sera from humans in endemic areas (*30*) and may play a role in protective immunity (*19*). This suggests that p41 is exposed to the host's immune system at a certain point of the infectious cycle, probably when the content of the rhoptry organelles is secreted onto the erythrocyte membrane (*31*). At this step, antibodies could inactivate the rhoptry proteins that are believed to participate in the invasion process (*7, 19*).

Certain features of the p41 sequence suggest two independent approaches to the control of malaria. First, the need for functional aldolase may reflect the strong conservation of the sequence among different isolates; it is unlikely that parasite mutants evolve that escape immune attack by protective antibodies against p41. Second, the common function of the enzyme yet the relatively high degree of sequence diversity between human and parasite aldolase suggests the possibility of finding compounds that specifically inhibit the parasite enzyme.

REFERENCES AND NOTES

1. W. A. Siddiqui, *Science* **197**, 388 (1977).
2. G. H. Mitchell, G. A. Butcher, W. H. G. Richards, S. Cohen, *Lancet* **i**, 1335 (1977).
3. L. H. Perrin *et al.*, *J. Clin. Invest.* **75**, 1718 (1985).
4. L. H. Perrin and R. Dayal, *Immunol. Rev.* **61**, 245 (1982).
5. R. F. Howard, H. A. Stanley, G. H. Campbell, R. H. Reese, *Am. J. Trop. Med. Hyg.* **33**, 1055 (1984).
6. C. Braun-Brenton *et al.*, *Mol. Biochem. Parasitol.* **20**, 33 (1986).
7. L. Schofield, *ibid.* **18**, 183 (1986).
8. R. R. Hall *et al.*, *Nature* **311**, 379 (1984).
9. R. A. Young and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1194 (1983).
10. R. A. Young *et al.*, *ibid.* **82**, 2583 (1985).
11. Affinity-purified p41 protein (*3*) was injected into rabbits according to standard immunization protocols. When parasite lysates are probed in immunoblots, a single band of 41 kD is detected by the serum.
12. W. D. Benton and R. W. Davis, *Science* **196**, 180 (1977).
13. The sequence data have been submitted to the European Molecular Biology Laboratory/GenBank data libraries under accession number J03084.
14. K. Tanabe, M. Mackay, M. Goman, J. G. Scaife, *J. Mol. Biol.* **196**, 273 (1987).
15. D. J. Kemp *et al.*, *Parasitology* **91**, 83 (1986).
16. Peptides were generated by digestion of 80 μg of affinity-purified p41 protein (*3*) with *Staphylococcus aureus* protease V8 and separated by reversed-phase high-performance liquid chromatography (HPLC) by using 0.1*M* trifluoroacetic acid and an acetonitrile gradient as the mobile phase. The amino acid sequences were determined on an Applied Biosystems 470 A gas phase sequencer.
17. C. Yanish-Perron, J. Vieira, J. Messing, *Gene* **33**, 103 (1985).
18. The Eco RI insert of clone M25-13 was subcloned in the proper orientation into pUC18 (*17*), resulting in pGF-2. A 1-liter overnight culture of *E. coli* transformed with pGF-2 was concentrated by centrifugation, and the bacterial pellet was dissolved in 5 ml of lysis buffer (*3*) supplemented with lysozyme (10 mg/ml). When lysis was apparent, further purification steps were carried out as described (*3*), yielding 5 μg of 41-kD recombinant protein. ELISA assays were performed as described (*22*).
19. L. H. Perrin, E. Ramirez, P. H. Lambert, P. A. Miescher, *Nature* **289**, 301 (1981).
20. K. G. Odink, S. C. Nicolls, A. J. Lockyer, Y. Hillman, *Mol. Biochem. Parasitol.* **10**, 55 (1984).
21. U. Gubler and B. J. Hoffman, *Gene* **25**, 263 (1983).
22. T. V. Huynh, R. A. Young, R. W. Davis, in *DNA Cloning: A Practical Approach*, D. M. Glover, Ed. (IRL Press, Washington, DC, 1985), vol. 1, pp. 49–78.
23. U. Certa, D. Rotmann, H. Matile, R. Reber-Liske, *EMBO J.* **6**, 4137 (1987).
24. The isolates came from East Africa (RO-58, RO-59, FCH 5-C2, and Palo Alto), West Africa (R-FCR 3, RO-33, Geneva no. 13, and RO-53), Central America (H-B3), South America (542), Asia (K1, CPG-1, T9/94, and NT-112), and the South Pacific (MAD-20).
25. J. Devereux, P. Haeberli, O. Smithies, *Nucleic Acids Res.* **12**, 387 (1984).
26. R. K. Wierenga *et al.*, *EMBO J.* **6**, 215 (1987).
27. C. Y. Lai and B. L. Horecker, *Essays Biochem.* **8**, 149 (1972).
28. R. W. McKee, in *Biochemistry and Physiology of Protozoa*, S. H. Hutter and A. Lwoff, Eds. (Academic Press, New York, 1951), vol. 1, pp. 251–322.
29. N. Saxena, V. C. Pandey, G. P. Dutta, S. Ghatak, *Indian J. Exp. Biol.* **24**, 502 (1986).
30. M. A. Gabra *et al.*, *Bull. WHO* **64**, 889 (1986).
31. L. H. Bannister, G. H. Mitchell, G. A. Butcher, E. D. Dennis, *Parasitology* **92**, 291 (1986).
32. L. H. Perrin, unpublished data.
33. K1 parasites were cultivated and extensively washed as described (*23*). The parasites were lysed by eight cycles of freeze-thawing. Lysis was monitored microscopically. Membranes and debris were removed by centrifugation at 12,000 rpm for 10 min at 4°C. Aliquots of this lysate or purified p41 protein (*3*) containing 50 units (1 unit is 1 μmol of dihydroxyacetone phosphate formed per minute and per milligram of protein at 37°C) of aldolase were assayed with a commercial aldolase kit (Sigma, procedure 752). For inhibition, 25 μl of rabbit antiserum to p41 (*11*) or preimmune serum were preincubated with the sample for 5 min at 25°C.
34. The increment in the preimmune serum control is due to aldolase and triose-phosphate isomerase activity present in rabbit serum.
35. Sizing of active p41 was performed on a 60-cm LKB TSK-G 3000 SW HPLC column connected to a 7.5-cm precolumn in 0.1*M* sodium phosphate, *p*H 7.5, at a flow rate of 0.4 ml/min. The column was calibrated with leucine aminopeptidase (326 kD), rabbit muscle aldolase (160 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), human immune interferon (32 kD), myoglobin (18 kD), and cytochrome c (12 kD). Proteins were detected after *o*-phthalaldehyde derivatization (*36*).
36. M. Roth, *Anal. Chem.* **43**, 880 (1971).
37. U. K. Laemmli, *Nature* **227**, 680 (1970).

38. We thank D. Doran for computer-assisted screening of the NBRF database; Y. Burki and D. Rotmann for technical assistance; S. LeGrice, K. Brenzel, and R. Gentz for discussions; and E. Kawashima for synthetic DNA adapters. Parts of this work were supported by the Swiss National Research Foundation.

22 December 1987; accepted 15 March 1988

Assembly of a Functional Immunoglobulin F_v Fragment in *Escherichia coli*

ARNE SKERRA AND ANDREAS PLÜCKTHUN*

An expression system was developed that allows the production of a completely functional antigen-binding fragment of an antibody in *Escherichia coli*. The variable domains of the phosphorylcholine-binding antibody McPC603 were secreted together into the periplasmic space, where protein folding as well as heterodimer association occurred correctly. Thus, the assembly pathway for the F_v fragment in *E. coli* is similar to that of a whole antibody in the eukaryotic cell. The F_v fragment of McPC603 was purified to homogeneity with an antigen-affinity column in a single step. The correct processing of both signal sequences was confirmed by amino-terminal protein sequencing. The functionality of the recombinant F_v fragment was demonstrated by equilibrium dialysis. These experiments showed that the affinity constant of the F_v fragment is identical to that of the native antibody McPC603, that there is one binding site for phosphorylcholine in the F_v fragment, and that there is no inactive protein in the preparation. This expression system should facilitate future protein engineering experiments on antibodies.

IMMUNOGLOBULINS (IGS) ARE A FAMILY of stable and similar molecules that can bind to a large number of different antigens. They constitute promising targets for investigating protein-ligand interactions since the overall folding of the domains seems to be independent of the structure of the binding site. Several three-dimensional structures of antibodies or their Fab fragments have been determined, and their common features have been compared (*1*). The essence of their architecture is a framework of fairly constant residues (arranged in a sandwich of β-sheets) linked by three hypervariable loops [complementarity-determining regions (CDR)] per chain that determine the specificity for antigen recognition. An early insight into enzyme catalysis by Pauling (*2*) was used to search for catalytic antibodies (*3*). An easy access to genetically engineered, functional antibody proteins would permit new approaches for studying antibody structure and function and the essentials of enzymatic catalysis (*4*).

Despite numerous investigations, the

Genzentrum der Universität München, Max-Planck-Institut für Biochemie, Am Klopferspitz, D-8033 Martinsried, FRG.

*To whom correspondence should be addressed.

expression of functional whole antibodies or functional antigen-binding fragments of antibodies has not been reported for any bacterial expression system, and the chance of designing such an expression system has been viewed pessimistically (*5, 6*). The expression of antibodies in yeast has been described (*7*), but only a small fraction of the expressed protein was functional. The purification of active antibodies or antibody fragments from yeast or any other microorganism has not been reported. In *E. coli*, the antibody protein could be produced only in a non-native state (*8*), and refolding experiments led to only a small percentage of correctly folded recombinant antibodies. Moreover, it is difficult to purify the native protein from non-native contaminants, which complicates accurate measurements of binding constants, folding yields, and spectral properties.

Other investigators preferred expression in cells of higher eukaryotes, thus permitting the production of functional antibodies (*5, 9, 10*). Yet none of these expression systems compares to *E. coli* in the ease of genetic manipulation, efficient transformation, fast growth, simple fermentation, and favorable economics. A bacterial expression system in which the chains assemble to form a functional complex in the same cell would permit the use of assays directly on bacterial colonies. One could then use positive selection methods for antigen binding and possible catalytic functions of the mutant antibodies. Apart from the investigation of the variable regions themselves, the possible replacement of the constant regions through genetic means by marker enzymes (*11*), toxins (*12*), or Ig regions from a different class (*13*) or from a different species (*14*) has attracted attention. The production of such hybrid antibodies may also be facilitated by a bacterial expression system.

In our search for an antibody model for investigations on binding and catalysis, we decided on an antibody with a known amino acid sequence and a three-dimensional structure that may also be close to a transition-state binding protein. Such a system is the myeloma protein McPC603 (*15, 16*), a phosphorylcholine-binding IgA from mouse. We decided to investigate the expression of the F_v fragment of McPC603 in *E. coli*. This fragment is the dimer of the V_L (115 amino acids) and V_H (122 amino acids) domains and contains the whole antigen binding site. Each domain has one intramolecular disulfide bond (connecting Cys-23 to Cys-94 in V_L and connecting Cys-22 to Cys-98 in V_H). There is no disulfide bond between the chains and no other free cysteine. We synthesized the genes for both the V_L and the V_H domain. The exact DNA sequence we synthesized, the synthesis methodology, and the logic of the sequence design are discussed elsewhere (*4*).

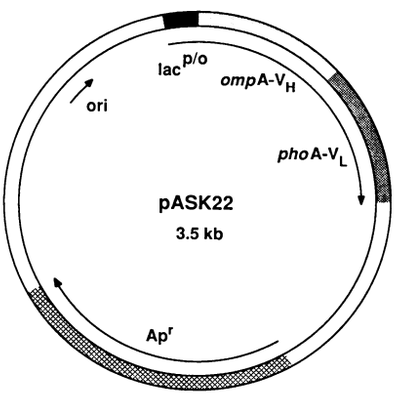

Fig. 1. Plasmid pASK22 for the coexpression and cosecretion of the V_L and V_H domain of McPC603 in *E. coli*. The plasmid contains the origin of replication (*ori*) and the ampicillin resistance gene (Ap^r) from the pUC family of plasmids (*29*). The V_H and the V_L domain are encoded as two separate proteins on the same transcription unit downstream from a *lac* promoter-operator (*lac*$^{p/o}$) (*29*), which is inducible by isopropyl-β-D-thiogalactoside (IPTG). The synthetic genes encoding the V_H and the V_L domain (*4*) are precisely fused to gene fragments encoding the signal sequence of the outer membrane protein A (*ompA*) (*19*) and the alkaline phosphatase (*phoA*) (*20*), respectively. Each coding region is preceded by a ribosomal binding site to ensure efficient translation initiation. Thus, both genes are arranged in a regulatable artificial dicistronic operon. The construction of the plasmid was performed by using standard DNA methodology (*30*).

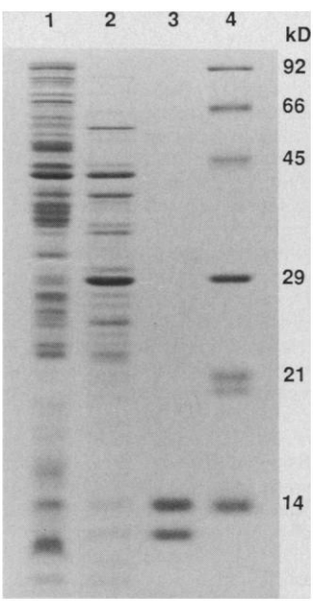

Fig. 2. Purification of the F_v fragment. An SDS-PAGE (14%) (*31*) stained with Coomassie brilliant blue is shown. (Lane 1) Total cell protein; (lane 2) periplasmic fraction; (lane 3) purified F_v fragment; and (lane 4) protein molecular size marker. For the purification, a culture of *E. coli* strain W3110 harboring plasmid pASK22 was grown in lactose broth (LB) medium containing ampicillin (100 mg/liter) to attain an absorbance at 550 nm of 0.5. After induction for 45 min by addition of isopropylthiogalactoside to a final concentration of 1 m*M*, the cells were harvested by centrifugation at 4000*g* for 10 min (at 4°C). A cell fractionation was carried out by resuspending the cell pellets in TES buffer (0.2*M* tris-HCl, *p*H 8.0; 0.5 m*M* EDTA; 0.5*M* sucrose) (10 ml per liter of original culture). The cells were then subjected to a mild osmotic shock by addition of TES, diluted 1:4 with H_2O, and containing 2 m*M* phosphorylcholine (15 ml per liter of original culture). After incubation on ice for 30 min, the suspension was centrifuged (5000*g*, 10 min) and the supernatant was centrifuged again (48,000*g*, 15 min). This supernatant, which contained all soluble periplasmic proteins (*32*), was concentrated by ultrafiltration with an Amicon YM 5 membrane to a volume of approximately 2.5 ml per liter of original culture and dialyzed against BBS buffer (0.2*M* borate/NaOH *p*H 8.0, 0.16*M* NaCl). This concentrated solution was applied to a phosphorylcholine affinity column (2.5-ml bed volume per 4 liters of bacterial culture) (*33*). After washing with BBS, pure F_v fragment was eluted with a solution of 1 m*M* phosphorylcholine in BBS. The typical yield of this not yet optimized procedure is approximately 0.2 mg of purified F_v fragment per liter of bacterial culture.

The expression system described herein is the result of attempts to reproduce in *E. coli* the folding and assembly pathway of antibodies in eukaryotic cells. In the eukaryotic plasma cell, the two chains of an antibody are separately transported from the cytoplasm to the lumen of the endoplasmic reticulum (ER) (*17*). This transport requires an NH_2-terminal signal sequence, which is cleaved off during or after the translocation event by a signal peptidase, to produce the mature protein. In the lumen of the ER, protein folding, formation of the disulfide bonds, and the association of individual chains to form the functional antibody take place (*17*). It is not clear yet which other proteins play an essential role in mediating these folding and assembly processes. In addition to these critical steps, which must be mimicked in the bacterial cell in order to obtain a functional F_v fragment, the antibodies are glycosylated in the lumen of the ER and Golgi apparatus and transported to the cell surface. Usually only the F_c is glycosylated, but this glycosylation is not required for antigen binding.

Our hypothesis was that the protein transport to the periplasm of *E. coli* is functionally equivalent to the transport of a protein to the lumen of the ER of a eukaryotic cell. We developed a system for expressing both chains in the same *E. coli* cell and secreting them together into the periplasm of *E. coli*. This system should permit the following critical steps in the assembly of a functional F_v fragment to occur: (i) synthesis of approximately stoichiometric amounts of both chains, (ii) transport of both precursor proteins to the periplasmic space, (iii) correct processing of both signal sequences, resulting in the same NH_2-termini as in the protein isolated from the mouse, (iv) fold-

ing to globular and soluble domains, (v) formation of the intramolecular disulfide bonds, and (vi) association of the two chains to form a heterodimer. Several examples (*18*) illustrate the secretion of heterologous monomeric proteins into the periplasm of *E. coli*, but it was not known whether folding and assembly of two different subunits can also occur to form a functional dimeric protein.

The expression vector we constructed is schematically drawn in Fig. 1. The genes, precisely fused to bacterial signal sequences (*19, 20*), are arranged in an artificial dicistronic operon. A homogeneous F_v fragment can be prepared from the periplasmic fraction of a cell lysate in a single step by affinity chromatography (legend to Fig. 2).

As can be deduced from the SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 2), the F_v fragment is completely pure. Both chains of the purified F_v fragment are present in a 1:1 molar ratio and have the sizes expected for the mature proteins (V_H, 13,600; V_L, 12,400). To confirm the correct cleavage of both signal sequences, the six amino-terminal amino acids of the two chains [V_H, NH_2-Glu-Val-Lys-Leu-Val-Glu; V_L, NH_2-Asp-Ile-Val-Met-Thr-Gln; (*15*)] were sequenced (*21, 22*). Both heterologous fusion proteins were properly cleaved by the bacterial signal peptidase, and there was no indication of either imprecise processing or any NH_2-terminal degradation.

We measured the affinity constant of the recombinant F_v fragment for phosphorylcholine by equilibrium dialysis (Fig. 3). The same conditions were used as in the determination of the affinity constant of native McPC603 isolated from mouse ascites (*23*). The value found for the F_v fragment ($1.21 \pm 0.06 \times 10^5 M^{-1}$) (Fig. 3) is identical (within experimental error) to that reported (*23*) for the native antibody ($1.6 \pm 0.4 \times 10^5 M^{-1}$). The Scatchard plot (Fig. 3) is linear and extrapolates to approximately 1 mole of hapten bound per mole of F_v fragment. This shows that there is one binding site per F_v fragment and that there is no inactive protein in the preparation.

We conclude that it is possible to express the F_v fragment of McPC603 as a fully functional and stable protein in *E. coli*. There was no previous indication of whether *E. coli* would be able to assemble a protein consisting of different subunits in the periplasm. *Escherichia coli* seems to assemble its own proteins by a different method. *Escherichia coli* penicillin-acylase, the best characterized protein that fits the definition of a soluble heterodimeric protein in the periplasm, is proteolytically processed from a single chain precursor in the periplasm (*24*). Our results indicate that folding and hetero-association of the variable domains is possible without known external help and is strongly favored in the periplasm of *E. coli*. Thus we could show that even when the assembly of two different chains is necessary for the formation of a functional protein, the transport to the periplasm of *E. coli* is functionally equivalent to the eukaryotic transport to the lumen of the ER. Further experimentation will be needed to clarify whether there is any role of homodimers (Bence-Jones proteins) (*25*) as assembly intermediates, or whether the correct heterodimer association is both kinetically and thermodynamically favored over homodimer formation. Most of the soluble V_L and V_H protein from the periplasmic fraction binds to the affinity column, indicating that it is correctly assembled to a heterodimer.

Our second result is that the F_v fragment of McPC603 has essentially the same affinity constant for phosphorylcholine as the intact antibody McPC603. This finding could not be expected a priori, since there is considerable debate about the functionality of F_v fragments (*26, 27*). The first accurate study of an F_v fragment focused on the dinitrophenol (DNP)-binding antibody MOPC-315. It revealed that the affinity constants for DNP were essentially identical for the F_v fragment and the Fab fragment (*26*). In a recent investigation of the human riboflavin-binding antibody Gar (*27*), a fragment consisting of V_H and the whole light chain was prepared. This fragment, which is comparable to an F_v fragment, has an affinity constant for riboflavin that is about three orders of magnitude lower than that determined for the native antibody. These results were contradictory, and it was not clear whether they are the consequence of true differences between antibodies (*28*) or are the result of experimental side effects (*27*).

We conclude that the F_v fragment of McPC603 is fully functional and can serve as a convenient model for studying antigen-antibody interactions, since the three-dimensional structure of the corresponding Fab fragment is known (*16*). We have devised an expression system not requiring any in vitro manipulations such as cleavage of fusion proteins, oxidation, or refolding. Furthermore, expression in a functional state permits the use of hapten binding for rapid and selective purification. The periplasmic location of the protein reduces both the potential protease degradation problem and the number of contaminating protein species to be separated. We believe that protein engineering of antibodies is greatly facilitated with this expression system.

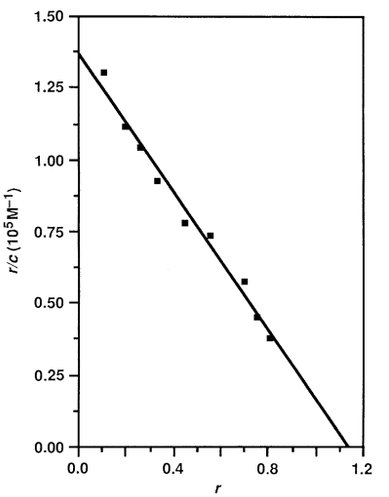

Fig. 3. Equilibrium dialysis data for the binding of phosphoryl[*methyl*-^{14}C]choline to the recombinant F_v fragment of McPC603. The equilibrium dialysis was carried out in a multicavity microdialysis chamber (Bel-Art Products) with a volume of about 100 μl on each side of the membrane. The chambers were filled with 50 μl of purified F_v fragment in BBS on one side and 50 μl of a solution of phosphoryl[*methyl*-^{14}C]choline (50 mCi/mmol, Amersham) in BBS on the other side. The concentration of the F_v fragment was determined to be 0.22 mg/ml from an A_{205} of 6.85 [using an extinction coefficient of $\epsilon_{1cm}^{1mg/ml} = 31$ for the average absorption of the peptide bond region in proteins at 205 nm (*34*)]. After equilibration for 22 hours at ambient temperature, samples (20 μl) from each solution were counted in 5 ml of Rotiszint 22 (Roth Biochemicals) using a Beckman LS 1801 scintillation counter. The data obtained are plotted according to Scatchard (*35*). An r denotes the fraction of antibody fragment with bound hapten, and c denotes the concentration of free hapten. The line was fitted by linear regression analysis. From the slope of this line an affinity constant of $K_a = 1.21(\pm 0.06) \times 10^5 M^{-1}$ is obtained.

REFERENCES AND NOTES

1. E. A. Kabat, *Adv. Protein Chem.* **32**, 1 (1978); L. M. Amzel and R. J. Poljak, *Annu. Rev. Biochem.* **48**, 961 (1979); D. R. Davies and H. Metzger, *Annu. Rev. Immunol.* **1**, 87 (1983).
2. L. Pauling, *Chem. Eng. News* **24**, 1375 (1946).
3. V. Raso and B. D. Stollar, *Biochemistry* **14**, 584 (1975); *ibid.*, p. 591; A. Tramontano, K. D. Janda, R. A. Lerner, *Science* **234**, 1566 (1986); S. J. Pollack, J. W. Jacobs, P. G. Schultz, *ibid.*, p. 1570; J. Jacobs, P. G. Schultz, R. Sugasawara, M. Powell, *J. Am. Chem. Soc.* **109**, 2174 (1987).
4. A. Plückthun, R. Glockshuber, I. Pfitzinger, A. Skerra, J. Stadlmüller, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
5. S. L. Morrison, *Science* **229**, 1202 (1985).
6. M. A. Boss and C. R. Wood, *Immunol. Today* **6**, 12 (1985).
7. C. R. Wood *et al.*, *Nature* **314**, 446 (1985).
8. S. Cabilly *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3273 (1984); M. A. Boss, J. H. Kenten, C. R. Wood, J. S. Emtage, *Nucleic Acids Res.* **12**, 3791 (1984).
9. S. L. Morrison and V. T. Oi, *Annu. Rev. Immunol.* **2**, 239 (1984).
10. S. Roberts and A. R. Rees, *Protein Eng.* **1**, 59 (1986).
11. M. S. Neuberger, G. T. Williams, R. O. Fox, *Nature* **312**, 604 (1984).
12. G. Möller, Ed., *Immunol. Rev.* **62** (1982).
13. M. S. Neuberger *et al.*, *Nature* **314**, 268 (1985).

14. P. T. Jones, P. H. Dear, J. Foote, M. S. Neuberger, G. Winter, *ibid.* **321**, 522 (1986).
15. M. Potter, *Adv. Immunol.* **25**, 141 (1977); R. M. Perlmutter *et al.*, *ibid.* **35**, 1 (1984); S. Rudikoff and M. Potter, *Biochemistry* **13**, 4033 (1974); S. Rudikoff, Y. Satow, E. Padlan, D. Davies, M. Potter, *Mol. Immunol.* **18**, 705 (1981).
16. D. M. Segal *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4298 (1974).
17. L. Hendershot, D. Bole, J. F. Kearney, *Immunol. Today* **8**, 111 (1987).
18. M. S. Briggs and L. M. Gierasch, *Adv. Protein Chem.* **38**, 109 (1986).
19. N. R. Movva, K. Nakamura, M. Inouye, *J. Biol. Chem.* **255**, 27 (1980).
20. H. Inouye, W. Barnes, J. Beckwith, *J. Bacteriol.* **149**, 434 (1982).
21. The two chains of the affinity-purified F_v protein were separated using SDS-PAGE and blotted onto an activated glass fiber sheet (*22*). The two bands corresponding to each of the two chains were each individually subjected to six cycles of gas-phase sequencing. In both sequenator profiles, the NH_2-terminal amino acids expected from precise cleavage of both signals were seen exclusively.
22. C. Eckerskorn, W. Mewes, H. Goretzki, F. Lottspeich, *Eur. J. Biochem.*, in press.
23. H. Metzger, B. Chesebro, N. M. Hadler, J. Lee, N. Otchin, in *Progress in Immunology: Proceedings of the 1st Congress of Immunology* (Academic Press, New York, 1971), pp. 253–267.
24. G. Schumacher, D. Sizmann, H. Haug, P. Buckel, A. Böck, *Nucleic Acids Res.* **14**, 5713 (1986).
25. J. R. Hobbs, *Essays Med. Biochem.* **1**, 105 (1975).
26. D. Inbar, J. Hochman, D. Givol. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2659 (1972).
27. J. Sen and S. Beychok, *Proteins* **1**, 256 (1986).
28. R. Huber and W. S. Bennett, *Nature* **326**, 334 (1987); M. Marquart and J. Deisenhofer, *Immunol. Today* **3**, 160 (1982).
29. C. Yanisch-Perron *et al.*, *Gene* **33**, 103 (1985).
30. A. Skerra and A. Plückthun, in preparation.
31. S. P. Fling and D. S. Gregerson, *Anal. Biochem.* **155**, 83 (1986).
32. We monitored the selectivity of the cell fractionation using β-lactamase as a periplasmic and β-galactosidase as a cytoplasmic marker enzyme. Under the conditions described, approximately 90% of total β-lactamase activity and less than 0.5% of total β-galactosidase activity were found in the periplasmic fraction.
33. B. Chesebro and H. Metzger, *Biochemistry* **11**, 766 (1972).
34. R. K. Scopes, *Protein Purification: Principles and Practice* (Springer, New York, 1982), pp. 241–242.
35. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
36. We thank C. Eckerskorn and F. Lottspeich for the NH_2-terminal protein sequencing and R. Glockshuber for the preparation of the phosphorylcholine affinity column. Supported by grant BCT0372 from the Bundesministerium für Forschung und Technologie to A.P., and by a predoctoral fellowship from the Stiftung Volkswagenwerk and the Fonds der Chemischen Industrie to A.S.

7 January 1988; accepted 18 March 1988

Escherichia coli Secretion of an Active Chimeric Antibody Fragment

MARC BETTER, C. PAUL CHANG, RANDY R. ROBINSON, ARNOLD H. HORWITZ

A chimeric mouse-human Fab protein that binds specifically to the human carcinoma cell line C3347 has been expressed and secreted from *Escherichia coli*. This molecule, which contains functionally assembled kappa and Fd proteins, binds as effectively to sites on the surface of C3347 cells as Fab fragments prepared proteolytically from whole chimeric or mouse antibody. The production in *Escherichia coli* of foreign heterodimeric protein reagents, such as Fab, should prove useful in the management of human disease.

THE BINDING-SELECTIVITY OF ANTIbody molecules makes them suited for applications as diverse as affinity chromatography, diagnostic reagents, and therapeutics in the detection and treatment of human diseases. Monoclonal antibodies are especially useful for these purposes because they can be prepared with homogeneous recognition specificities targeted at virtually an unlimited number of antigenic determinants. The protein domains that confer these antigen recognition determinants can be proteolytically separated from the remainder of the molecule and still retain their antigen-binding ability. This portion of an antibody (Fab) is roughly one-third the size of an intact immunoglobulin G (IgG) (about 48 kD) and exhibits monovalent antigen binding. The similar $F(ab')_2$ portion retains divalent antigen-binding capacity and contains both recognition domains linked by two interchain disulfide bridges. Antibodies differ, however, in their susceptibility to proteolytic cleavage, and preparations can be heterogeneous. The relatively simple structure of a Fab (5 disulfide bonds) compared to an intact antibody (16 disulfide bonds) and the therapeutic usefulness of Fab molecules make them attractive targets for production by microbial fermentation after appropriate protein engineering. Here we discuss the expression of a mouse-human chimeric Fab in *Escherichia coli*, that is, a molecule that contains the variable regions (antigen recognition domains) from a mouse monoclonal antibody and the C_H1 and C_κ constant regions from a human IgG1 antibody.

Each protein chain of a Fab has two intrachain disulfide bonds that stabilize functional domains, and a single cysteine involved in interchain disulfide linkage. *Escherichia coli* has been used to produce individual immunoglobulin chains internally that are not properly folded (*1, 2*), or individually secreted chains (*3*); however, for *E. coli* to assemble the truncated heavy chain (Fd) and κ into the correct heterodimeric molecule, both chains must be translated simultaneously and secreted. This operation would then mimic the cognate immunoglobulin assembly process.

The chimeric L6 antibody (*4*), directed toward a ganglioside antigen expressed on the cell surface of many human carcinomas (*5*), has been described. This antibody, prepared from the culture supernatant of an Sp2/0 transfectoma cell line, is expressed from the cDNA copies of the chimeric L6 IgG γ and κ chain genes. The cDNA clones for these two chimeric genes were used as the starting point for expression of a Fab molecule in mammalian cells and bacteria.

A termination codon was introduced into the chimeric heavy chain gene at amino acid 228 by site-directed mutagenesis (*6*) (Fig. 1a) in a manner that introduced a Bcl I restriction site. A similar step introduced a restriction site, Sst I, into the coding region at the processing cleavage site of the native heavy chain leader peptide and the mature heavy chain. Site-directed mutagenesis was

Table 1. Binding activity of bacterial Fab to human carcinoma cells. Target cells were incubated for 30 min at 4°C with each antibody or Fab, followed by incubation with fluorescein isothiocyanate (FITC)–labeled goat antibody against human κ for the bacterial Fab, FITC-labeled goat antibody against mouse IgG for the L6 mouse antibody, FITC-labeled goat antibody against mouse κ for L6 mouse Fab, or goat antibody against human IgG for the chimeric L6 antibody. We determined antibody binding to the cell surface by using a Coulter model EPIC-C cell sorter. FITC-labeled antibodies were obtained from TAGO.

Antibody	Binding ratio*	
	C3347 cells L6+	T51 cells L6−
Mouse L6	95	1
Sp2/0 chimeric L6	116	1
Bacterial L6 Fab	54	1
Mouse L6 Fab†	16	1

*The binding ratio is the number of times brighter a test sample is than a control sample treated with FITC-conjugated second antibody. Quantitative differences in binding to C3347 cells probably reflect the relative activity of individual FITC-conjugates. Data shown are from one of two similar binding assays. †Prepared by enzymatic digestion of mouse L6 antibody with papain.

International Genetic Engineering, Inc. (INGENE), 1545 17th Street, Santa Monica, CA 90404.