (TCR) domain, we have cloned the cDNA encoding a TCR Vα from a hybridoma with specificity for the human immunodeficiency virus (HIV) envelope glycoprotein 120–derived peptide P18-I10 (RGPGRAFVTI) bound to the murine major histocompatibility complex (MHC) class I molecule, H-2D^d. This cDNA was then expressed in a bacterial vector, and protein, as inclusion bodies, was solubilized, refolded, and purified to homogeneity. Yield of the refolded material was from 10 to 50 mg per liter of bacterial culture, the protein was soluble at concentrations as high as 25 mg/ml, and it retained a high level of reactivity with an anti-Vα2 monoclonal antibody. This domain was monomeric both by size exclusion gel chromatography and by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Circular dichroism spectra indicated that the folded Vα domain had secondary structure similar to that of single immunoglobulin or TCR domains, consisting largely of β sheet. Conditions for crystallization were established, and at least two crystal geometries were observed: hexagonal bipyramids that failed to diffract beyond ~6 Å, and orthorhombic crystals that diffracted to 2.5 Å. The dimerization of the Vα domain was investigated further by solution nuclear magnetic resonance spectroscopy, which indicated that dimeric and monomeric forms of the protein were about equally populated at a concentration of 1 mM. Thus, models of TCR-mediated T cell activation that invoke TCR dimerization must consider that some Vα domains have little tendency to form homodimers or multimers.

The TCR is a heterodimeric cell surface glycoprotein consisting of disulfide-linked α and β chains expressed clonally on mature T lymphocytes bearing the coreceptor molecules CD4 or CD8 (1). These molecules bind a noncovalent complex consisting of an antigenic or self peptide and an MHC-encoded class I or class II molecule expressed on the surface of APC (2). Activation of CD8 or CD4-expressing T lymphocytes is controlled by the interaction of the TCR with the MHC–peptide complex, and this molecular interaction plays a critical role in the initiation and perpetuation of T lymphocyte responses to foreign antigens or to dysregulated self antigens, such as those expressed by tumor cells (3, 4). The discrimination that a TCR makes in identifying an MHC molecule or antigenic peptide is exquisite. Single–amino acid substitutions in either the MHC or the peptide ligand can eliminate or drastically diminish the cellular response mediated by a specific TCR (5). Despite an ever-expanding battery of amino acid sequence data generated from the cloning and sequencing of TCR cDNAs and genes, our understanding of the relationship of the structure of TCR to the binding and cellular signaling activity of these molecules is rudimentary. The three-dimensional x-ray crystallographic structures of an MHC class II ligand for a TCR, human HLA-DR1 (6), complexed with a peptide from influenza virus hemagglutinin (7), that of MHC class II complexed with a superantigen (8), and that of a complex of the MHC class II molecule HLA-DR3 complexed with the class II-associated invariant chain peptide (CLIP)[1] fragment of the invariant chain (9), all consistently show a dimer of dimers. These observations provide the basis for a model in which the formation of superdimers of the MHC–peptide complexes on cell surfaces plays a critical role in the ability to activate the T cell (6, 10).

[1] *Abbreviations used in this paper:* CD, circular dichroism; CLIP, class II associated invariant chain peptide; IPTG, isopropyl β-D-thiogalactoside; NMR, nuclear magnetic resonance; RU, resonance unit; SPR, surface plasmon resonance.

Recently, a three-dimensional x-ray crystallographic structure of a TCR-β chain derived from an MHC class II–restricted, influenza virus–specific T cell hybridoma was reported, demonstrating the similarity of the TCR basic fold to that of the immunoglobulins (11). In addition, the three-dimensional structure of a single Vα4.2 domain derived from a TCR with specificity for the mouse MHC class II molecule I-A^u complexed with a myelin basic protein–derived peptide has been described (12). In solution, this particular Vα domain forms homodimers as assessed by their behavior in gel filtration chromatography (13), and in the crystal, it forms an Fv-like dimer, and the homodimers form tetramers through crystal contacts. This tendency of the Vα4.2 to form superdimers led to a modification of the MHC-based model in that it supported a critical role of the α chain in the TCR dimerization needed for signaling (12).

As part of a continuing effort to understand the relationship of structure to function of TCR, their interaction with MHC–peptide complexes, and the potential role of TCR dimerization in initiation of signaling events, we have begun to clone and express domains and combining sites from TCR restricted to particular MHC–peptide complexes. In this article we report the cloning of a cDNA encoding a Vα domain from a T cell hybridoma that is activated by the HIV-IIIB envelope glycoprotein 120–derived peptide, P18-I10 (RGPGRAFVTI), complexed to the mouse MHC I molecule, H-$2D^d$. This domain has been expressed efficiently as inclusion bodies in *Escherichia coli*, and the solubilized protein has been quantitatively refolded, purified, and characterized serologically and by circular dichroism (CD). Conditions for crystallization have been established, and the domain, soluble at high concentration in low-salt buffers, has been analyzed for dimerization by nuclear magnetic resonance (NMR).

Materials and Methods

Cloning of Rearranged TCR Vα Genes. B4.2.3 (14), a T cell hybridoma with specificity for the HIV-IIIB envelope glycoprotein 120 peptide P18-I10 (RGPGRAFVTI in the single-letter amino acid code) presented in the context of the MHC class I molecule H-$2D^d$, was used as the source for the cloning of TCR cDNAs. Total cellular RNA was extracted from B4.2.3 with the RNAgents Total RNA Isolation System (Promega Corp., Madison, WI) following the manufacturer's protocol. mRNA was then isolated by adsorption onto an oligo(dT)-biotinylated probe (PolyATtract mRNA Isolation System; Promega Corp.). TCR-Vα region–specific cDNA was synthesized and cloned as described (15). Briefly, cDNA synthesis by reverse transcriptase (RT-MLV; GIBCO BRL, Gaithersburg, MD) was primed with specific synthetic oligonucleotides complementary to α chain constant region sequences upstream of the respective J-C junction. After second-strand synthesis, synthetic linkers were added to the 5′ end and inserts were amplified by PCR and cloned into the BlueScript KS^+ vector (Stratagene Inc., La Jolla, CA). DNA sequence of all inserts was determined by the dideoxy method using Sequenase (United States Biochemical Corp., Cleveland, OH). The Vα sequence was compared with sequences in GenBank using BLASTn (16) and showed a perfect match from the 5′ flanking region to the end of Vα with the sequence previously reported as Vα 2.6 (17, 18). The J region was from Jα38 (19).

Expression and In Vitro Refolding of VαJα Protein. The B4.2.3 Vα domain construct was prepared by PCR amplification of the cloned α chain cDNA. The primers used to generate Vα short and Vα long were 5′-GGAATTCCATATGGTGAGTGGCCAGCAGGAG-3′ (5′ LONG OLIGO), 5′-GGAATTCCATATGCAGCAGGTGAGACAAAGTCC-3′ (5′ SHORT OLIGO), and 5′-CGCGGATCCTCATGGATTTACTACCAGACTTGTCCC-3′ (3′ OLIGO). PCR was carried out with deep-vent polymerase (New England Biolabs, Beverly, MA) through 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, and extension at 72°C for 3 min. PCR products were isolated by agarose gel electrophoresis and purified using QIAEX (Qiagen, Inc., Chatsworth, CA). The PCR products were cleaved with the relevant restriction enzymes and cloned into the pET-3a vector (Novagen Inc., Madison, WI [20]). The integrity of the cloned fragment was verified by nucleotide sequence determination. The Vα2.6J38 sequence reported here has been deposited in GenBank (accession number U63546).

E. coli strain BL21(DE3) cells were transformed with pET3a constructs containing the TCR-Vα domain gene, and were grown in 500 ml to midlogarithmic phase (OD_{600} = 0.6–0.8) in Luria-Bertani (LB) broth containing carbenicillin (200 μg/ml) at 37°C. TCR-Vα domain production was induced by addition of 0.5 mM isopropyl β-D-thiogalactoside (IPTG). After incubation for 3 h, the cells were centrifuged, washed with TE buffer (100 mM Tris, 2 mM EDTA, pH 8.0), and resuspended in 20 ml of TE containing hen egg lysozyme (0.25 mg/ml). After incubation at 4°C overnight, cell lysis was completed by sonication in the presence of 0.1% (wt/vol) sodium deoxycholate (DOC). Detergent-insoluble inclusion bodies were pelleted in a rotor (model SS-34; Sorvall, DuPont Instruments, Wilmington, DE) at 15,000 rpm for 30 min, washed twice in TE buffer containing 0.1% DOC, and washed again with TE buffer lacking DOC three times.

The B4.2.3 TCR-Vα domain was refolded from inclusion bodies using a modification of published procedures (21, 22). Briefly, the partially purified inclusion bodies were dissolved in 10 ml of 6 M guanidinium hydrochloride, 0.3 M dithiothreitol, 2 mM EDTA, and 0.1 M Tris, pH 8. After incubation at room temperature for 1–2 h, protein was dialyzed against 6 M guanidinium hydrochloride (pH 2) at 4°C. Mixed disulfide bonds were produced by adding an equal volume of 6 M guanidinium hydrochloride, 0.4 mM oxidized glutathione, 4 mM EDTA, and 0.2 M Tris, pH 8, to the denatured protein in solution. After incubation at room temperature for 4 h, the solution was dialyzed against 6 M guanidine hydrochloride, 0.1 M Tris, and 2 mM EDTA, pH 8, at 4°C. The protein was then diluted 1:100 into 0.4 M arginine, 0.4 mM reduced glutathione, 2 mM EDTA, and 0.1 M Tris, pH 8. After incubation at 6–10°C with stirring for 4 d, the renatured protein was extensively dialyzed against 20 mM glycine, pH 9. In some cases, the Vα domain was refolded by a modification of the method of Kurucz et al. (23). Inclusion bodies were solubilized in 2% sodium lauryl sarcosinate (SLS), 50 mM Tris HCl, pH 8.0, and oxidized by addition of 50 μM $CuSO_4$. This was made 6 M in urea, and SLS was removed on Dowex 1 (AG 1-X8; Bio-Rad Laboratories, Hercules, CA). Protein was then diluted 1:100 into 0.4 M arginine, 0.1 M Tris, pH 8, and 2 mM EDTA. This was then dialyzed against glycine as described above. Protein refolded by either protocol was then partially purified and concentrated by ion-exchange chromatography on a HiTrap Q column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) equilibrated in 20 mM glycine, pH 9, and step eluted with PBS. Further purifica-

tion was achieved with size exclusion chromatography on a Sephadex 75L column (Pharmacia Biotechnology Inc.) and isocratic elution in PBS. The homogeneous peak was collected and concentrated by centrifugal ultrafiltration with Centricon 3 (Amicon, Inc., Beverly, MA) membranes. The yield of purified protein varied between 10 and 50 mg from 1 liter of bacterial culture. Vα proteins refolded by either protocol were identical as assessed by SDS-PAGE, gel chromatography, antibody reactivity, and CD spectra. Crystals were obtained under the same conditions with material refolded either way.

Real-Time Surface Plasmon Resonance. Binding of TCR proteins to antibodies was evaluated by surface plasmon resonance using a Pharmacia BIAcore™ (Pharmacia Biosensor AB, Uppsala, Sweden). All binding experiments were performed at 25°C. mAbs and TCR domains were coupled through amino groups by conventional methods as previously described at pH 4.5–5.5 (24, 25). TCR domains and mAbs were diluted into HBST (20 mM Hepes, pH 7.1, 150 mM NaCl, 3.4 mM EDTA, and 0.005% Tween 20) and injected over either antibody or TCR domain–coupled biosensor surfaces, as described in detail elsewhere (26). The surfaces were regenerated by exposure to 50 mM phosphoric acid. Rat anti–mouse Vα2 mAb (clone B20.1 [27]) and anti–mouse Vβ7 (clone TR310 [28]) were purchased from PharMingen (San Diego, CA).

Analysis of Kinetics Binding Data. Text files of data gathered from experiments were imported into IGOR Pro (WaveMetrics, Lake Oswego, Oregon) running on a Macintosh computer, and the binding curves (sensorgrams) were fit to single and in some cases double exponential expressions (O'Shannessy et al., 1993). Rate constants for dissociation kinetics were determined based on sensorgrams collected from washout experiments performed at a flow rate of 100 μl/min, a rate shown to minimize the effects of rebinding. Curve fitting was performed using a single exponential function descriptive of one kinetic class of the dissociating complexes: $B_t = B_0 \exp(-k_{off}t)$, where B_t is binding at time t, B_0 is binding at time 0, and k_{off} is the apparent dissociation rate constant. For the determination of the association rate constants, the time scale was adjusted to zero at the beginning of the injection of the fluid phase analyte, and the association phase of the sensogram was fit to the exponential function: $B_t = B_m (1-\exp(k_{obs}t))$, where B_m is the maximal level of binding in resonance units (RU), and $k_{obs} = (k_{on}c) + k_{off}$. c is the molar concentration of injected analyte, and k_{on} is the apparent association rate constant in units of $M^{-1}\ s^{-1}$. Sensorgrams in the association phase were collected over a range of c, and k_{on} was determined from the linear plot of c versus k_{obs}.

CD. CD spectra were measured in a spectropolarimeter (model J500C; Jasco Inc., Easton, MD) equipped with a DP500N data processor at 25°C. Protein concentrations were 100 μg/ml in a 1-mm pathlength quartz cuvette. Data were digitized and downloaded for curve fitting as described before (29). Spectra were analyzed in terms of secondary structure using CONTIN (30).

Crystallization. Screening for crystallization conditions was performed in hanging drops using the sparse matrix screen of Jancarik and Kim (31) as implemented in Crystal Screen (Hampton Research, Riverside, CA). The most reproducible conditions were with 85–90% 4.0 M Na formate, pH 7.

Nuclear Magnetic Resonance Spectroscopy. The ^1H NMR spectra of solutions of Vα short protein at concentrations ranging from 0.25 to 1.0 mM in PBS were recorded at 600 MHz, 25°C. The transverse relaxation rates for backbone amides in well-structured regions of a protein are relatively uniform, and values measured for the overlapping amides in the 9.3–9.5-ppm region were used to determine the rotational correlation time, τ_c, of the protein. The empirical relation $\tau_c = 10^{-9}/(5 \times T_2)$ s^2 was used for calculating τ_c from the transverse relaxation time, T_2 (32).

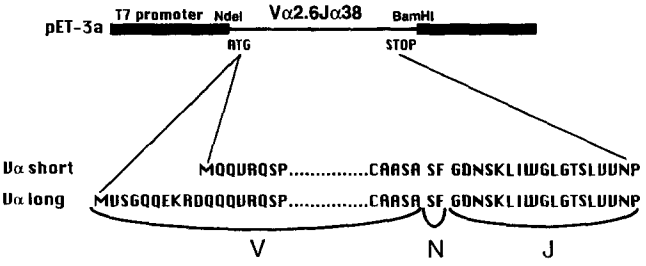

Figure 1. Structure of Vα long and Vα short expression vectors. A schematic of the pET3a subclones of Vα short and Vα long is shown. Partial amino acid sequences emphasizing the NH_2 termini, the N regions of the encoded proteins, and the J segments are shown.

Results

Cloning and Expression of TCR-Vα Domain. The nucleotide sequence of the cDNA encoding the Vα domain of the B4.2.3 hybridoma indicated that the hybridoma expressed Vα2.6Jα38 with the junctional sequence shown in Fig. 1. To confirm that this sequence represented that which was used by the B4.2.3 hybridoma, the hybridoma cells were examined by surface immunofluorescence using Vα2-specific mAb B20.1, confirming that they express a receptor of this Vα family (data not shown). Because of ambiguity in the location of the codon encoding the NH_2-terminal amino acid residue of the mature Vα chain (17, 18), we engineered two different methionine initiation codons (ATG) in the positions indicated in Fig. 1, and the resulting proteins have been referred to as Vα long and Vα short. A single-chain three-domain TCR with specific binding and biological activity uses the Vα short NH_2-terminal sequence, suggesting that this is the natural length of the molecule (Plaksin, D., P. McPhie, and D.H. Margulies, manuscript in preparation).

Both Vα long and Vα short proteins were expressed at high levels (~50 μg/ml bacterial culture) after IPTG induction of bacteria containing the indicated plasmids (Fig. 2 *A*). To obtain natively folded protein, inclusion bodies were either solubilized in guanidinium hydrochloride and refolded by controlled reoxidation of disulfide bonds and renaturation or solubilized in detergent, oxidized, and refolded as detailed in Materials and Methods. After concentration and partial purification by ion exchange chromatography, concentrated protein was further purified by size exclusion chromatography on a Superdex 75L (Pharmacia Biotech, Piscataway, NJ) column (Fig. 2 *B*). As can be seen in the chromatogram, even at this stage of purification, there was no convincing indication of a noncovalent dimer of the Vα domain, although a rare chromatogram (such as that shown) revealed a suggestion of a dimer at a level insufficient for quantitation. When purified protein was concentrated further by ultrafiltration to levels as high as 2 mM and analyzed again on either Superdex 75L or Superdex

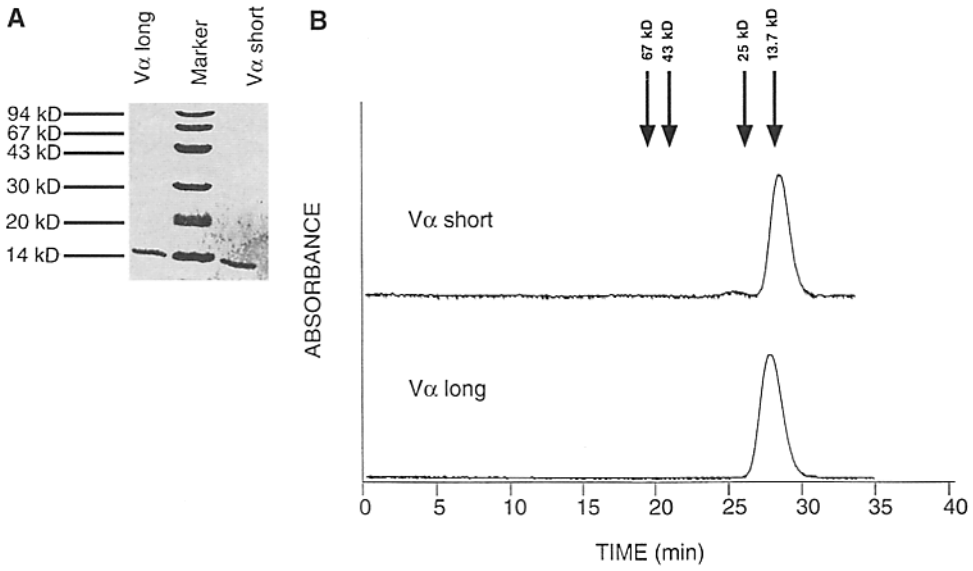

Figure 2. Homogeneity of refolded Vα domains. (*A*) SDS-PAGE of purified Vα2 domains. Both Vα long and Vα short were expressed in *E. coli* as described in Materials and Methods, and protein was refolded and purified through both ion exchange and size exclusion chromatographic steps as described. The TCR domains were examined after reduction of disulfide bonds in 20% polyacrylamide gel containing SDS. The molecular size markers are indicated in kilodaltons. (*B*) Size exclusion chromatography under native conditions. Samples of the refolded Va domains were analyzed by size exclusion chromatography in PBS on Superdex 75L at a flow rate of 0.5 ml/min. Positions of molecular size standards are indicated in kilodaltons. Absorbance at 280 nm is plotted in arbitrary units.

200L columns in PBS, no indication of a dimer was observed.

Circular Dichroism Reveals Secondary Structure Similar to That of Ig V Domains. To evaluate the nature of the secondary structure of the refolded Vα domains, protein purified through both ion exchange and size exclusion chromatography steps was examined by CD spectroscopy (Fig. 3). Spectra of both Vα long and Vα short showed characteristic minima at 215 nm consistent with a largely β sheet structure. Spectra were analyzed in terms of secondary structure using the CONTIN program (30). The Vα long showed 41 ± 10% β sheet and 4 ± 5% α helix; the Vα short had 38 ± 9% β sheet and 5 ± 5% α helix. These spectra are similar to those reported for a Vα4.2 TCR domain (33) and to those described for single-chain Fv TCR (34, 35). These all have similarities to the spectra of Ig V domains and Fab fragments (36–40).

Reactivity with Vα2-specific mAb. To confirm that the refolded protein was in a native state, we analyzed the antibody reactivity to the isolated Vα2.6 domains by surface plasmon resonance (SPR). The specific mAb B20.1 (anti-Vα2) was covalently linked to the dextran-modified gold surface of an SPR biosensor chip, and the domains (Vα short and long, respectively) were injected in solution (Fig. 4). Binding of the purified Vα long and Vα short proteins to the immobilized antibody was clearly observed as the steady, time dependent increase in RU. The dissociation rate constant k_{off} was obtained by curve fitting to the washout phase, 0.032 (± 0.001) s^{-1} (which corresponds to a $t_{1/2}$ of 21 s). Association phase data were collected over a range of

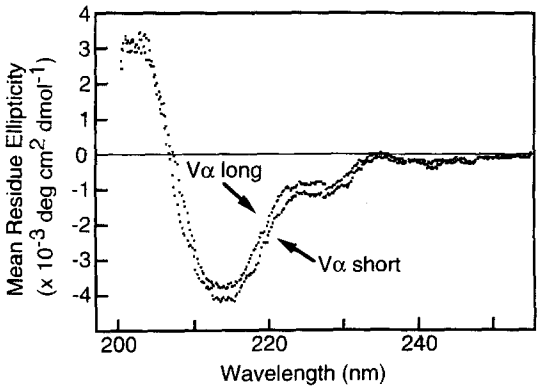

Figure 3. CD spectra of refolded Vα domains. CD spectra were collected as described in Materials and Methods at 25°C. Spectra of Vα long and Vα short are indicated.

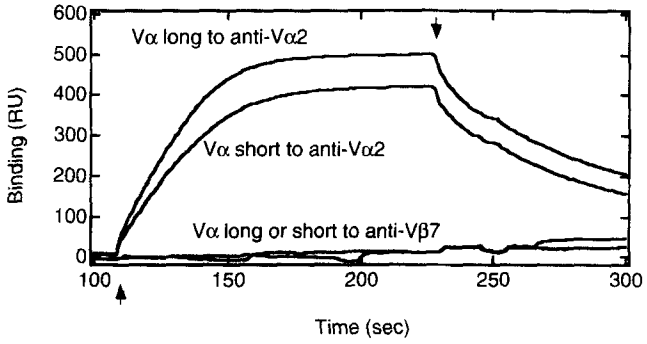

Figure 4. Vα2 domains bind to anti-Vα2 but not anti-Vβ7 mAb. Anti-Vα2 or anti-Vβ7 mAb was coupled to a biosensor chip (~8,000 RU of each were coupled) as described in Materials and Methods. Vα long or Vα short was injected over the two mAbs at a flow rate of 10 μl/min, and binding was detected by SPR. The upward-pointing arrow indicates the initiation of the wash-on (association) phase, and the downward arrow indicates the initiation of the wash-out (dissociation) phase.

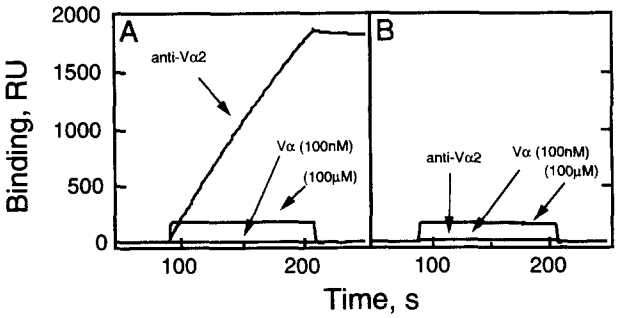

Figure 5. Failure to detect dimerization in SPR assay. (*A*) Vα short was immobilized on a biosensor surface (to level of ~3,000 RU) as described in Materials and Methods and exposed to either the Vα short domain in solution or an anti-Vα2 mAb as indicated at a flow rate of 10 μl/min. *B* shows a blank surface. The concentration of anti-Vα2 was 100 nM, and the concentration of the solution phase Vα short domain was injected at a concentration of either 100 nM or 100 μM.

concentration, and the concentration-dependent k_{obs} were obtained by curve fitting as described in Materials and Methods. These were plotted against concentration, fit to a linear regression ($r = 0.999$) and yielded a k_{on} of 2.37×10^6 $M^{-1}s^{-1}$. (The k_{off} obtained from the y intercept of the c versus k_{obs} plot also was equal to 0.032 s^{-1}). The calculated equilibrium dissociation constant was $K_d = 1.35 \times 10^{-8}$ M. The Vα2 domains also did not bind to the control anti-Vβ7 mAb, as expected. The Vα2 domain was also immobilized to the surface, and the specific anti-Vα2 mAb bound tightly, with a much longer $t_{1/2}$, due to the bivalency of the reaction (see Fig. 5 *A*).

Crystallization and Preliminary X-Ray Analysis. We screened for conditions that supported the crystallization of both Vα long and Vα short. Using a sparse matrix screen (31), we found that Vα long crystallized in both a hexagonal bipyramid form and in an orthorhombic form. The hexagonal bipyramids did not diffract to more than 6 Å, whereas the orthorhombic crystals diffracted to 2.5 Å. Preliminary x-ray diffraction analysis suggests that the orthorhombic crystals have unit cell parameters $a = 56.1$ Å, $b = 78.4$ Å, $c = 89.1$ Å. Space group $P2_12_12_1$ was indicated by systematic absences in the principle zones. The Matthews coefficient V_M (41) was 8.1 $Å^3$/dalton. Assuming the partial specific volume of the protein in the crystal to be 0.74 cc/g (the average value for most globular proteins), there are probably four Vα molecules in the asymmetric unit and a solvent content of ~40%.

Analysis of Dimerization. Having established the purity, native configuration, and homogeneity of the Vα domains, we asked whether we could detect dimers even at high concentration using techniques potentially more sensitive than gel filtration chromatography. Because the SPR method permits the detection of molecular interactions of K_d as high as 10^{-4} M under some conditions, we immobilized the Vα short domain to a biosensor chip, and then exposed the immobilized protein to very high concentrations of the Vα domain in solution. As shown in Fig. 5, even at concentrations as high as 100 μM, no binding to the immobilized Vα was observed. The level of the RU signal observed is due to the refractive index change due to the high concentration of protein, and was no different when these same protein solutions were passed over a control surface (Fig. 5 *B*). As a positive control, a very low concentration (100 nM) of the anti-Vα2 mAb bound the immobilized Vα domain very well (Fig. 5 *A*).

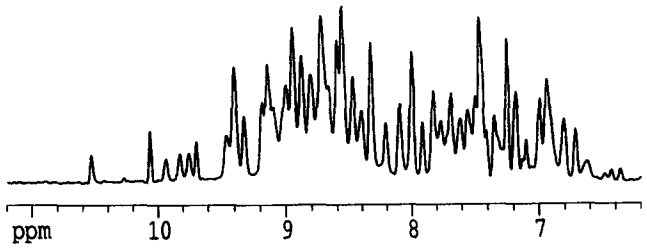

Figure 6. ^1H NMR spectrum of the amide region of Vα short, recorded from a 0.25-mM solution at 600 mHz, 25°C. The transverse relaxation rates for backbone amide protons were used to determine the rotational correlation time, τ_c, of the protein, as described in Materials and Methods.

Finally, we have examined the dimerization characteristics of the Vα short domain by solution NMR spectroscopy. For macromolecules, the transverse relaxation rate (T_2^{-1}) of the ^1H resonances increases linearly with the rotational correlation time of the protein. The rotational correlation time is a direct function of the molecular size of the protein. Because the amide region of the ^1H NMR spectrum of Vα short is well behaved (see Fig. 6), we have used the measured values of T_2, determined as a function of concentration, to assess the dimerization. At a concentration of 1 mM, the backbone amide protons of Vα short relax relatively uniformly at a rate of 48 ± 2 s^{-1}. This rate decreases to 36 ± 2 s^{-1} at 0.5 mM, and 33 ± 2 s^{-1} at 0.25 mM. A relaxation rate of 33 s^{-1} corresponds to a rotational correlation time of 6.6 ± 0.6 ns (32), which is close to the value expected for a nearly spherical globular protein of ~14 kD at 25°C. (Vα short has a calculated molecular weight of 12,688 daltons, and that determined by SDS-PAGE and size exclusion gel chromatography is ~14 kD [see Fig. 2]). The increase in rotational correlation time observed at higher concentrations is indicative of a rapid exchange between monomeric and dimeric forms of the protein, with the monomeric and dimeric states roughly equally populated at 1 mM. Thus, the K_d for the dimerization reaction, $2(V\alpha) \rightleftharpoons (V\alpha)_2$, is estimated to be 1 ± 0.5 mM.

Discussion

We report here the biochemical, serological, and biophysical properties of a murine TCR Vα domain expressed as inclusion bodies in *E. coli*. Using minor modifications of standard refolding protocols, the inclusion bodies are easily solubilized, and the single intrachain disulfide bond is readily formed. No covalent dimers or multimers were observed. Upon removal of denaturant and transfer to native conditions, the protein exhibits a CD spectrum consistent with being a single-Ig–like domain. The refolded domain

binds to the single available Vα2-specific mAb, and this epitope is sensitive to thermal denaturation in native buffer (data not shown). Thus, in addition to other Ig domains that have been expressed as inclusion bodies in *E. coli* (42), we have now demonstrated the utility of expressing the TCR-Vα domain in this manner. At lower yield, some TCR Fv constructs have also been expressed as inclusion bodies (22, 34, 43). This approach is similar to that used in the expression of antibody (44–47) and Vα (33) domains in *E. coli* using bacterial leader peptides to direct the newly synthesized protein to the periplasmic space, but offers the advantage that the preparation of the inclusion bodies is a simple first step in the purification of the protein. The yields we have achieved are excellent and compare favorably with the maximum amounts that can be prepared from bacterial cells in systems that exploit the high-level expression of a single mRNA by virtue of the use of the highly processive T7 RNA polymerase (20). The major disadvantage with intracellular expression as inclusion bodies is that the insoluble protein must be solubilized and refolded; however, at least in the case of this single-domain TCR-Vα, refolding can be performed efficiently. This domain crystallizes readily, an indication of its purity. In addition, such homogeneous preparations of TCR-Vα domains should prove invaluable in immunization for family- and subfamily-specific mAbs.

The ability to produce such large amounts of homogeneous and soluble TCR-Vα domain has permitted us to examine in detail the nature of the dimerization in solution. The Vα domain that we report here only dimerizes in solution at very high concentration, with a dimerization constant estimated from ^{1}H NMR spectroscopy of 1 mM. This weak dimerization is in marked contrast to that of the Vα4.2 domain studied by Fields et al. (12, 13), which purifies as a 25-kD homodimer in solution. Two distinct types of Vα dimerization should be considered: one that forms the V_H/V_L-like Fv dimer through the largely hydrophobic interactions of the Vα domains which may be influenced by amino acid differences in different Vαs; and a second that is the result of two Vα dimers to form $(V\alpha/V\alpha)_2$ superdimers. The weak dimerization of the Vα2.6 domain that we observe in solution is presumably that of the first type, and the strong dimerization noted by Fields et al. in solution is also likely to be similar. The model proposed by Fields et al. (12) is that the structure of the TCR promotes the formation of a cell surface $(V\alpha V\beta)_2$ tetramer as part of an early signal in T cell activation. Because the Vα4.2 domain they studied dimerizes in solution (presumably through the V_HV_L-like interface observed in the crystal), and because its crystals reveal a dimer of dimers that contact through the C″ strands of the Vαs, these authors have suggested that the dimerization of the complete cell surface TCR is largely mediated through Vα. Structural evidence consistent with this view is that the folding topology of the β strands of the Vα4.2 domain pairs the C″ strand with the D strand, an organization distinct from that of typical Ig V domains in which the C″ strand hydrogen bonds to C′. The reorganization of these strands in the Vα4.2 crystal structure allows the packing of the Vα domains, which would be sterically impeded in Ig V domains or Vβ. Although we cannot yet address the issue of crystal packing in the Vα2.6 domain we describe here, the fact that we observed no dimer at reasonable concentrations in solution and required concentrations of at least 0.5 mM to detect some by ^{1}H NMR suggests that not all Vα domains have the same tendency to form the V_HV_L-like dimer. The issue of dimerization of molecules confined to the cell surface is more complex than that of molecules in solution, and weak molecular interactions as measured with soluble domains may prove significant for cell surface receptors (48, 49). Thus, whether TCR dimerization is mediated, enabled, or enhanced by conserved interactions through Vα will require additional knowledge of the structure of Vα2.6 as well as of complete TCR and TCR–MHC–peptide complexes. Inspection of the amino acid sequence of the Vα2.6 domain in the region of the C″ and D strands indicates that this Vα differs significantly from the Vα4.2 of Fields et al. (12). Whether the Vα2.6 domain exhibits the same strand pairing as Vα4.2 and whether its decreased ability to dimerize reflects structural differences in the C″ and D strands remain to be determined. The Vα2.6 domain that we have examined is derived from an MHC class I–restricted TCR, whereas the Vα4.2 domain studied by Fields et al. (12) is from an MHC II–restricted TCR. It thus remains a formal possibility that dimerization potential through Vα reflects the MHC class specificity of the TCR, though evidence suggesting a role of MHC I dimers in T cell activation (50) and against MHC I versus MHC II bias of TCR repertoire (51) would argue against such an explanation.

Thus, our characterization of a Vα2.6 domain derived from a TCR specific for an MHC class I (H-2D^d)–restricted, viral peptide–specific (RGPGRAFVTI) complex raises the possibility that TCR dimerization through the Vα domain is not an obligate property of this domain, but, perhaps like the dimerization of Ig V_L domains (45, 52, 53), reflects unique differences between particular molecules. Additional structural and binding studies of cell membrane components involved in T cell signaling should help clarify the contribution of the Vα domain.

We thank Sergei Khilko and Lisa Boyd for their help, David Segal for advice on protein refolding, and Misha Sitkovsky and Kannan Natarajan for their comments on the manuscript.

D. Plaksin was supported by a Fogarty Fellowship. This work was supported by a grant from the AIDS-Targeted Anti-Viral Program of the Office of the Director of the National Institutes of Health to A. Bax.

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Received for publication 14 May 1996 and in revised form 15 July 1996.

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