Acta Cryst. (1994). D50, 64–70

Crystallization of Two Monoclonal Fab Fragments of Similar Amino-Acid Sequence Bound to the Same Area of Horse Cytochrome *c* and Interacting by Potentially Distinct Mechanisms*†

By Ronald Jemmerson‡ and Susan Buron

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455, *USA*

Ruslan Sanishvili and Emanuel Margoliash

Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois, Chicago, Illinois 60607, *USA*

and Edwin Westbrook and Mary Westbrook

Biological and Medical Research Division, Argonne National Laboratory, 9700 *S. Cass Avenue, Argonne, Illinois* 60439, *USA*

(*Received* 14 *April* 1993; *accepted* 26 *August* 1993)

Abstract

The mouse monoclonal antibodies (mAb), 2E5.G10 and 1F5.D1, are specific for horse cytochrome *c* and appear to bind the same epitope, since their heavy (H) and light (L) chains are functionally interchangeable. Comparison of the amino-acid sequences suggests that slightly different interactions may be involved in antigen recognition. In addition, the H chains differ at only a few amino-acid residues from the H chain of a rat cytochrome *c*-specific mAb suggesting that specificity for one protein over another may be determined by these amino-acid differences. To address these possibilities, the three-dimensional structures of the Fab portions of the mAb bound to cytochrome *c* are being determined by X-ray diffraction analysis. Here we describe the preparation and crystallization of the two complexes with horse cytochrome *c*. The complex of the Fab fragment of 2E5.G10 with horse cytochrome *c* yielded crystals of X-ray diffraction quality under two sets of conditions; in both the space group was $P2_1$. The corresponding complex of 1F5.D1 under one of these conditions crystallized in the $P2_12_12_1$ space group. Three-dimensional X-ray data for these two complexes have been collected with nominal resolutions of 2.86 and 2.48 Å, respectively.

1. Introduction

The three-dimensional structures of several protein Ag–Fab complexes have been determined by X-ray crystallography (Amit, Mariuzza, Phillips & Poljak, 1986; Colman *et al.*, 1987; Sheriff *et al.*, 1987; Padlan *et al.*, 1989; Tulip *et al.*, 1989; Bentley, Boulot, Riottot & Poljak, 1990). The characteristics of antibody binding observed in these studies are strikingly similar, providing a generalized concept of the chemical nature of native protein Ag–Ab interaction (Davies, Sheriff & Padlan, 1988). This includes: (1) a relatively large surface (750 Å^2) in which the antigen residues are located; (2) contribution of one or more amino-acid residues from each of the six CDR's of the Ab; (3) relatively minor changes in the conformation of Ab and Ag, however significant enough to suggest a degree of induced fit in some cases (Davies & Padlan, 1992); and (4) major contributions of hydrogen bonding and van der Waals interactions to the energy of binding.

While crystallographic studies of Ab–Ag complexes to date have revolutionized our view of these interactions, there are many questions that remain. Of particular interest to us is the molecular basis for Ab specificity, *i.e.* what is the physical and chemical information within the structure of an Ab that enables it to bind a particular protein Ag with high

* Supported by National Science Foundation grant DMB-9019181 and Council for Tobacco Research grant 3282 to RJ, National Institutes of Health grant AI-12001 to EM and Department of Energy contract W31-109-ENG-38 to EW.

† Abbreviations used in this paper: Ab, antibody; Ag, antigen; CDR, complementarity-determining region; Fab, antigen binding fragment of immunoglobulin; Fc, constant fragment of immunoglobulin; IgG, immunoglobulin G; H, heavy chain; H1, CDR1 of the H chain; H3, CDR3 of the H chain; L, light chain; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PEG, polyethylene glycol; SDS, sodium dodecyl sulfate; SDS–PAGE, polyacrylamide gel electrophoresis in SDS.

‡ To whom correspondence should be addressed at: Department of Microbiology, Box 196, University of Minnesota Health Sciences Center, 420 Delaware Street SE, Minneapolis, Minnesota 55455, USA.

© 1994 International Union of Crystallography
Printed in Great Britain – all rights reserved

ISSN 0907-4449 © 1994

affinity, yet not even recognize a variant of the Ag that differs by a single amino-acid residue in the antigenic determinant. We are approaching this problem by analyzing Ab recognition of natural and recombinant variants of cytochrome *c*. mAb have been prepared against several cytochromes *c* and those binding the same region have been compared on the basis of their primary structures (Goshorn, Retzel & Jemmerson, 1990). Although some diversity in Ab structure was observed, several examples were found where Ab to the same region on different cytochromes *c* were similar in the amino-acid sequences of the H or L chain, differing only at a few residues. This suggested that those few changes encode the specificity for a particular cytochrome *c* variant.

To examine directly whether this is the case and to determine which residue changes in the Ab are important for the variant specificity, the detailed three-dimensional structures of the Ab–Ag complexes are needed. Therefore, we attempted to prepare crystals of cytochrome *c* complexed to the Fab of some of these Ab for use in X-ray diffraction studies. Of the six complexes tried, crystals have been obtained, varying in quality, from five of them. Here we report on the crystallization of two of these complexes (2E5.G10 Fab–horse cytochrome *c* and 1F5.D1–horse cytochrome *c*). The H and L chains of these Ab are functionally interchangeable (Jemmerson, Mueller & Flaa, 1993) indicating that they bind the same site on horse cytochrome *c* and when examined for binding a panel of cytochrome *c* variants, they show similar fine specificities (Jemmerson & Johnson, 1991). While the L chains differ by only five amino-acid residues, the H chains differ by 15 in the variable (V) region, eleven of these within the CDR. Comparison of 2E5.G10 and 1F5.D1 to a third mAb specific for a different cytochrome *c* (rat) suggests that H1 and/or H3 are important in determining the specificity and that, in particular, an aspartic acid residue in the horse cytochrome *c*-specific Ab could be important, perhaps in forming a salt bridge with a lysine in the Ag, which is known to be critical for binding (Jemmerson *et al.*, 1993). Interestingly, the aspartic acid residues thought to be involved in this interaction are at different residue positions in both H1 and H3 of 1F5.D1 and 2E5.G10. Thus, although these two mAb bind the same site they may do so in slightly different ways.

2. Experimental

Cytochrome c purification

Horse cytochrome *c* was purchased from Sigma Chemical Co. (St Louis, MO). The protein (250 mg) was dialyzed against 65 m*M* sodium phosphate pH 7.5 and chromatographed on a 1.5 × 50 cm column of CM-Sephadex (Pharmacia, Piscataway, NJ) in the same buffer. After 4 d all of the deamidated forms had passed through the column, polymeric cytochrome *c* detected at the top of the column was removed and the native cytochrome *c* was then eluted from the column with 2 *M* NaCl dissolved in the running buffer. The purified cytochrome *c* (approximate yield 75%) was dialyzed against PBS (136 m*M* sodium chloride, 2.7 m*M* potassium chloride, 8 m*M* dibasic sodium phosphate and 1.5 m*M* monobasic potassium phosphate, pH 7.2) and stored frozen.

mAb purification

The mAb were obtained as previously described (Goshorn *et al.*, 1990). Hybridomas secreting the mAb were grown in the peritoneum of ten pristane-primed BALB/c mice, yielding a total of approximately 50 ml ascites fluid. The mAb were precipitated in cold 50% saturated ammonium sulfate, centrifuged at 3500 g for 20 min and washed once. The precipitates were dissolved in 5–10 ml 40 m*M* sodium phosphate pH 8.0 and dialyzed against the same buffer with several solution changes. The mAb were purified by chromatography through 30 ml DEAE-Sephacel (Pharmacia) packed in a 50 ml syringe and equilibrated in 40 m*M* sodium phosphate pH 8.0. Between 70 and 100 mg of each mAb were obtained.

Fab preparation

The mAb were treated with papain to isolate the Fab (Porter, 1959). The purified mAb were dialyzed against 0.1 *M* potassium phosphate pH 7.2, containing 1.25 m*M* 2-mercaptoethanol and 1.25 m*M* disodium ethylenediaminetetraacetate. Typically, the mAb were treated with 2% (by weight) papain (Sigma) for 2 h at 30 K and with 2% more enzyme for an additional 4 h with continuous mixing. The treatment was terminated by the addition of iodoacetamide to 4 m*M*. The digested material was dialyzed against 5 m*M* potassium phosphate pH 8.0 and chromatographed on 30 ml DEAE-Sephacel packed in a 50 ml syringe. The Fab of the two mAb studied eluted in the running buffer while the Fc remained bound. The Fab were dialyzed against 20 m*M* ethanolamine pH 9.5 and chromatographed on 30 ml Q-Sepharose Fast Flow (Pharmacia) packed in a 50 ml syringe in the same buffer (Boulot *et al.*, 1988). Fab monomers, Fab dimers and any intact mAb were separated by a linear gradient over 400 ml of 0–0.5 *M* potassium chloride in the chromatography buffer. The Fab-containing fractions were pooled and dialyzed against PBS.

Fab–cytochrome c complexes

The Fab were concentrated by centrifugation using a Centriprep 10 concentrator (Amicon, Beverly, MA). They were then incubated overnight at 277 K with 2–3-fold molar excess ferric horse cytochrome *c*. The Fab–cytochrome *c* complexes were separated from free cytochrome *c* by gel filtration chromatography on a 1.5 × 50 cm column of Sephadex G-100 in PBS. The complexes were dialyzed against distilled water and concentrated to 10–15 mg ml^{-1} total protein using a Centricon 10 concentrator (Amicon) and stored at 277 K with 0.05% sodium azide. Purity of the Fab and Fab–cytochrome *c* complexes was assessed by SDS–PAGE, disulfide-bonded polypeptides being reduced with 2-mercaptoethanol (2.5%). The gels were stained with Coomassie blue.

Crystal preparation

Crystals of the Fab–cytochrome *c* complexes were obtained in hanging drops by the vapor-diffusion method. Briefly, 0.7 ml of the precipitating solution was added to wells of a 24-well microtiter plate. The wells were sealed using vacuum grease and a glass coverslip from which was suspended 7 μl of one of the Fab–cytochrome *c* complexes (10–15 mg ml^{-1}) dissolved in 7 μl of the precipitating solution. Initially, 50 different solutions varying in pH, salt and precipitant (Crystal Screen, Hampton Research, Riverside, CA) were screened for their ability to facilitate crystal formation (Jancarik & Kim, 1991). Further variations in pH, salt and precipitant concentration from the initial conditions which showed evidence of crystal formation were then examined to optimize the conditions.

Crystal diffraction studies

Crystallographic parameters of all crystal forms discussed here were characterized by precession photography. We used a Supper precession camera mounted on a Diffractis 588 X-ray generator with a 1.5 kW sealed Cu tube (Enraf–Nonius). Cu *Kα* radiation was selected with a nickel filter, and data were recorded on Kodak direct-exposure Scientific Imaging Film (Charles Supper Company).

Three-dimensional X-ray data have been collected on a Siemens/Xentronics multiwire area detector (Durbin *et al.*, 1986), mounted on a Huber four-circle goniostat. The X-ray source was a Rigaku RU200 rotating-anode generator, fitted with a copper anode, and operated at 5 kW. Cu *Kα* radiation was selected with a graphite monochromator. Diffraction data were reduced with the *XENGEN* program package (Howard *et al.*, 1987). For three-dimensional data collection from monoclinic crystals, we collected 900 data frames, each 0.2° of crystal rotation about the crystallographic *c* axis, 250 data frames of rotation about the *b* axis, and merged them. Each data frame required 180 s of X-ray exposure time, and the detector was swung 10° away from the direct X-ray beam. For the orthorhombic $P2_12_12_1$ crystal form, 500 data frames were recorded by rotating about one orientation, 250 frames at an orientation orthogonal to the others, and these data were merged. Each frame required 120 s exposure time. The detector was swung 15° from the direct X-ray beam.

3. Results

Preparation of cytochrome c–Fab complexes

The method described by Boulot *et al.* (1988) provided the basis for mAb purification and Fab preparation. However, instead of HPLC, classical chromatography methods were employed. The mAb were purified by ion-exchange chromatography and not Ag affinity chromatography to avoid the possibility of denaturing the mAb during their elution from Ag-coated beads. The mAb were not entirely pure after passage through DEAE-Sephadex; however, any contaminants were removed during the preparation and purification of Fab. We found that ion-exchange chromatography on Q-Sepharose Fast Flow of the Fc-cleared papain digest of the mAb sometimes allowed for the isolation of Fab monomers, Fab dimers and intact IgG. This is most apparent with IgG1 which is not as sensitive to papain digestion as other IgG isotypes. In Fig. 1(*a*), chromatography of a partial papain digest of the mAb 2E5.G10 (IgG1,*κ*) is shown. For this experiment the mAb was treated with 1.5% (by weight) papain for 6 h at 310 K. Three fractions of IgG-related materials were obtained. From SDS–PAGE of reduced and non-reduced fractions (Fig. 1*b*) they were identified as follows: A, Fab monomer; B, Fab dimer; and C, IgG.

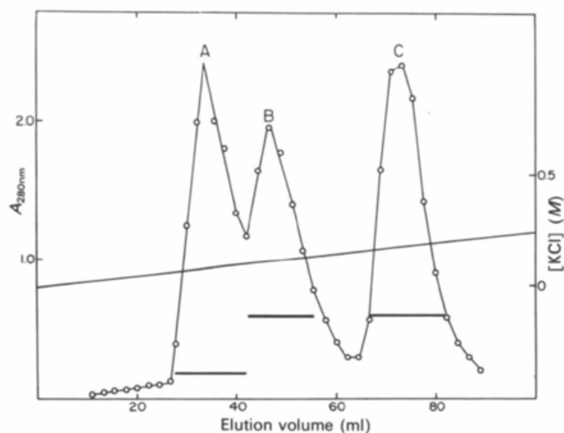
By increasing the papain concentration to 4% in the digestion of 2E5.G10 IgG, the intact IgG (fraction C) was eliminated, but Fab dimer remained in amounts approximately equal to that of the Fab monomer. Apparently for IgG1, papain can cleave on either side of the inter-heavy-chain disulfide bonds. Cleavage on the distal side results only in dimer formation and cleavage on the proximal side, in monomer. The mAb 1F5.D1 (IgG2a,*κ*) was completely cleaved to Fab monomer.

Incubation of the purified Fab monomers with excess horse cytochrome *c* resulted in the formation of equimolar complexes which were isolated by gel filtration on Sephadex G-100. The molar ratio of horse cytochrome *c* to Fab was 0.96 for the complex

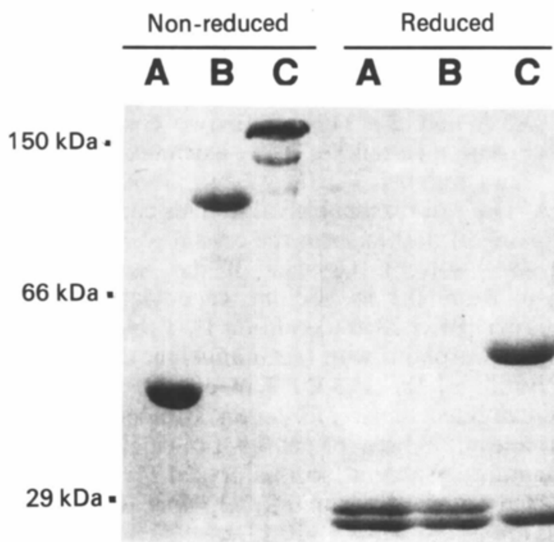
with 2E5.G10 Fab and 1.0 for the complex with 1F5.D1. These ratios were calculated using a millimolar extinction coefficient of 75 at 280 nm for the Fab, and extinction coefficients of 106.1 m*M* at 410 nm and 23.2 at 280 nm for cytochrome *c* in the ferric state. Slight variations from a molar ratio of 1.0 in the complexes may reflect differences between the actual extinction coefficients for the Fab and that which was assumed in the calculations.

Crystallization of horse cytochrome c–Fab complexes

Crystals of differing size and shape were observed for both complexes under a variety of conditions. For the 2E5.G10 Fab–cytochrome *c* complex, two sets of conditions, listed in Table 1, yielded crystals of sufficient size and quality for diffraction study. For the 1F5.D1 Fab–cytochrome *c* complex, one of these conditions was also productive (condition II). Photographs of crystals of the 2E5 Fab–cytochrome *c* complex are shown in Fig. 2(*a*). Crystals of the 2E5 Fab–cytochrome *c* complex, grown under condition II (Table 1) exhibited similar morphology. Crystals

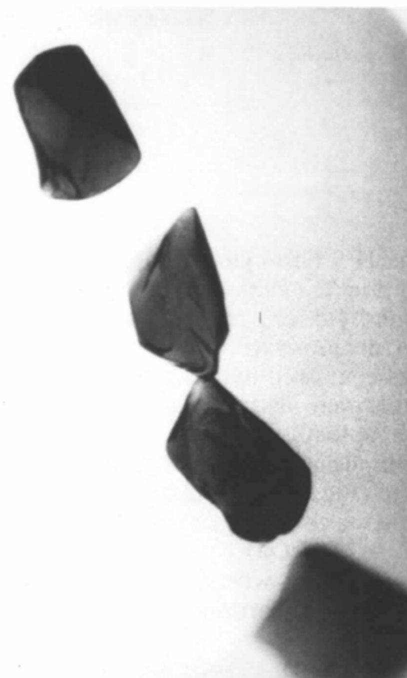


(*a*)



(*b*)

Fig. 1. Chromatography of papain-cleaved mAb 2E5.G10 on Q-Sepharose Fast Flow (*a*) and identification of the peaks by SDS–PAGE (*b*). Peak A is Fab monomer; peak B is Fab dimer; and peak C is IgG.



(*a*)

(*b*)

Fig. 2. Crystals of the complex of 2E5.G10 Fab with horse cytochrome *c* formed in 0.1 *M* sodium cacodylate pH 6.5, 20% PEG 3350 and 0.2 *M* ammonium acetate (*a*), and of the complex of 1F5.D1 Fab with horse cytochrome *c* formed in 0.1 *M* sodium cacodylate pH 6.5, 20% PEG 3350 (*b*). The crystals shown in (*a*) are described in Table 1 (crystallization condition I). Their largest dimension is 0.4 mm. Each hexahedron in (*b*) is 0.5 mm long.

Table 1. *Summary of preliminary X-ray study of crystals of two Fabs complexed with horse cytochrome c*

Complex	Crystallization conditions	Space group and lattice parameters (Å, °)	No. of molecules in unit cell and per asymmetric unit	Matthews number, V_m, and volume, V_{prot}, occupied by protein in crystal (%)
2E5.G10–cytochrome *c*	I	$P2_1$		
	0.2 *M* NH_4 acetate	$a = 97.7$	4	2.4
	0.1 *M* Na cacodylate	$b = 78.8$		
	pH 6.5	$c = 83.6$	2	51
	20% PEG 3350	$\alpha = \gamma = 90$, $\beta = 113$		
2E5.G10–cytochrome *c*	II	As above	As above	As above
	0.1 *M* Na cacodylate			
	pH 6.5			
	20% PEG 3350			
1F5.D1–cytochrome *c*	II	$P2_12_12_1$		
		$a = 81.0$	4	2.48
		$b = 110.1$		
		$c = 74.5$	1	50
		$\alpha = \beta = \gamma = 90$		

of the 1F5 Fab–cytochrome *c* complex grown under condition II (Table 1) appeared as elongated hexahedrons (0.5 long and 0.2 mm diameter) (Fig. 2*b*).

To demonstrate that the crystals of these complexes contained both cytochrome *c* and Fab, several crystals were dissolved in 1.0% SDS, reduced with 2-mercaptoethanol and electrophoresed in SDS–PAGE using 12.5% acrylamide gels. From the results (Fig. 3), it is clear that all crystals of both complexes contain Fab (two chains) and cytochrome *c* (M_r = 12.5 kDa).

To examine whether contamination of the Fab monomer–cytochrome *c* complex with Fab dimer–cytochrome *c* complex might affect crystal formation, 2E5.G10 Fab monomer–cytochrome *c* was incubated with 0.1, 1 or 10% 2E5.G10 Fab dimer–cytochrome *c*. The mixed solutions were subjected to the same conditions which yielded diffractable crystals of 2E5.G10 Fab monomer–cytochrome *c*. Within 1 week, crystals similar in appearance formed, whether 2E5.G10 Fab dimer–cytochrome *c* were present or not even in concentrations as high as 10% (results not shown).

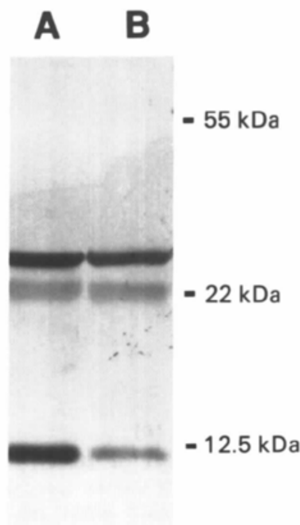


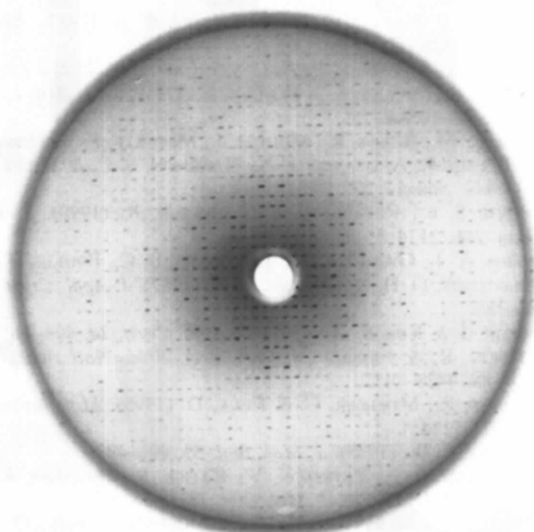
Fig. 3. Identification of proteins present in the crystals of the 2E5.G10 Fab–horse cytochrome *c* complex (lane A) and the 1F5.D1–horse cytochrome *c* complex (lane B) by SDS–PAGE. The gel was run under reducing conditions.

Characterization of the crystals and X-ray data collection

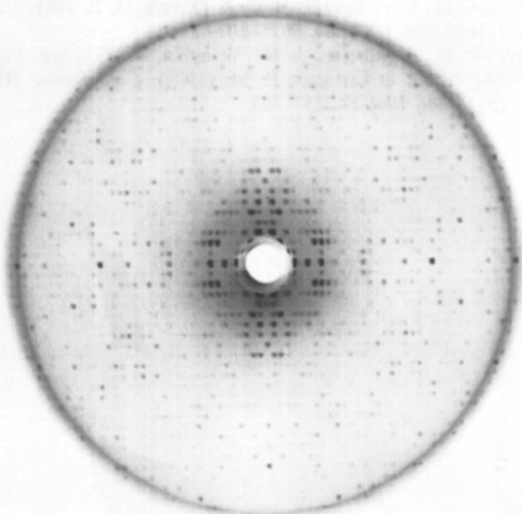
Fig. 4(*a*) shows the (0*kl*) zone of crystal form I of the 2E5.G10–cytochrome *c* complex (Table 1). This crystal form exhibited 2/*m* diffraction symmetry and $k = 2n$ systematic absences on the (0*k*0) line, so it belongs to space group $P2_1$, with $a = 97.7$, $b = 78.8$, $c = 83.6$ Å and $\beta = 113°$. With two complete Fab–cytochrome *c* complexes in its asymmetric unit, the Matthews number V_m (Matthews, 1968) would be 2.4 Å^3 Da^{-1}, a reasonable value. This corresponds to a packing of molecules in the crystal of 51% protein and 49% solvent. Crystals of the same complex grown from 0.1 *M* sodium cacodylate pH 6.5, 20% (*w*/*v*) PEG 3350 (condition II, Table 1), proved to be isomorphous with this monoclinic crystal form.

Crystals of the 1F5.D1 Fab–cytochrome *c* complex exhibited *mmm* diffraction symmetry and the odd integer indices of all its principal axes were systematically absent, so this crystal form could be assigned to space group $P2_12_12_1$. Its lattice dimensions are $a = 81.01$, $b = 110.13$, $c = 74.54$ Å. Fig. 4(*b*) shows the (0*kl*) zone of this crystal form. With one molecular complex per asymmetric unit, this crystal form has a V_m of 2.47 Å^3 Da^{-1}. The results of our crystal characterizations described above are summarized in Table 1.

The $P2_1$ crystals of the 2E5.G10 Fab–cytochrome *c* complex, obtained either in the presence or the absence of salt, and the $P2_12_12_1$ crystals of the 1F5.D1 Fab–cytochrome *c* complex, were stable in the X-ray beam and suitable for three-dimensional data collection. Full three-dimensional data were collected and processed from a single crystal for each complex. The final statistics for data collection are summarized in Table 2.



(*a*)



(*b*)

Fig. 4. X-ray photographs of 0*kl* zones for (*a*) monoclinic crystals of 2E5.G10 Fab–horse cytochrome *c* complex grown from 0.2 *M* ammonium acetate, 0.1 *M* sodium cacodylate pH 6.5, 20% (*w*/*v*) PEG 3350, and (*b*) orthorhombic crystals of 1F5.D1 Fab–horse cytochrome *c* crystals grown from 0.1 *M* sodium cacodylate pH 6.5, 20% PEG 3350. The crystal-to-film distance was 75 mm, precession angle $\mu = 15°$ and exposure time 12 h.

Table 2. *Summary of crystallographic data sets for complexes of Fab 1F5.D1 and 2E5.G10 with horse cytochrome c*

Complex	Resolution (Å)	No. of unique reflections	Completeness of data (%)	$\langle I/\sigma \rangle$	R_{sym}* (%)
2E5.G10 with horse cytochrome *c*	2.86	23 605	All data: 90.8 At 2.86 Å: 99.8	11.3	9.3
1F5.D1 with horse cytochrome *c*	2.48	20 393	All data: 86.0 At 2.63 Å: 95.6	14.3	6.7

* $R_{sym} = \sum |I_{obs} - I_{avg}| / \sum I_{avg}$.

4. Discussion

The antigenic specificities of the two mAb 2E5.G10 and 1F5.D1 whose Fabs we have crystallized in complex with horse cytochrome *c*, are similar to each other and to that of a third mAb, E8, which was also elicited against horse cytochrome *c* (Carbone & Paterson, 1985). This mAb is similar to 2E5.G10 and 1F5.D1 in the amino-acid sequences of both H and L chains and its Fab has been crystallized in complex with horse cytochrome *c* (Mylvaganam, Paterson, Kaiser, Bowdish & Getzoff, 1991). None of these three mAb are clonally related and only 2E5.G10 and 1F5.D1 were obtained from the same mouse. These three mAb provide the opportunity to examine functional differences among mAb binding the same region on a protein antigen.

The Fab–Ag complexes described in this report are two of the six complexes that we have attempted to crystallize. We have, in fact, obtained crystals of varying quality for five complexes. However, to date only the crystals of the complexes of 2E5.G10 and 1F5.D1 with horse cytochrome *c* have been determined to be of the quality to collect X-ray diffraction data. Variation of the conditions for crystallization may lead to higher quality crystals for the other complexes. From what has been published regarding the crystallization of Ab–Ag complexes, it appears that no more than 1 in 20 Fab fragments have been crystallizable (Boulot *et al.*, 1988). We have not yet attempted to crystallize our Fab free from cytochrome *c*. It is possible that complexation with cytochrome *c* may facilitate the crystallization process. It is also possible that the method of Fab preparation and purification we employed removed any impurities that could have interfered with the crystallization of Fab in other laboratories. Since for several IgG1 we have found both Fab dimer and

monomer present after papain cleavage, we considered the possibility that any dimer present in a Fab monomer preparation could affect crystallization. However, addition of the complex of the Fab dimer of 2E5.G10 bound to horse cytochrome *c* to the complex of Fab monomer–cytochrome *c* did not alter the time course of crystallization nor the visual quality of the crystals. This was the case whether the dimer represented as little as 0.1% of the total Fab or as much as 10%.

Although 2E5.G10 and 1F5.D1 are similar in primary structure and, in complex with cytochrome *c*, crystallize under similar conditions, there are noted differences. The 2E5.G10 Fab complex crystallizes within less than 2 weeks, quite reproducibly, while the 1F5.D1 Fab complex requires 4 weeks or longer. Furthermore, the crystal lattices are distinct. The symmetry group for the 2E5.G10 Fab–cytochrome *c* complex is $P2_1$ and for the 1F5.D1 Fab–cytochrome *c* complex is $P2_12_12_1$. This is the same symmetry group observed for crystals of the E8–horse cytochrome *c* complex that formed under similar conditions (Mylvaganam *et al.*, 1988).

The authors are grateful to Dr Karl W. Volz of the Department of Microbiology and Immunology, The University of Illinois at Chicago, for kindly providing the X-ray facility used for precession characterization studies of these crystal forms, and to Drs William Gleason and Leonard Banaszak, University of Minnesota, for their advice, assistance and encouragement during the early phase of this work. The photographic assistance of Tim Leonard and secretarial support of Jan Smith and Carmen Orendain are much appreciated.

References

AMIT, A. G., MARIUZZA, R. A., PHILLIPS, S. E. V. & POLJAK, R. J. (1986). *Science*, **233**, 747–753.

BENTLEY, G. A., BOULOT, G., RIOTTOT, M.-M. & POLJAK, R. J. (1990). *Nature (London)*, **348**, 254–257.

BOULOT, G., GUILLON, V., MARIUZZA, R. A., POLJAK, R. J., RIOTTOT, M.-M., SOUCHON, H., SPINELLI, S. & TELLO, D. (1988). *J. Cryst. Growth*, **90**, 213–221.

CARBONE, F. R. & PATERSON, Y. (1985). *J. Immunol.* **135**, 2609–2616.

COLMAN, P. M., LAVER, W. G., VARGHESE, J. N., BAKER, A. T., TULLOCH, P. A., AIR, G. M. & WEBSTER, R. G. (1987). *Nature (London)*, **326**, 358–363.

DAVIES, D. R. & PADLAN, E. A. (1992). *Curr. Biol.* **2**, 254–256.

DAVIES, D. R., SHERIFF, S. & PADLAN, E. A. (1988). *J. Biol. Chem.* **263**, 10541–10544.

DURBIN, R. M., BURNS, R., MOULAI, J., METCALF, P., FREYMANN, D., BLUM, M., ANDERSON, J. A., HARRISON, S. C. & WILEY, D. C. (1986). *Science*, **232**, 1127–1132.

GOSHORN, S. C., RETZEL, E. & JEMMERSON, R. (1990). *J. Biol. Chem.* **266**, 2134–2142.

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.

JANCARIK, J. & KIM, S. H. (1991). *J. Appl. Cryst.* **24**, 409–411.

JEMMERSON, R. & JOHNSON, J. G. (1991). *Proc. Natl Acad. Sci. USA*, **88**, 4428–4432.

JEMMERSON, R., MUELLER, C. & FLAA, D. (1993). *Mol. Immunol.* **30**, 1107–1114.

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

MYLVAGANAM, S. E., PATERSON, Y., KAISER, K., BOWDISH, K. & GETZOFF, E. D. (1991). *J. Mol. Biol.* **221**, 455–462.

PADLAN, E. A., SILVERTON, E. W., SHERIFF, S., COHEN, G. H., SMITH-GILL, S. J. & DAVIES, D. R. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 5938–5942.

PORTER, R. R. (1959). *Biochem. J.* **73**, 119–126.

SHERIFF, S., SILVERTON, E. W., PADLAN, E. A., COHEN, G. H., SMITH-GILL, S. J., FINZEL, B. C. & DAVIES, D. R. (1987). *Proc. Natl Acad. Sci. USA*, **84**, 8075–8079.

TULIP, W. R., VARGHESE, J. N., WEBSTER, R. G., AIR, G. M., LAVER, W. G. & COLMAN, P. M. (1989). *Cold Spring Harbor Symp. Quant. Biol.* **54**, 257–263.