Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 3830–3834, May 1993
Immunology

A bacterially expressed single-chain Fv construct from the 2B4 T-cell receptor

ISTVAN KURUCZ, CAROLINA R. JOST, ANDREW J. T. GEORGE, SARAH M. ANDREW, AND DAVID M. SEGAL

Experimental Immunology Branch, National Cancer Institute, Building 10, Room 4B17, National Institutes of Health, Bethesda, MD 20892

Communicated by David R. Davies, January 19, 1993 (received for review November 9, 1992)

ABSTRACT **A single-chain Fv construct of the 2B4 T-cell receptor has been made and expressed in *Escherichia coli* as bacterial inclusion bodies. After solubilization in 6 M guanidine hydrochloride and formation of mixed disulfides with glutathione, the protein was refolded by diluting out the denaturant and allowing intramolecular disulfide bridges to form by disulfide exchange. Approximately 65–100 mg of refolded protein was obtained from 1 liter of bacterial culture, an appreciable fraction of which was monomeric in nondenaturing solvents. This protein bound to three monoclonal antibodies specific for allotypic or idiotypic determinants on the native 2B4 variable region but did not bind several other anti-T-cell-receptor monoclonal antibodies that lacked such specificity. These experiments show that T-cell-receptor variable regions, like the V regions of antibodies, can form a well-behaved single-chain Fv molecule and provide large amounts of recombinant single-chain Fv T-cell receptor that can be used to study T-cell function.**

Our current knowledge of the molecular structure of the antigen-specific portion of the T-cell receptor (TCR) is based upon its striking homology in amino acid sequence with antibodies (1). Three-dimensional structures of antibodies and antibody–antigen complexes are known (2, 3), and it is likely that the TCR structure is very similar (4, 5). However, in spite of the homology between the two classes of molecules, TCRs and antibodies recognize different types of antigens. In general, an animal can produce antibodies that bind to any foreign antigen (6). In contrast, the TCRs of an animal recognize only peptide antigens embedded in a deep groove formed by the N-terminal domains of class I or class II molecules of the major histocompatibility complex (7, 8). Unlike antibodies, where characterization is facilitated by their soluble nature, TCRs exist exclusively as transmembrane receptors and are, therefore, difficult to obtain in the large amounts needed for structural and functional characterization. Several attempts have been made to obtain genetically engineered soluble TCRs in biochemically significant quantities. These include the production of TCR chains truncated immediately prior to the transmembrane portion (9), of hybrid molecules composed of TCR and immunoglobulin (10, 11), and of genetically altered forms that can be easily cleaved from the plasma membrane (12, 13).

Recently, several groups have demonstrated that recombinant proteins can be produced from antibody molecules by joining the C terminus of the heavy chain variable (V) region to the N terminus of light chain V region (or vice versa) by using a polypeptide spacer (14, 15). Such constructs, known as single-chain Fv (sFv) constructs, have binding activities similar to those of the native antibodies (16). Because of the similarity between antibodies and TCRs, it seems likely that active sFv constructs from TCRs could also be produced. Therefore, we and others (17–19) have generated TCR–sFv molecules, with the expectation that such constructs will have the antigen binding properties and structures of V portions of the native receptors. In this paper we have produced a soluble TCR–sFv from the TCR of the 2B4 T-cell hybridoma, specific for a C-terminal peptide of cytochrome *c* bound to I-E^k (20). The recombinant protein was expressed in large amount as bacterial inclusion bodies, and after *in vitro* refolding, this TCR–sFv existed as a monomeric globular protein in solution and was indistinguishable antigenically from the V regions of the native TCR.

MATERIALS AND METHODS

Cell Lines and Antibodies. 2B4 cells were obtained from Jonathan Ashwell (National Cancer Institute). 2B4 is a T-cell hybridoma specific for a C-terminal peptide of pigeon cytochrome *c* bound to I-E^k (20). The 2B4 TCR uses the V region genes $V_\alpha 11$ and $V_\beta 3$ (21, 22). The following monoclonal antibodies (mAbs) were used: A2B4 (anti-2B4 clonotype) (23) (a kind gift from Jonathan Ashwell) and H57 (anti-C_β) (24), KT65 (anti-$V_\alpha 8$) (25), RR8.1 (anti-$V_\alpha 11$) (26), KJ25 (anti-$V_\beta 3$) (27), RR4.7 (anti-$V_\beta 6$) (28, 29), F23.1 (anti-$V_\beta 8$) (30, 31), and RR 3.15 (anti-$V_\beta 11$) (32, 33) (kindly provided by Richard Hodes, National Cancer Institute). The anti-myc-peptide antibody myc1 9E10.2 (34) was purchased from the American Type Culture Collection. Alkaline phosphatase and fluorescein isothiocyanate-conjugated antibodies against mouse, rat, and hamster IgG were purchased from Southern Biotechnology Associates (Birmingham, AL).

Bacterial Strains, Plasmids, and Oligonucleotides. Bluescript SK(+) (pBS) (35) and *Escherichia coli* XL-1 Blue (36) were purchased from Stratagene. Plasmid pET11d and bacterial strain BL21DE3 (37) were provided by F. W. Studier (Brookhaven National Laboratory). Oligonucleotides were synthesized using an Applied Biosystems 391 DNA synthesizer and were purified on oligonucleotide purification cartridges (model OPD 400771; Applied Biosystems).

SDS/PAGE and Column Chromatography. SDS/PAGE was done using a Pharmacia PhastSystem with 12.5% gels. Gels were stained with Coomassie brilliant blue G250. Size-exclusion chromatography was performed with a Pharmacia FPLC system. A Superdex 75 HR 10/30 column was used for analytical purposes, and a 1.6 × 55 cm column of preparative-grade Superdex 75 was used for purifications.

TCR–sFv Construct. The 2B4 TCR–sFv construct was prepared by PCR amplification of 2B4 cDNA as follows: mRNA was isolated from $\approx 10^8$ 2B4 cells by using the FastTrack mRNA isolation kit (Invitrogen, San Diego). At the time of isolation of mRNA, 2B4 cells expressed high levels of TCR and responded to nominal antigen. Reverse transcription was carried out in a 20-μl reaction mixture containing 1 μg of mRNA, all four dNTPs (each at 0.5 mM) (Perkin–Elmer/Cetus), 40 units of RNase inhibitor, 200 units

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Abbreviations: TCR, T-cell receptor; sFv, single-chain Fv; V, variable; C, constant; V_α, C_α, etc., α chain V region, α chain C region; mAb, monoclonal antibody.

of Moloney murine leukemia virus reverse transcriptase, 4 μl of 5× reverse transcription buffer (all from GIBCO/BRL), and 2.5 μM 3′ primer. The primers used to generate V_α and V_β cDNA were ATATACTGCAGATCTTTTAACTGGTACACAGC and ATATAAAGCTTTCACTAAGTCACATTTCTCAGATCCCT, respectively. V_α and V_β constructs were produced by PCR amplification of the homologous cDNA using the 3′ primers described above and the following 5′ primers: for V_α, ATATAGGATCCGGCATGGGAGATCAGGTGGAGCAGAG, and for V_β, ATATACTGCAGAATTCAAAAGTCATTCAGAC.

PCR was carried out with 25 cycles (denature at 94°C, 30 sec; anneal at 50°C, 1 min; extend at 72°C, 1 min). *Taq* polymerase and PCR buffer were obtained from Perkin–Elmer/Cetus. PCR products were isolated by agarose gel electrophoresis and purified using Geneclean (Geneclean II kit; Bio 101, La Jolla, CA). The PCR products were then cleaved with the relevant restriction enzymes (all purchased from GIBCO/BRL; *Bam*HI and *Pst* I for V_α and *Pst* I and *Hin*dIII for V_β, to cleave restriction sites incorporated into primers) and cloned separately into pBS. Finally, V_α and V_β were incorporated into the same vector by linking the two at the *Pst* I site. The resulting plasmid, designated pBS-αβ-6, contained an insert encoding V_α linked to the N terminus of V_β by the first 14 residues of C_α. mRNA generated from the T7 promoter of pBS-αβ-6 produced a protein of the correct molecular weight by using a rabbit reticulocyte lysate *in vitro* translation system (Promega). The sequence of the insert was determined using the dideoxynucleotide sequencing method with a Sequenase Version 2.0 kit (United States Biochemical).

The insert was further modified by PCR amplification and inserted into the pET11d expression vector. PCR amplification of pBS-αβ-6 was done using the following primers: for V_α, 5′ primer, ATATACCATGGGAGATCAGGTGGAGCAGAGTCCT, and 3′ primer, TGAGCCTCCACCCCCGGATCCACCCCCGCCAGACCCTCCGCCACCATCTTTTAACTGGTACACAGC. These primers incorporate an *Nco* I site and a start codon at the 5′ end and a *Bam*HI site and sequences encoding a portion of the $(G_4S)_3$ linker (14) at the 3′ end. For V_β, the 5′ and 3′ primers were GGTGGCGGAGGGTCTGGCGGGGGTGGATCCGGGGGTGGAGGCTCAAATTCAAAAGTCATTCAGACT and ATATAGGATCCTCACTAATTCAGATCCTCTTCTGAGATGAGTTTTTGTTCTGAGGAGACGGTAGTCACATTTCTCAGATCCTC, respectively. These primers incorporate *Bam*HI sites at both 5′ and 3′ ends of V_β and the remaining portion of the $(G_4S)_3$ linker at the 5′ end. In addition, the 3′ primer contains two stop codons and encodes a peptide from the *myc* oncogene that is recognized by the myc1 9E10.2 mAb. After digestion with *Nco* I and *Bam*HI, the V_α product was ligated into the *Nco* I and *Bam*HI sites of pET11d. This vector was then cleaved with *Bam*HI and the V_β product (after digestion with *Bam*HI) was ligated. After transformation, colonies were selected in which the V_β construct was in the correct orientation, as judged by restriction mapping and PCR analysis.

Protein Expression. BL21DE3 cells were transformed with pET11d containing the TCR–sFv construct and were grown to midlogarithmic phase (OD_{600} = 0.8–1.0) in ampicillin (50 μg/ml)-containing LB broth at 37°C. TCR–sFv production was then induced by addition of 1 mM isopropyl β-D-thiogalactoside (InoVar Chemicals, Gibbstown, NJ). After a 2-h incubation, bacterial RNA synthesis was blocked by adding rifampicin (0.2 mg/ml; Sigma), and the cultures were shaken for an additional hour. Initially, pellets from 2-ml cultures from single clones were lysed in SDS sample buffer and subjected to SDS/PAGE. One clone that produced large amounts of protein as judged by Coomassie staining and by Western blot analysis with the anti-peptide antibody was chosen for further experiments. For larger-scale production of the 2B4 TCR–sFv, this clone was grown and induced as described above in ≈500 ml. The cell pellet was washed once with TE buffer (100 mM Tris/2 mM EDTA, pH 8.0) and resuspended at a density of 1 g (wet bacteria) per 10 ml of TE containing hen egg lysozyme (1 mg/ml). After incubation at 4°C overnight, cell lysis was completed by sonication in the presence of 0.1% sodium deoxycholate (SDC). Inclusion bodies were pelleted at 22,500 × *g* for 30 min and washed twice in TE buffer containing 0.1% SDC followed by three more washes in TE buffer without SDC.

Refolding of the Recombinant Protein. 2B4 TCR–sFv was refolded using a procedure similar to that of Buchner and Rudolph (38). Briefly, partially purified inclusion bodies [0.3–0.5 g (wet weight)] were dissolved in 6–10 ml of 6 M guanidine hydrochloride/0.3 M dithiothreitol/2 mM EDTA/0.1 M Tris, pH 8.0. After incubation at room temperature for 1–2 h, protein was dialyzed exhaustively against 6 M guanidine hydrochloride (pH 2.0) at 4°C. Mixed disulfide bonds were produced by adding an equal volume of 6 M guanidine hydrochloride/0.4 M oxidized glutathione/4 mM EDTA/0.2 M Tris, pH 8.0, to the denatured protein solution. After a 2- to 3-h incubation at room temperature, the solution was dialyzed against 6 M guanidine hydrochloride/0.1 M Tris/2 mM EDTA, pH 8, at 4°C. The protein was then diluted to a final concentration of 20 μg/ml or less (assuming an A_{280} value of 1.3) into 0.4 M arginine hydrochloride/0.4 mM reduced glutathione/2 mM EDTA/0.1 M Tris, pH 8.0. After incubation at 10°C for 3–4 days, the renatured protein was concentrated by ultrafiltration with an Amicon stirred cell using PM10 or YM10 Diaflo ultrafilter membranes. The yield of protein at this stage varied between 65 and 100 mg from 1 liter of bacterial culture, as determined by A_{280} measurements.

ELISA. Plastic plates (Immulon 1; Dynatech) were incubated with a fixed amount or with serial dilutions of antigen and blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS). Probing antibodies were applied, usually in the form of hybridoma tissue culture supernatant, incubated, washed, and labeled with an alkaline phosphatase-labeled secondary antibody (goat anti-hamster, mouse, or rat; Southern Biotechnology Associates). After addition of *p*-nitrophenyl phosphate substrate (Sigma), A_{405} values were recorded with an ELISA reader (Titertek Multiscan MCC/340; Flow Laboratories). Cross-reaction of the secondary antibody with the antigen was always checked by omitting the probing antibody. In one experiment, the TCR–sFv was reduced and alkylated prior to analysis. The protein was denatured in 8 M urea/0.1 M Tris, pH 8, and either reduced for 30 min at room temperature with a 0.1 vol of 0.1 M dithiothreitol in 0.1 M Tris (pH 8) and then treated with 0.1 vol of 0.2 M iodoacetamide in the same buffer for 30 min at room temperature or first reduced and then alkylated. Samples were diluted in borate-buffered saline and analyzed by the ELISA described above.

Flow Cytofluorometric Analysis. 2B4 cells were labeled with TCR-specific mAbs and stained with fluorescein isothiocyanate-conjugated secondary antibodies (1–2 μg per 10^6 cells). Cells were analyzed with a Becton Dickinson FACscan using the CONSORT 30 program.

RESULTS

sFv–TCR Construct. The genetic construct used to generate an sFv from the 2B4 TCR is shown in Fig. 1*A*. The construct encodes the 2B4 V_α domain followed by a 29-residue spacer that links the C terminus of V_α to the N terminus of V_β. The linker consists of 14 α chain constant region (C_α) residues followed by a 15-residue flexible spacer, $(Gly_4\text{-}Ser)_3$, that has commonly been used in constructing sFv proteins from antibodies (14). In addition, the construct encodes a peptide tag (derived from the human *myc* oncogene) joined to 7 C_β residues at its C-terminal end that allows

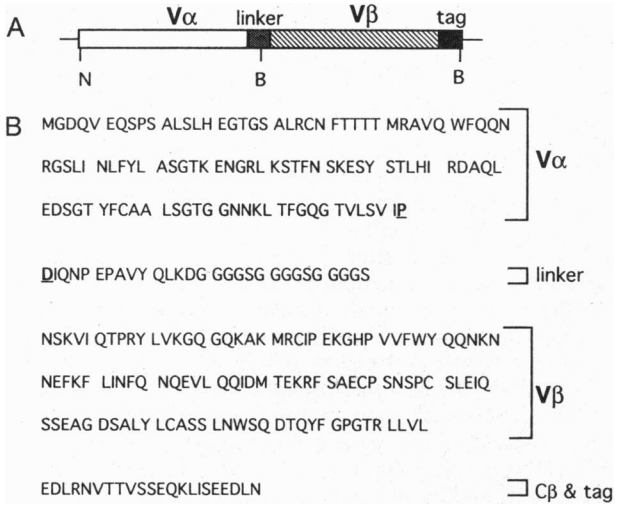

FIG. 1. Schematic representation and deduced amino acid sequence of the 2B4 TCR–sFv construct. (*A*) Diagram of the TCR–sFv. The 3′ end of V_α is connected to the 5′ end of V_β by a linker region, encoding 14 residues of C_α and a 15-residue flexible spacer, $(Gly_4\text{-}Ser)_3$. A region encoding a 15-aa residue tag derived from the human *myc* oncogene has been inserted 3′ to a short portion of C_β at the 3′ end of V_β. N, *Nco* I site; B, *Bam*HI sites. (*B*) Deduced amino acid sequence of the TCR–sFv construct. The P and D residues indicated by boldface type that is underlined were previously reported as being R and V (21). Otherwise V and C regions residues match reported sequences (21, 22) exactly.

the protein to be identified by a peptide-specific antibody (mycl 9E10.2). The sequence of the insert is shown in Fig. 1*B*. The V regions were found to be identical to the published 2B4 sequence (21, 22), except for a 3-nt difference in V_α, which would result in 2-aa residue differences. Repeated amplification, cloning, and sequencing of V_α from functionally active 2B4 cells suggested that our sequence is correct and not due to a PCR error.

Expression and Partial Purification of the Recombinant 2B4 TCR–sFv. Bacteria containing the 2B4 TCR–sFv in pET11d produce a significant fraction of their total protein as the sFv (30.1 kDa) after induction with isopropyl β-D-thiogalactoside (Fig. 2, lane 2). The TCR–sFV was partially purified by washing the bacterial pellet with a detergent solution, thus taking advantage of the insolubility of the protein in inclusion bodies. This procedure produced recombinant protein that was at least 50% pure (Fig. 2, lane 3).

***In Vitro* Refolding of the 2B4 TCR–sFv.** Inclusion bodies were solubilized in 6 M guanidine hydrochloride, and disulfide bonds were fully reduced. Starting with this totally reduced and denatured material, mixed disulfide bonds were formed between the SH groups of the protein and glutathione, and the modified protein was allowed to gain its native conformation by diluting it into buffer containing 0.4 M arginine as a cosolvent, under conditions that allow the reshuffling of S–S bonds. After *in vitro* refolding, the majority of the recombinant protein migrated on an SDS gel more rapidly than the reduced protein (Fig. 2, lanes 4 and 5), showing that intramolecular disulfide bonds had formed. Minor bands that migrated just behind the major peak (Fig. 2, lane 4) probably represent less-compact forms of the protein with incorrectly paired disulfide residues. In addition, higher molecular mass bands (Fig. 2, lane 4) result from interchain disulfide bridges involving TCR–sFv molecules and contaminating proteins that contain sulfhydryl groups.

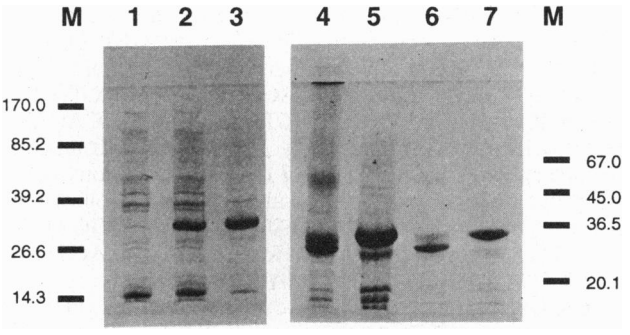

FIG. 2. SDS/PAGE analysis of TCR–sFv at different stages of production. Lanes: 1, lysate from uninduced bacteria; 2, lysate from bacteria induced with isopropyl β-D-thiogalactoside; 3, partially purified inclusion bodies (lanes 1–3 were run under reducing conditions); 4 and 5, refolded concentrated TCR–sFv, nonreduced and reduced, respectively; 6 and 7, refolded TCR–sFv after purification by size-exclusion chromatography, nonreduced and reduced, respectively (12.5% gels were stained with Coomassie blue); M, molecular mass markers identified in kDa.

The refolded native protein was next analyzed by size-exclusion chromatography under nondenaturing conditions. The elution profile of the refolded TCR–sFv (Fig. 3*A*) contained three peaks of aggregated protein, and a major peak eluting with an apparent molecular mass of ≈36 kDa, in the range expected for the monomeric sFv. The monomer peak was isolated using preparative size-exclusion chromatography and reanalyzed by the same method (Fig. 3*B*) and by SDS/PAGE (Fig. 2, lanes 6 and 7). By these criteria, the refolded purified TCR-sFv forms a stable monomeric protein in dilute solution (Fig. 3*B*) that is largely free of contaminating proteins and contains only a minor portion of misfolded material (Fig. 2, lane 6). We have found the monomeric refolded 2B4 sFv to be stable at concentrations below ≈1 mg/ml not only in the presence of the cosolvent but also in physiological buffers such as PBS and (for a limited time) in low-ionic-strength buffers such as 10–20 mM Tris or Hepes.

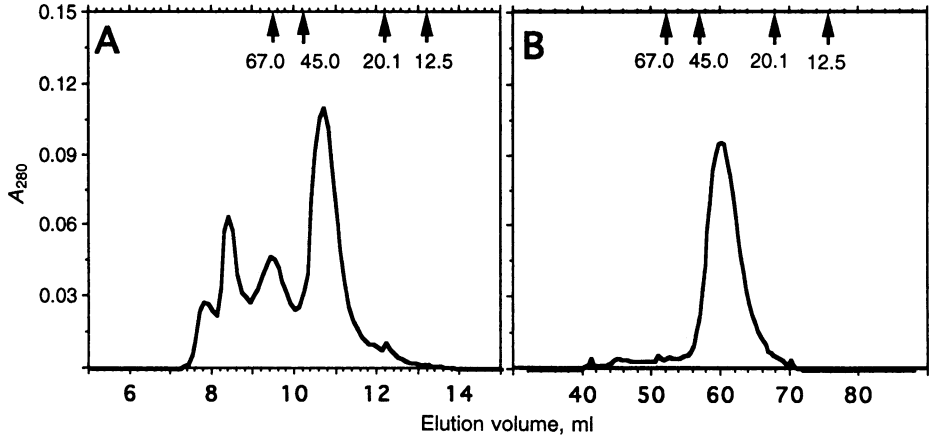

FIG. 3. Size-exclusion chromatography elution profiles of refolded TCR–sFv. (*A*) Elution profile of refolded TCR–sFv on a Superdex 75 HR 10/30 analytical column prior to purification. (*B*) TCR–sFv was purified on a 1.6 × 55 cm Superdex 75 preparative column and rerun on the same column. The elution buffer in both profiles was 0.4 M arginine in 0.1 M Tris/2 mM EDTA, pH 8.0. Sample volumes and flow rates were 0.2 ml and 0.7 ml/min in *A* and 2.0 ml and 2 ml/min in *B*, respectively. Molecular masses were calibrated with bovine serum albumin (67 kDa), ovalbumin (45 kDa), soybean trypsin inhibitor (20.1 kDa), and horse cytochrome *c* (12.5 kDa) under identical conditions.

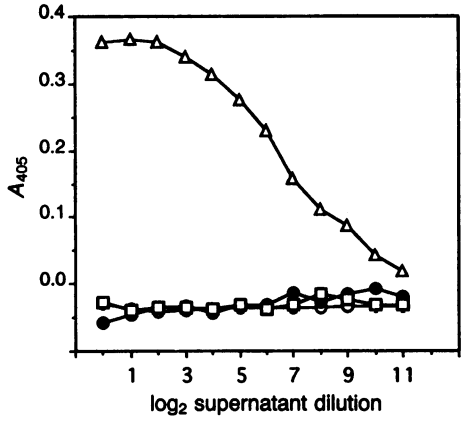

FIG. 4. Binding of anti-TCR mAbs to the 2B4 TCR–sFV. 2B4 TCR–sFv protein was immobilized in plastic microtiter wells and then treated with RR3-15 anti-V_β11 mAb (○), RR4-7 anti-V_β6 mAb (●), RR8.1 anti-V_α11 mAb (△), and KT65 anti-V_α8 mAb (□). All mAbs were rat IgG, and goat anti-rat IgG coupled to alkaline phosphatase was used as the developing reagent. The 2B4 TCR contains V_α11 and V_β3 allotypic determinants.

At higher concentrations, however, the protein tends to aggregate (data not shown).

Antigenic Properties of the Recombinant TCR–sFV. To characterize the TCR–sFv antigenically, immunoassays were carried out using a panel of antibodies. Examples of binding data are shown in Fig. 4, indicating the clear distinction between positive and negative binding. A summary of all our binding data is given in Table 1. These data show that the TCR–sFv was recognized by all three antibodies (anti-V_α11, anti-V_β3, and anti-2B4 idiotype antibodies) that bound to the V region of the 2B4 TCR but was not recognized by any other TCR-specific antibody included in our panel. Reduction and alkylation of the 2B4 TCR–sFv abolished its ability to bind the A2B4 antibody (Fig. 5), suggesting that this antibody recognizes a conformationally dependent epitope. The reduced, but not alkylated, TCR–sFv rapidly regained its ability to bind A2B4 after being immobilized on plastic (Fig. 5) in an oxidizing environment, as expected. The data of Table 1 and Figs. 4 and 5 show that the recombinant 2B4 TCR–sFv is antigenically indistinguishable from the native 2B4 V region.

DISCUSSION

In this study we have produced an sFv recombinant protein from the 2B4 TCR. We chose this particular TCR because its nucleotide sequence was known (21, 22), several mAbs were available that bound the receptor (23, 26, 27), its functional properties were well characterized using the 2B4 T-cell hybridoma (39, 40), and soluble versions of the intact extracellular portion of the receptor have been described (10, 12). The 2B4 TCR–sFv was produced in bacteria as inclusion bodies and after refolding gave 65–100 mg of protein from a liter of bacterial culture of which 20–30 mg was monomeric sFv, as estimated by gel filtration. This protein was antigenically indistinguishable from the V region of the native receptor (Table 1 and Figs. 4 and 5).

Table 1. Recognition of 2B4 TCR and TCR–sFv by TCR-specific antibodies

Antibody	Specificity	2B4 cells	TCR-sFv
H57	C_β	+	−
KT65	V_α8	−	−
RR8-1	V_α11	+	+
KJ25	V_β3	+	+
RR4-7	V_β6	−	−
F23.1	V_β8	−	−
RR3-15	V_β11	−	−
A2B4	Anti-2B4 clonotype	+	+
9E10	Anti-peptide tag	ND	+

Binding of antibodies to 2B4 cells was determined by flow cytometry analysis and to 2B4 TCR–sFv was determined by ELISA. ND, not done.

The main problem we encountered during production of the soluble TCR–sFv was in the refolding process. Bacterial expression systems produce large amounts of protein rapidly and provide a convenient means for altering amino acid sequences for structural and functional studies. However, bacteria lack the sophisticated protein-folding machinery found in eukaryotic cells. Bacteria apparently do have a protein disulfide isomerase by which they can correctly form disulfide links in the oxidizing atmosphere of the periplasmic space (41). sFv proteins, containing an appropriate leader sequence, are directed to the periplasmic space, where the leader peptide is removed and disulfide pairing occurs (42). However, such proteins are not always properly folded and often must be denatured and refolded in dilute solution to obtain active material (16, 43).

In the procedure described here, we have not included a leader sequence in our construct, and massive amounts of protein accumulated as misfolded non-disulfide-linked material in cytoplasmic inclusion bodies. Protein was produced by the pET11d expression vector, which uses the bacteriophage T7 RNA polymerase and the T7 promoter to drive protein synthesis (37). The T7 polymerase elongates polypeptide chains about five times faster than the endogenous bacterial polymerase and is insensitive to rifampicin, which blocks the bacterial RNA polymerase. Therefore, in the presence of rifampicin, the bacteria produce large amounts of sFv that can easily be recovered in high yield with at least 50% purity, but in a nonnative form. The rapid production of such material is the major advantage of using bacterial inclusion bodies to produce sFv. The method we used to refold this material was adapted from one described by Buchner and Rudolph (38) to

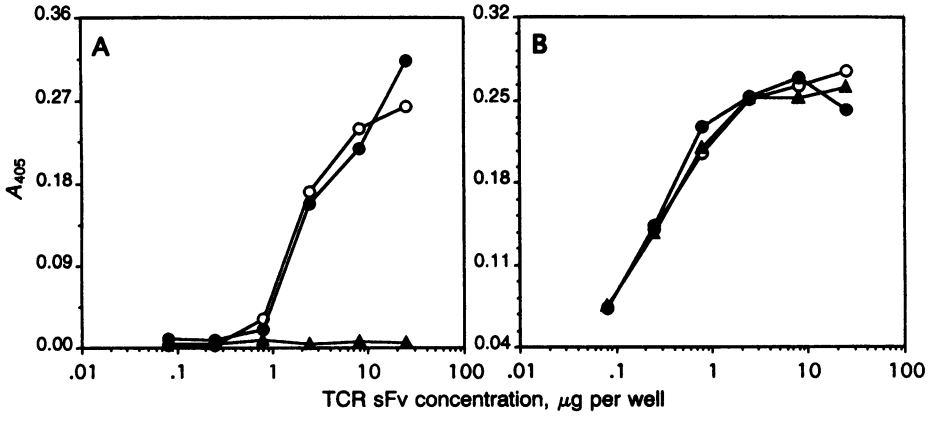

FIG. 5. Recognition of TCR–sFv by A2B4 requires intact disulfide bonds. TCR–sFvs were denatured in 8 M urea and then reduced with dithiothreitol and allowed to reoxidize (○), treated with iodoacetamide (●), or reduced with dithiothreitol and alkylated with iodoacetamide (▲). Samples were then diluted in borate-buffered saline, adsorbed to ELISA plates, and tested for the ability to bind A2B4 (anti-2B4 clonotype) (*A*) or 9E10 (anti-peptide tag) (*B*) mAbs. The binding of 9E10 to the three protein samples is a control showing that all three bound similarly to the ELISA plate.

refold recombinant Fab fragments. The protein that we obtained using this procedure contained a large fraction of material eluting as a monomeric globular protein by size-exclusion chromatography in nondenaturing solution. In other studies (A.J.T.G., unpublished data), we found a good correlation between the ability of an anti-dinitrophenol–sFv to bind antigen and to exist as a monomeric protein in solution.

Four mAbs that were predicted to be specific for the sFv actually bound to our construct, thus confirming that our bacterial product was in fact the 2B4–sFv. One antibody was directed against the C-terminal peptide that we incorporated into the construct as an analytical tag. The binding of this antibody to the construct showed that the sFv message was completely translated. Antibodies against $V_\alpha 11$, $V_\beta 3$, and a 2B4 idiotope showed that the construct also contained the expected V-region epitopes. It is not known to what extent the binding of these latter antibodies indicated that the recombinant protein was in its proper configuration. However, we were able to demonstrate that reduction and alkylation of the recombinant protein abolished its binding to the A2B4-clonotype-specific antibody, suggesting that this antibody recognized a conformational epitope on the TCR sFv.

Three other groups have reported the production TCR–sFv constructs, all in bacterial secretion systems. Novotny *et al.* (17) produced a TCR–sFv from the RFL3.8 fluorescein-specific T-cell clone. After refolding, this TCR–sFv was found to be relatively insoluble and site-directed mutagenesis was used to replace some of the hydrophobic residues that, according to predictions, were exposed on the surface of the molecule. The mutated TCR–sFv was soluble, bound to fluorescein isothiocyanate-coupled Sepharose, and was recovered by elution with free fluorescein. Ward (19) recently produced a TCR–sFv derived from an encephalitogenic T-cell hybridoma (1934.4). This protein was secreted directly into the medium by the bacteria and contained a β-pleated sheet structure, by CD analysis, as expected for a properly folded immunoglobulin-like domain. Finally, Soo Hoo *et al.* (18) produced a TCR–sFv from the 2C CTL clone, specific for H-2L^d, that after *in vitro* refolding yielded a protein that could be purified from an anti-2C idiotype affinity column but failed to bind to H-2L^d molecules.

The TCR–sFv described here exists as a monomeric protein in nondenaturing solution. Misfolded proteins have a strong tendency to aggregate in solution (44), and our observation that the 2B4 sFv is monomeric, with its antigenic identity to the native TCR, is a strong indication that this TCR–sFv is in a properly folded state. The 2B4 TCR, like other TCRs, recognizes a complex cell surface antigen (39, 40), probably with low affinity (10, 45), and binds to a number of well-defined superantigens (46, 47). Our 2B4–sFv construct provides sufficient quantities of soluble protein to measure such interactions, and we have recently found (I.K., unpublished data) that the 2B4 sFv does, in fact, have binding activity.

We thank Drs. A. M. Weissman and R. J. Hodes for critically reading the manuscript. We also thank Dr. J. Ashwell for the 2B4 and A2B4 hybridomas and Dr. F. W. Studier for providing the pET11d plasmid and the BL21DE3 cells. Finally, we thank Dr. Hodes for kindly supplying several of the TCR-specific antibodies.

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