Acta Cryst. (1994). D**50**, 535-542

Crystallization, Sequence and Preliminary Crystallographic Data for Transmission-Blocking Anti-Malaria Fab 4B7 with Cyclic Peptides from the Pfs25 Protein of *P. falciparum*

BY ENRICO A. STURA,* ANGRAY S. KANG, RANDY S. STEFANKO AND JULIO C. CALVO

Department of Molecular Biology, The Scripps Research Institute, 10666 *North Torrey Pines Road, La Jolla, California* 92037, *USA*

DAVID C. KASLOW

Laboratory of Malaria Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, *USA*

AND ARNOLD C. SATTERTHWAIT

Department of Molecular Biology, The Scripps Research Institute, 10666 *North Torrey Pines Road, La Jolla, California* 92037, *USA*

(*Received* 3 *December* 1993; *accepted* 31 *January* 1994)

Abstract

X-ray quality crystals of an Fab fragment from a transmission-blocking monoclonal antibody 4B7 (MAb 4B7) against a sexual stage protein Pfs25 of *Plasmodium falciparum* were grown as uncomplexed and peptide-complexed forms. Initially, the intact immunoglobulin was crystallized because cleavage with pepsin or papain did not produce a homogeneous product. Further proteolytic trials with elastase produced a suitable Fab fragment from which crystals have been obtained, both for the free Fab and in complex with cyclic peptides and in the presence of linear peptides. While linear peptides bind to MAb 4B7, cyclic peptides modeled on a predicted β-hairpin loop of the third EGF-like domain of Pfs25 bind better and readily co-crystallize with the Fab. The genes for the variable domain of the Fab have been cloned, sequenced and the primary amino-acid sequence for the complete Fab deduced. This work explores the use of glycerol as an additive and the modification of the peptide sequence outside the epitope for improving in the crystallization. Data sets have been collected from crystals of several Fab-peptide complexes and from uncomplexed Fab to resolutions ranging from 2.4 to 3.3 Å. The packing arrangements of several crystal forms have been determined by molecular replacement, and refinement of their three-dimensional structures is in progress. The three-dimensional structure of this Fab complexed with the various peptides will aid in an understanding of the mode by which this antibody recognizes and prevents transmission of the malaria parasite.

* To whom all correspondence should be addressed.

Abbreviations

Monoclonal antibody, MAb; antigen-binding fragment of immunoglobulin, Fab; immunoglobulin, IgG; epidermal growth factor, EGF; complementarity determining region, CDR; dithiothreitol, DTT; phenylmethylsulfonyl fluoride, PMSF; polyethylene glycol, PEG; *N*-aminoglycine, Z; 5,5-dimethoxypentanoic acid, J.

Introduction

Malaria is a disease in which transmission of the parasite from the human host to the mosquito vector is a target for vaccine research (Vermeulen *et al.*, 1985; Kaslow *et al.*, 1988; Barr *et al.*, 1991). Antibodies, such as the murine monoclonal antibody 4B7 (MAb 4B7), directed against a 25 kDa antigen on the surface of sexual stages of the parasite can block completely the transmission of the parasite from vertebrate host to the mosquito vector (Barr *et al.*, 1991). The induction of transmission-blocking antibodies could be important in preventing the proliferation of escape mutants and drug-resistant strains of the parasite. Drug resistance is a hallmark of the malaria parasite that currently infects over 100 million persons annually. Transmission-blocking vaccines may be administered in conjunction with drug therapy, or as part of a multi-stage vaccine, both to infected and uninfected individuals, as part of a global program for the control of malaria. It is expected that structural studies of transmission-blocking antibodies, especially when complexed to target antigens, will aid in the development of such transmission-blocking vaccines.

MAb 4B7 recognizes and blocks the action of Pfs25, a protein expressed on the surface of sexual stages of the malaria parasite, *Plasmodium falciparum*, as it develops in the midgut of the mosquito (Barr *et al.*, 1991; Vermeulen *et al.*, 1985). The epitope recognized

by MAb 4B7 has been defined as lying within the ILDTSNPVKT sequence contained in the third EGF-like domain of the Pfs25 molecule (Stura, Satterthwait, Calvo, Stefanko, Langeveld & Kaslow, 1994). Several cyclic peptides have been synthesized to mimic the predicted β-hairpin of this EGF-like domain. The cyclic peptides have been shown to bind better to 4B7 than their linear counterparts and to co-crystallize readily with the Fab. The combination of X-ray structural studies and peptide design may provide a unique way to understand neutralization of the parasite by monoclonal antibodies. The three-dimensional structure of the epitope bound to neutralizing antibodies will provide a template for the synthesis of novel antigens which may be better at stimulating a neutralizing response against the parasite. We report here the crystallization and sequence determination of neutralizing Fab 4B7 alone and in complex with cyclic peptides.

Materials and methods

Elastase cleavage of MAb 4B7

MAb 4B7 was isolated from ascites fluid by ammonium sulfate precipitation, purified, and typed as described by Stura, Satterthwait, Calvo, Stefanko, Langeveld & Kaslow (1994). Cleavage of this IgG2a (κ light chain) with either papain or pepsin failed to yield material sufficiently homogeneous for crystallization. Trials with elastase were performed using ratios of 0.5-5%(*w*/*w*) elastase to IgG (5 mg ml^{-1}). Incubation was carried out at 310 K for 1-15 h. A 1 mg ml^{-1} PMSF solution in ethanol was used to stop the reaction. The PMSF solution was added in the ratio 1:10 (*v*/*v*) to the elastase/antibody reaction mixture. Aliquots were analyzed for complete digestion by SDS-PAGE; optimal conditions were determined to be 5%(*w*/*w*) elastase to IgG (5 mg ml^{-1}) for 9 h. The undigested IgG was then separated over a Sephacryl S-300 size exclusion column, with a buffer consisting of 0.1 *M* sodium acetate, 0.2 *M* NaCl, 0.02% NaN_3. Further purification of the Fab using a Mono-S column (Pharmacia) on an HPLC system with 0.05 *M* sodium acetate pH 5.0, and elution with 1 *M* NaCl under the same buffer conditions (purification conditions as for the IgG preparation; Stura, Satterthwait, Calvo, Stefanko, Langeveld & Kaslow, 1994) yielded material for crystallization trials. The elution profile consisted of 80% of the Fab which is not bound to the column, and 20% of which elutes later as a single peak with 180 m*M* NaCl (referred to as 'bound').

Synthetic peptides

Peptides and peptide analogues corresponding to sequence Ile122-Val133 from Pfs25 were prepared by solid-phase peptide synthesis. Each linear peptide was blocked at the N-terminus by acetylation and at the C-terminus by amidation. Linear peptide synthesis was carried out using an Advanced ChemTech 350 multiple-peptide synthesizer on Rink amide resin (NovaBiochem, 0.46 meq g^{-1}) using a standard Fmoc synthesis strategy, where amino acids were coupled with hydroxybenzotriazole and diisopropyl carbodiimide. The side chains of Fmoc amino acids were protected by butyl (Asp, Thr, Ser), Boc (Lys) or trityl (His) groups. Cyclic peptides were synthesized on solid support using *N*-aminoglycine (Z) and 5,5-dimethoxypentanoic acid (J) to form the hydrazone link (Arrhenius & Satterthwait, 1990; Chiang, Cabezas, Calvo & Satterthwait, 1994). Peptides were cleaved from the resin and protecting groups removed by treatment with trifluoroacetic acid:water (95:5) for 1 h and precipitated with ether. Each peptide was purified to >98% homogeneity on a RP-18 column (Vydac 201TP1022, 2.2 × 25 cm, 10 µm particles) using HPLC and a 0-100% water/acetonitrile gradient containing 0.1% trifluoroacetic acid. Each peptide was identified by FAB mass spectroscopy.

RNA isolation and cDNA synthesis

Hybridoma 4B7 cells (10^6) were pelleted, and the total RNA was isolated (Kang, Burton & Lerner, 1991) and used as a template for the cDNA synthesis for the V_H and κ chains with the following primers: MV_H, 5′-CTG GGT CAT CTG GAG CTC GGC CAG TGG ATA GAC AGA TGG GGG TGT CGT TTT GGC-3′ and κ, 5′-GCG CCG TCT AGA ATT AAC ACT CAT TCC TGT TGAA-3′, using SuperScript Kit (BRL). Briefly, 7 µg of total RNA was mixed with 60 pmol of primer, heated to 343 K for 10 min and immediately cooled on ice. 2 µl of RNase inhibitor, 10 µl of 5× synthesis buffer, 8 µl of dNTP mix (to give final concentration of 200 µ*M* of each NTP), 5 µl of 0.1 *M* DTT, and 1 µl of BRL SuperScript RT (200 U µl^{-1}) were added, and the reaction was made up to 50 µl with DEPC-treated water. The reaction was allowed to proceed at room temperature for 10 min and then at 315 K for 50 min. The reaction was terminated by incubation at 363 K for 5 min, then placed on ice for 10 min, followed by the addition of 1 µl RNase H and incubation at 310 K for 20 min. PCR amplification was performed in a 100 µl reaction mixture as described earlier, using V_H 1(5′-AGG TCC AGC TGC TCG AGT CTG G-3′), and the MV_H-chain primer for the heavy chain, and V_L 5,6(5′-CCA GAT GTG AGC TCG TGA TGA CCC AGA CTC CA-3′ Lc 5, 5′-CCA GAT GTG AGC TCG TCA TGA CCC AGT CTC CA-3′) Lc 6, and the κ-chain primers for the light chain (Kang *et al.*, 1991). The PCR amplified V_H 400 bp fragment was cloned into pComb8 digested with XhoI/SacI: The κ-chain 700 bp fragment was digested with SacI/ XbaI and cloned into pComb8 digested with SacI/SpeI. The complete nucleotide sequence of the V regions of the H and L chain were determined from double-stranded DNA by automated DNA sequencing (Applied Biosystems) and the protein sequence deduced. Protein sequencing of

the amino terminus of each chain facilitated the choice of amplification primers and subsequent correction for residues introduced by the cloning sites.

Crystallization procedures

Reagents for crystallization were ammonium sulfate, sodium phosphate, potassium phosphate and sodium citrate from Fisher Scientific, polyethylene glycols (PEGs) 600, 2000 4000 and 10 000 from Fluka Chemical Corporation, PEG 8000 from Baker Chemical Company and imidazole and malic acid from Sigma Chemical Company, and glycerol was enzyme grade from Fisher Scientific. Imidazole malate buffer was made by mixing 2 *M* imidazole solution with a 2 *M* malic acid solution to achieve the desired pH and diluted tenfold to buffer the PEG solutions.

Crystallization conditions were screened, and crystals were grown by the vapor-diffusion method (for other standard crystallization techniques, see McPherson, 1982), using multiwell sitting-drop vapor-diffusion plates (Stura *et al.*, 1989; Stura & Wilson, 1990, 1991). The screening consisted of a limited initial search or footprint (Stura, Nemerow & Wilson, 1992) with ammonium sulfate, sodium and potassium phosphate, sodium citrate and PEG of molecular weight 600, 4000 and 10 000 [see also Wilson, Rini, Fremont, Fieser & Stura (1991) and Stura, Fieser & Wilson (1993) for further details about antibody preparation, cleavage and crystallization]. Crystallization conditions were screened for the free Fab and for various complexes of the Fab with peptides of different sequences (Table 1). Seeding was used to optimize crystallization conditions and to produce large crystals (Stura & Wilson, 1990, 1991). Sitting drops consisted typically of 2.5 µl of protein to which the same volume of reservoir solution was added. The completed trays were allowed to equilibrate in a constant temperature incubator at 295.5 K from overnight to up to 5 d prior to seeding.

Because of the large number of potential peptide complexes that could be crystallized, rather than applying the standard approach where footprint screens are set up for the free Fab and for each of the peptide complexes, the results obtained from the crystallization of the 4B7 IgG-peptide complex (Stura, Satterthwait, Calvo, Stefanko, Langeveld & Kaslow, 1994) were used to minimize the initial screening with the Fab-peptide complexes (see *Reverse Screening*, Stura, Satterthwait, Calvo, Kaslow & Wilson, 1994).

Data collection

Data were collected using a Siemens proportional counter multiwire area detector mounted on Elliott GX-18 and Siemens rotating-anode X-ray generators operating at 40 kV, 55 mA with a 100 µm focal cup and at 50 kV, 80 mA with a 300 µm focal cup, respectively. Both generators were equipped with Franks focusing mirrors (Harrison, 1968). Collimation devices, small beamstop and a helium cone designed and constructed in our laboratory have been used for facilitating the alignment procedures and to reduce background scatter.

Results and discussion

Cyclic peptides

Cyclic Pfs25 peptides were synthesized by replacing predicted hydrogen bonds formed between main-chain amide proton and the amide carbonyl O atom (NH···O=C*R*NH) with a hydrazone covalent hydrogen-bond mimic (N—N=CHCH$_2$CH$_2$). Since on average every other amino acid in globular proteins engages in main-chain-to-main-chain hydrogen bonding, this chemistry is generally applicable to the synthesis of conformationally constrained peptides (Chiang *et al.*, 1991). It also provides a flexible method for fine tuning peptides to fit better to receptors since the position of the hydrogen-bond mimic can be varied within the cyclic peptide. Pfs25 has been recognized as an EGF-like protein on the basis of sequence homology and cysteine patterns (Kaslow *et al.*, 1988). Modeling of constrained Pfs25 peptides was based on the solution structure of EGF-like domains derived from NMR studies (Cooke *et al.*, 1987; Kline *et al.*, 1990). The linear peptide recognized by MAb 4B7 corresponds to a predicted β-hairpin in the third EGF-like domain of Pfs25 (Kaslow *et al.*, 1988; Stura, Satterthwait, Calvo, Stefanko, Langeveld & Kaslow, 1994). Two prototypical cyclic peptides corresponding to predicted β-hairpin conformations were synthesized by replacing main-chain hydrogen bonds with covalent hydrogen-bond mimics at the base of the loop (Fig. 1). The predicted conformations were based on NMR structures for EGF-like domains and predictions made by *GORBTURN* that identifies tetrapeptide sequences with propensities for specific reverse-turn types (Wilmot & Thornton, 1988, 1990). Cyclic peptide C2, [N=CH(CH$_2$)$_3$CO]ILDTSNPVKTGVG(NCH$_2$CO)G, (Fig. 1) was designed to mimic the prototypical EGF β-loop which locates two cysteines directly opposite one another at the base of the loop. Cyclic peptide C1, [N=CH—(CH$_2$)$_3$CO]ILDTSNPVKTG(NCH$_2$CO)G, is based on a prediction which places TSNP at the apex of the loop (Fig. 1). The SNP sequence corresponds to the most frequent amino acids found in the i + 1 to i + 3 positions in a type VIII turn in proteins (Wilmot & Thornton, 1990) and TSNP is predicted to be a non-specific turn by the program *GORBTURN* (Wilmot & Thornton, 1988). Both cyclic peptides show significantly higher affinities for 4B7 than the corresponding linear peptides and C1 binds better than C2 in ELISA (Satterthwait *et al.*, in preparation). Since C1 is composed from fewer amino acids than C2, the

increased binding may be due to conformational fine tuning resulting in better shape complementarity within the MAb 4B7 binding pocket. Cyclic peptides have proved instrumental in this co-crystallization and also for an anti-HIV-1 Fab (Stura *et al.*, in preparation) which may reflect improved affinities.

Nucleic acid and protein sequences of variable domains

The nucleotide and derived amino-acid sequences of the heavy- and light-chain variable domains of 4B7 are shown in Fig. 2, with sequence numbers consistent with Kabat, Wu, Perry, Gottesman & Foeller (1991). Amino-acid residues 1-5 of the heavy chain and 1-7 of the light chain were obtained by N-terminal amino-acid sequencing. The light-chain variable-domain sequence belonged to the murine $V_K V$ subgroup with 93.5% sequence identity with BXW-16′CL (Kabat *et al.*, 1991; Kofler *et al.*, 1988). The heavy-chain variable-domain sequence most closely matches the murine $V_H III(A)$ subgroup, as defined by Kabat *et al.* (1991) with an 80% sequence identity with the DFL16.2 (Clarke *et al.*, 1985) germline gene from the BALB/c murine strain.

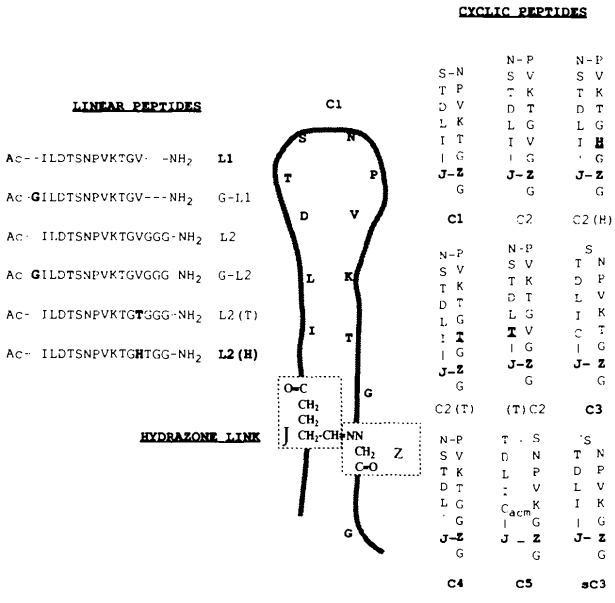

Fig 1. Sequences of linear and cyclic peptides used in co-crystallization experiments with Fab 4B7. The residue J is equivalent to formyl glycine while Z is equivalent to glycine. A covalent hydrogen mimic, CH=N, replaces hydrogen bonds which are predicted to form between the formyl oxygen and the amide proton of glycine. By substituting J and Z at different positions in the peptide chain, the peptide can be forced to adopt different conformations which in turn affect the crystallization. Linear peptide L1 corresponds best with cyclic peptide C1, whereas peptide G-L2 corresponds closely to the cyclic peptide C2. Residues denoted in bold have been modified to aid in the crystallization. Co-crystals of C1 are obtained readily under a wide range of PEG concentrations and pH, while others required more stringent conditions. X-ray data have been collected for co-crystals of the peptides for which are denoted in bold, *i.e.* **L1** but not G-L1.

Crystallization of free Fab 4B7

Crystallization of the uncomplexed Fab was achieved by scanning though a wide pH range around the conditions under which crystals of the cyclic peptide complexes were obtained. Polyethylene glycols from M_r 2000 to 10 000 were found to yield crystals. Two forms are obtained (Fig. 3*d*), both using protein at 10 mg ml^{-1} concentration, with 13-16% PEG, 0.2 *M* imidazole malate. The first form consists of flat plates radiating out from an initial nucleus under a range of pH 5.5-8.0, which were found unsuitable for structural studies. The second form consists of prismatic crystals which grow up to 0.3 × 0.25 × 0.2 mm in size. The flat plates nucleate more readily than the prismatic crystals which nucleate

Fig. 2. Nucleotide and predicted amino-acid sequence of the heavy- and light-chain variable regions of antibody 4B7. CDR regions, as defined by Kabat *et al.* (1991), are in bold type. Numbering is according to standard convention. Regions determined by N-terminal sequencing are in parentheses. The heavy chain belongs to the VHIII(A) and the light chain to the V_k(V) subgroups. The differences with the sequence of antibody 17/9 used in the molecular-replacement structure determination are also shown; although amino-acid identity is only 71% for V_H and 56% for V_L, many of the changes are conservative. MAb 4B7 is an anti-protein antibody, the L1 and H3 loops of 4B7 are shorter than for the anti-peptide antibody 17/9.

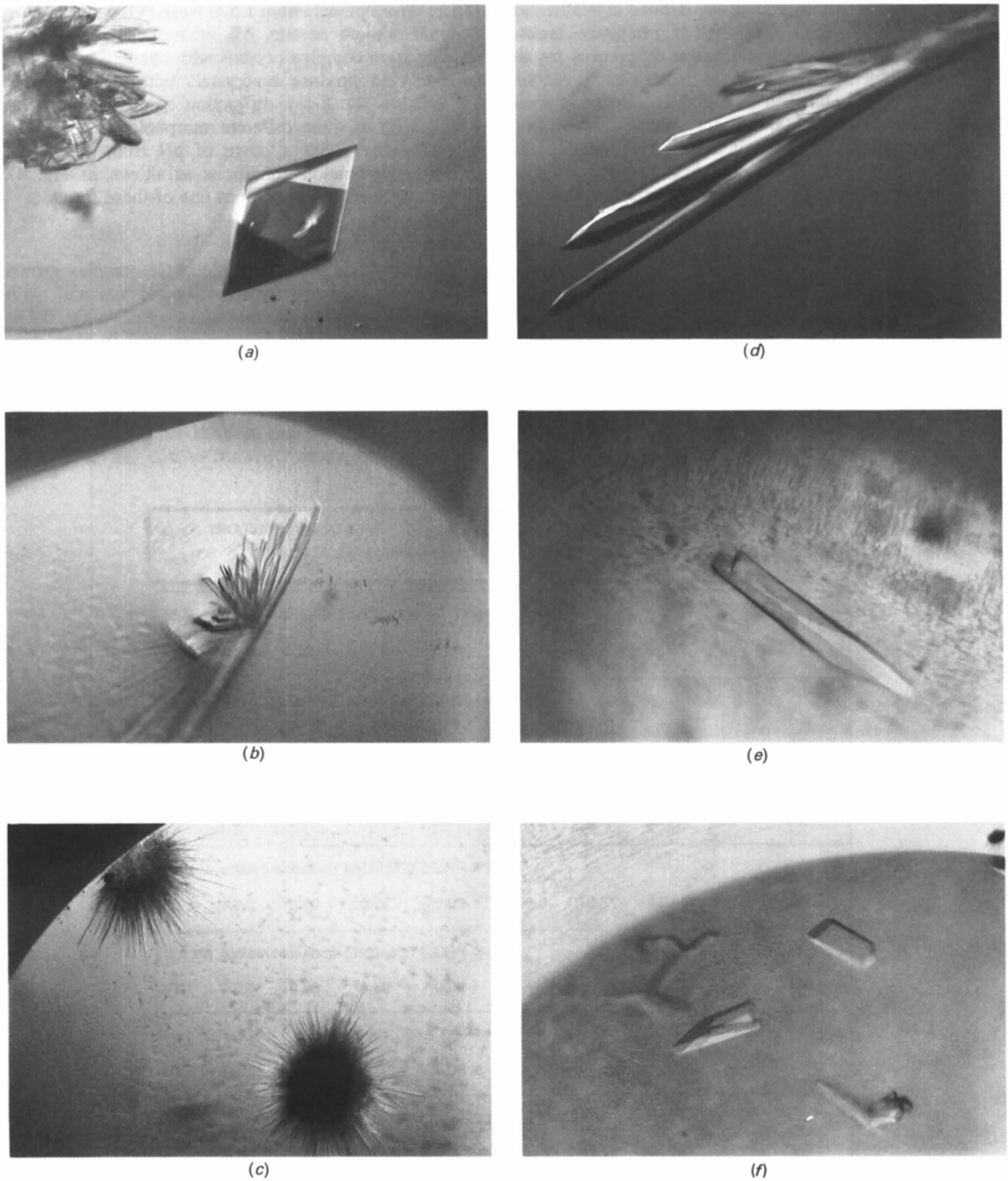

Fig. 3. Photomicrographs of crystals showing a range of morphologies for the crystals of Fab 4B7 and peptide complexes. Crystals of the free Fab 4B7 obtained in two distinct morphologies are shown in (*a*). Fab4B7-C3 and -C4 complexes (Fig. 4) seeded from the prismatic crystals like the one shown in (*a*) are morphologically similar to the thin clustered plates on the right. Complexes with cyclic peptides C2(T), C1, C2, C2(H) are shown in (*b*), (*d*), (*e*) and (*f*), respectively. Crystals of the C5 and sC3 are similar to those in (*f*), but more rounded in shape. Crystals of the complex with the cyclic peptide C1 diffract to 2.75 Å (data complete to only 3.1 Å, Fig. 4), while those with C2 and the other peptides of the C2 series diffract at present to only about 5 Å. Polycrystalline needles, shown in (*c*), unsuitable for X-ray structural studies have been obtained for the linear peptide L2 from 1. 8 *M* phosphate with 6% ethanol. Crystals with linear peptides diffracting to high resolution have been obtained as shown in Fig. 4.

and grow only within a narrowly defined crystallization range, 14-15% PEG 8000, 0.2 *M* imidazole malate, pH 7.5-8.0. There are some minor differences for the various molecular weight PEGs. Nucleation is reduced with PEG 2000 and there is a greater amount of phase separation with PEG 8000. The 'unbound' fraction from the Mono-S purification nucleated more readily and gave prismatic crystals. Seeding has not been necessary to obtain large crystals of this form. Growth is complete within 5 d.

Crystallization of Fab 4B7 linear peptide complexes

Footprint screens (Stura, Nemerow & Wilson, 1992) were also set up for Fab 4B7 (10 mg ml^{-1}) complexed with linear peptides L1 and L2 (Fig. 1). Small hexagonal-sided flat plates were obtained for L1 from 0.75 *M* sodium citrate, 5 m*M* sodium borate, pH 8.5 (condition 6A in the footprint screen; Stura, Nemerow & Wilson, 1992). Thin needles in a polycrystalline clump were obtained for L2 from an additive screen (under development) from 1.5 *M* NaH_2PO_4 and K_2HPO_4, 100 m*M* sodium acetate, 6% ethanol, pH 5.5. Cross-seeding from complex crystals with cyclic peptides was necessary to produce co-crystals with linear peptide L1 suitable for X-ray diffraction studies. Co-crystals with L2(H) in three different morphologies nucleated spontaneously under a range of pH from 4.5 to 8.0 (other crystallization conditions as shown in Fig. 4). Data have been collected from one of these forms.

Preliminary X-ray studies

Single crystals of Fab 4B7-loop C1 complex grown from Fab at 7 mg ml^{-1} from the 'bound' material, with a reservoir solution consisting of 14% PEG 8000, 0.2 *M* imidazole malate, pH 7.5, were mounted in glass capillaries and analyzed on a Siemens multiwire detector, at both 15 and 18 cm crystal-to-film distance, using a helium path. A data set has been collected to 2.95 Å (complete to 3.1 Å) and reduced using the *XENGEN* package of programs (Howard *et al.*, 1987). Crystals

PEPTIDE COMPLEX				CRYSTALLIZATION CONDITIONS				
space group	a	b	c	α	β	γ	D_{min}	R_{sym}
C1				14% PEG 8,000, 0.2M imidazole malate, pH 7.5*				
$P3_112$	76.2Å	76.2Å	135.7Å	90.0°	90.0°	120.0°	3.1Å	9.8%
free				15% PEG 8,000, 0.2M imidazole malate, pH 7.5*				
C2	149.8Å	73.6Å	60.7Å	90.0°	96.8°	90.0°	2.45Å	4.1%
C3				10% PEG 8,000, 0.2M imidazole malate, pH 5.5				
C2	148.6Å	73.5Å	60.1Å	90.0°	97.2°	90.0°	2.6Å	6.1%
C4				12% PEG 8,000, 0.2M imidazole malate, pH 5.5				
C2	148.0Å	73.3Å	59.7Å	90.0°	97.0°	90.0°	2.5Å	6.3%
C5				11% PEG 8,000, 0.2M imidazole malate, pH 6.5				
C2	71.6Å	74.2Å	104.7Å	90.0°	93.4°	90.0°	3.3Å	7.5%
sC3				14% PEG 8,000, 0.2M imidazole malate, pH 5.5*				
C2	71.1Å	73.3Å	106.2Å	90.0°	93.3°	90.0°	2.9Å	2.7%
L1				15% PEG 8,000, 0.1M NaCl, 0.2M imidazole malate, pH 8.0				
C2	105.3Å	77.1Å	146.1Å	90.0°	95.3°	90.0°	2.7Å	8.1%
L2(H)				15% PEG 8,000, 0.2M imidazole malate, pH 5.5-7.5				
C2	150.5Å	74.0Å	61.1Å	90.0°	96.7°	90.0°	2.5Å	3.4%

Fig. 4. Lattice parameters, crystallization conditions and data-collection statistics for uncomplexed Fab 4B7 and complexes with cyclic and linear peptides.

grown with 1% glycerol as an additive appeared to be better ordered but showed high susceptibility to radiation damage, with a loss in diffraction from 2.7 to 6 Å in 2-3 h. The change in temperature from the constant environment of the incubator at 295.5 K to the lower, variable, room temperature (about 290 K) used during data collection may have had an influence on the loss of crystalline order.

Crystals of the free Fab 4B7, grown from 10 mg ml^{-1} Fab from the 'unbound' fraction, under identical crystallization conditions to those for the complex with C1, are morphologically different from those of the complex and diffract to better than 2.45 Å resolution, with less decay due to radiation damage.

Space-group determination and molecular packing

The crystals of the Fab 4B7-loop C1 complex belong to the trigonal space group $P3_121$ with lattice parameters $a = b = 77.0$, $c = 136.9$ Å. The lattice was determined using the refine program of the *XENGEN* package (Howard *et al.*, 1987) and further confirmed using *XDS* (Kabsch, 1988). The symmetry was confirmed from data reduction. The crystals contain one molecule in the asymmetric unit with a Matthews coefficient (Matthews, 1968) of 2.07 Å^3 Da^{-1} and the 3_1 or 3_2 screw axis is clearly evident from the absences along the 00l axis. The axis was later determined to be 3_1 from the molecular-replacement solution of the structure using *MERLOT* (Fitzgerald, 1988) and *X-PLOR* (Brünger, Kuriyan & Karplus, 1987; Brünger, 1990). A molecular-replacement solution, carried out as previously described (Wilson *et al.*, 1991), was obtained without difficulty using Fab 17/9 (Rini, Schulze-Gahmen & Wilson, 1992) (sequence homology: 71% V_H, 56% V_L; Fig. 2) which was found to be the best search model.

Crystals of the free Fab were face-centered monoclinic *C*2 with cell dimensions $a = 149.8$, $b = 73.6$, $c = 60.7$ Å, $\beta = 96.79°$ as determined from the refine program of the *XENGEN* package (Howard *et al.*, 1987) and confirmed using *XDS* (Kabsch, 1988). A data set complete to 2.7 Å has been collected from these crystals. At present only 50% of the intensities collected between 2.7 and 2.5 Å are statistically significant ($I/\sigma > 1$). The molecular packing for the free antibody in this cell and the location of the crystal contacts have been determined.

It interesting to note that many of the crystals grow in the same space group, *C*2, but with different cell lattice parameters (Fig. 4). Preliminary molecular-replacement solutions have been obtained for the other complexes listed in Fig. 4 and refinement of several of these structures has been initiated.

We would like to acknowledge Dr Thomas A. Collett for providing the initial protocol for elastase cleavage, the excellent technical assistance of Warren Densley and Gail Fieser for antibody production, Kirsten L. Gaarder for cloning and analysis of the DNA sequences and Dr Ian Wilson (IAW) and Daved Fremont for helpful discussions. This work was supported in part by funding from USAID contract DPE-5979-A-00-1035-00 to ACS and a Department of Defence contract DAAL-03-92-G-0215 to ASK. ASK is a recipient of a Cancer Research Institute/Partridge Foundation Investigators Award. EAS and RSS are partially supported on NIH grants GM-38419, GM-46192 and AI-23498 (to IAW). This is publication 7804-MB from The Scripps Research Institute.

References

ARRHENIUS, T. & SATTERTHWAIT, A. C. (1990). *Peptides: Chemistry, Structure and Biology. Proceedings of the* XI *American Peptide Symposium*, edited by J. RIVER & G. MARSHALL, pp. 870-872. Leiden: ESCOM.

BARR, P. J., GREEN, K. M., GIBSON, H. L., BATHURST, I. C., QUAKYI, I. A. & KASLOW, D. C. (1991). *J. Exp. Med.* **174**, 1203-1208.

BRÜNGER, A. T. (1990). *Acta Cryst.* A**46**, 46-57.

BRÜNGER, A. T., KURIYAN, J. & KARPLUS, M. (1987). *Science*, **235**, 458-460.

CHIANG, L.-C., CABEZAS, E., CALVO, J. C. & SATTERTHWAIT, A. C. (1994). In *Peptides* 1994. *Proceedings of the* XIII *American Peptide Symposium*, edited by R. HODGES & J. A. SMITH. Leiden: ESCOM.

CHIANG, L.-C., CABEZAS, E., NOAR, B., ARRHENIUS, T., LERNER, R. A. & SATTERTHWAIT, A. C. (1991). *Peptides* 1990. *Proceedings of the* XXI *European Peptide Symposium*, edited by E. GIRALT & D. ANDREU, pp. 465-467. Leiden: ESCOM.

CLARKE, S. H., HUPPI, K., RUEZINSKY, D., STAUDT, L., GERHARD, W. & WEIGERT, M. (1985). *J. Exp. Med.* **161**, 687-704.

COOKE, R. M, WILKINSON, A. J., BARON, M., PASTORE, A., TAPPIN, M. J., CAMPBELL, I. D., GREGORY, H. & SHEARD, B. (1987). *Nature (London)*, **327**, 339-341.

FITZGERALD, P. D. M. (1988). *J. Appl. Cryst.* **21**, 273-278.

HARRISON, S. C. (1968). *J. Appl. Cryst.* **1**, 84-90.

HOWARD, A. J., GILLILAND, G. L., FINZEL, B. C., POULUS, T. L., OHLENDORF, D. H. & SALEMME, F. R. (1987). *J. Appl. Cryst.* **20**, 383-387.

KABAT, E. A., WU, T. T., PERRY, H. M., GOTTESMAN, K. S. & FOELLER (1991). Editors. *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda: US Department of Health and Human Services.

KABSCH, W. (1988). *J. Appl. Cryst.* **21**, 916-924.

KANG, A. S., BURTON, D. R. & LERNER, R. A. (1991). *Methods: a Companion to Methods in Enzymology*, Vol. 2, pp. 111-118. New York: Academic Press.

KASLOW, D. C., QUAKYI, I. A., SYING, C., RAUM, M. G., KEISTER, D. B., COLIGAN, J. E., MCCUTCHAN, T. F. & MILLER, L. H. (1988). *Nature (London)*, **333**, 74-75.

KLINE, T. P., BROWN, F. K., BROWN, S. C., JEFFS, P. W., KOPPLE, K. D. & MÜLLER, L. (1990). *Biochemistry*, **29**, 7805-7813.

KOFLER, R., STROHAL, R., BALDERAS, R. S., JOHNSON, M. E., NOONAN, D. J., DUCHOSAL, M. A., DIXON, F. J. & THEOFILOPOULOS, A. N. (1988). *J. Clin. Invest.* **82**, 852-860.

MCPHERSON, A. (1982). *Preparation and Analysis of Protein Crystals*. New York: Wiley.

MATTHEWS, B. W. (1968). *J. Mol. Biol.* **33**, 491-497.

RINI, J. M., SCHULZE-GAHMEN, U. & WILSON, I. A. (1992). *Science*, **255**, 959-965.

STURA, E. A., FIESER, G. G. & WILSON, I. A. (1990). *Immunomethods*, **3**, 164-167.

STURA, E. A., JOHNSON, D. L., INGLESE, J., SMITH, J. M., BENKOVIC, S. J. & WILSON, I. A. (1989). *J. Biol. Chem.* **26**, 9703-9706.

STURA, E. A., NEMEROW, G. N. & WILSON, I. A. (1992). *J. Cryst. Growth*, **122**, 273-285.

STURA, E. A., SATTERTHWAIT, A. C., CALVO, J., KASLOW, D. C. & WILSON, I. A. (1994). *Acta Cryst.* D**50**, 448-455.

STURA, E. A., SATTERTHWAIT, A. C., CALVO, J., STEFANKO, R., LANGEVELD, J. P. & KASLOW, D. C. (1994). *Acta Cryst.* D**50**, 556-562.

STURA, E. A. & WILSON, I. A. (1990). *Methods: a Companion to Methods in Enzymology*, Vol. 1, pp. 38-49. New York: Academic Press.

STURA, E. A. & WILSON, I. A. (1991). *J. Cryst. Growth*, **110**, 270–282.

VERMEULEN, A. N., PONNUDURAI, T., BECKERS, P. J. A., VERHAVE, J. P., SMITS M. A. & MEUWISSEN, J. H. E. T. (1985). *J. Exp. Med.* **192**, 1460.

WILMOT, C. M. & THORNTON, J. M. (1988). *J. Mol. Biol.* **203**, 221-232.

WILMOT, C. M. & THORNTON, J. M. (1990). *Protein Eng.* **3**, 479-493.

WILSON, I. A., RINI, J. M., FREMONT, D. H., FIESER, G. G. & STURA, E. A. (1991). *Methods Enzymol.* **203**, 153-176.