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tion of more complex carbonyl compounds for formaldehyde ( $\text{H}_2\text{CO}$ ) may lead to the direct formation of acyclic nucleoside analogs at the C-8 position of adenine. Glycoaldehyde, for example ( $\text{CHO}-\text{CH}_2\text{OH}$ ), may produce a dihydroxyl analog of potential interest in chemical evolution studies.

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- The HCN concentrations used in this and in other studies can similarly not be regarded as "prebiotic." Solutions of 0.1 to 1M are chosen to produce a measurable set of products within a reasonable time. That such concentrations would not have been possible in a prebiotic ocean can be appreciated from the fact that the total nitrogen now present at the surface of the Earth would produce no more than a 0.2M solution if totally converted to HCN and dissolved in the present oceans. High concentrations of HCN can, however, be produced by freezing very dilute solutions, and this process has been proposed to have operated on the prebiotic Earth (1).
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## Generation of a Catalytic Antibody by Site-Directed Mutagenesis

ENOCH BALDWIN AND PETER G. SCHULTZ\*

A hybrid Fv fragment of the dinitrophenyl-binding immunoglobulin A (IgA), MOPC315, has been generated by reconstituting a recombinant variable light chain ( $V_L$ ) produced in *Escherichia coli* with a variable heavy chain ( $V_H$ ) derived from the antibody. The Tyr<sup>34</sup> residue of  $V_L$  was substituted by His in order to introduce a catalytic imidazole into the combining site for the ester hydrolysis. The His mutant Fv accelerated the hydrolysis of the 7-hydroxycoumarin ester of 5-(2,4-dinitrophenyl)-aminopentanoic acid 90,000-fold compared to the reaction with 4-methyl imidazole at pH 6.8 and had an initial rate that was 45 times as great as that for the wild-type Fv. The hydrolyses of aminopropanoic and aminohexanoic homologs were not significantly accelerated. Thus a single deliberate amino acid change can introduce significant catalytic activity into an antibody-combining site, and chemical modification data can be used to locate potential sites for the introduction of catalytic residues.

TWO GENERAL APPROACHES HAVE emerged for the development of selective biological catalysts: genetic modification of enzyme active sites and chemical modification of biological or synthetic receptors with catalytic groups. These efforts have not only resulted in the generation of novel catalysts but have also provided a greater understanding of the mechanisms of enzymatic reactions. Recently, we and others have demonstrated that antibody-combining sites are also attractive starting points for the generation of selective catalysts, since they bind ligands with high

affinity and specificity (1). A number of strategies have been applied to the generation of antibodies that catalyze a variety of reactions with rate enhancements of  $10^2$  to  $10^6$  (2-5). Site-directed mutagenesis should prove useful for increasing the activity of these catalytic antibodies, or for the stepwise evolution of antibody-combining sites into efficient selective catalysts. We describe the substitution of a catalytic His residue for Tyr at position 34 of the light chain of the 2,4-dinitrophenyl (DNP)-binding antibody MOPC315 (6, 7). Tyr<sup>34L</sup> was previously shown to be in close proximity to the binding site by affinity labeling experiments (8). The His mutant binds DNP ligands one-half to one-eighth as tightly as the wild type, but catalytically hydrolyzes DNP-coumarin ester

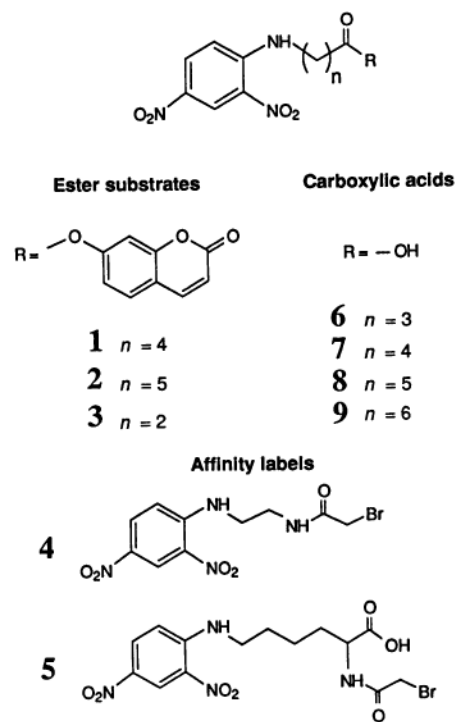


Fig. 1. Structures of ligands, substrates, and affinity labels.

145 times as rapidly, with a rate enhancement of  $\sim 90,000$  compared to 4-methylimidazole.

The MOPC315 antibody binds a variety of DNP ligands with association constants of  $10^3$  to  $10^7 M^{-1}$  (8, 9) and can be proteolyzed with pepsin to yield functional Fab' or Fv fragments (10). The Fv fragment (26 kD) is a heterodimer consisting of two peptides,  $V_H$  (14 kD) and  $V_L$  (12 kD), and contains all of the sequences necessary for folding of the binding domain and recognition of the DNP hapten. Although the atomic coordinates for MOPC315 have not been determined, magnetic resonance spectroscopy (11-13) and affinity labeling (8) studies have provided some information concerning binding site structure.

Because imidazole acts as a nucleophilic catalyst for the hydrolysis of carboxylate esters in aqueous solutions, introduction of a His at the appropriate position in the combining site of MOPC315 should result in a catalytic antibody with specific hydrolytic activity towards DNP-containing esters. Data from chemical modification experiments were used to target residues for substitution with His. Although 14 potentially reactive side chains occur in the hypervariable region (2 His, 2 Lys, 3 Arg, and 7 Tyr), DNP-containing affinity labels alkylate primarily two residues, Tyr<sup>34L</sup> and Lys<sup>52H</sup> (4, 8). The reactivity of each residue strongly depends on the number of atoms between the DNP ring and the electrophilic carbon of the affinity reagent; Tyr<sup>34L</sup> is alkylated

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most efficiently by **4**, whereas Lys<sup>52H</sup> reacts with **5** (Fig. 1). Pollack *et al.* have previously exploited this data by using cleavable affinity labels to introduce a thiol (**4**), and subsequently a catalytically active imidazole (**5**) into the MOPC315 combining site at position 52H. We chose Tyr<sup>34L</sup> for mutagenesis since it appeared that a His<sup>34L</sup> side chain would be well situated to catalyze the hydrolysis of esters **1** through **3** (Fig. 1) (**4**, **5**). The role of Tyr<sup>34L</sup> in DNP binding has not been clearly established: affinity labeling of the Tyr hydroxyl to form the 2-keto-5-thiopentyl ether prevents binding of ligands (**4**), perhaps by blocking the entrance to the site, while nitration has no effect (**13**). Tyr<sup>34L</sup> is conserved in murine  $\lambda$  light chains and is found frequently in  $\kappa$  chains and in heavy chains at the analogous position (position 33) (**22**). We also substituted Phe at position 34L to assess the contribution of the Tyr hydroxyl group to hapten binding.

In order to generate mutant MOPC315 combining sites, we chose to express the V<sub>L</sub> portion of the Fv domain of the antibody in *Escherichia coli*. Eukaryotic disulfide-containing proteins are often produced in *E. coli* in a reduced insoluble form and must be subsequently renatured to the native state (**14**). Reduced and separated V<sub>H</sub> and V<sub>L</sub> peptides of MOPC315 have been recombined to yield a stable Fv (**10**). The single disulfide

bond in V<sub>H</sub> or V<sub>L</sub> can be formed by oxidation of the reduced forms prior to reconstitution. The simplicity and efficiency of reconstitution makes the Fv a desirable target for expression of an antibody-combining site in *E. coli*. We exploited this property to produce a hybrid Fv, in which recombinant V<sub>L</sub> produced in *E. coli* is reconstituted with V<sub>H</sub> derived from MOPC315 IgA. Expression of functional Fv (**15**) and Fab (**16**) fragments in bacteria by secretion has also been reported.

A gene encoding the first 115 amino acids of MOPC315 light chain (**22**) was designed and chemically synthesized (Fig. 2A), and expressed as a fusion with the  $\lambda$  cII gene (**17**) (Fig. 2B). The NH<sub>2</sub>-terminal extension of the cII-V<sub>L</sub> hybrid was removed by site-specific cleavage with factor Xa (**18**). The liberated V<sub>L</sub> peptide was reconstituted with antibody-derived V<sub>H</sub>, and the resulting Fv was purified by gel filtration and affinity chromatography (Fig. 3). Antibody-derived V<sub>H</sub> was prepared by denaturing ion-exchange chromatography of authentic MOPC315 Fv, which was obtained from pepsin digestion of purified IgA, and subsequent affinity chromatography. Pepsin treatment afforded two major V<sub>H</sub> peptides and a small amount of incompletely digested V<sub>H</sub>-containing fragments, which copurified. Reconstitution experiments carried out in the

**Table 1.** Rate constants,  $K_{cat}$ , for hydrolysis of substrates **1** through **3** by Fv(Y34H<sub>L</sub>) according to the assay conditions described in Fig. 4B.

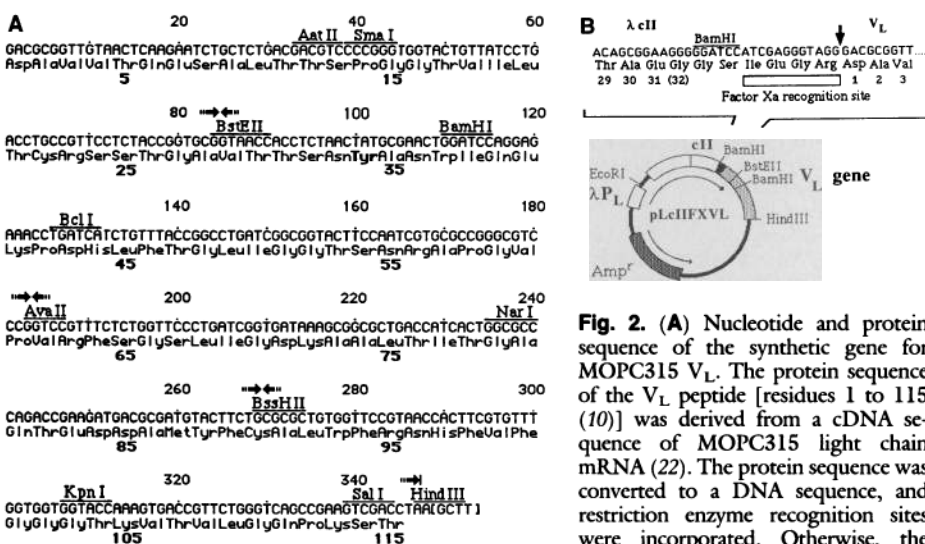
| Ester | Fv(Y34H <sub>L</sub> ) | Fv(315) | Fv(Y34F <sub>L</sub> ) |
|-------|------------------------|---------|------------------------|
| 1     | 0.18 ± 0.03*           | 0.004   | 0.004                  |
| 2     | 0.015                  | 0.005   |                        |
| 3     | <0.005                 |         |                        |

\*SE ( $n = 3$ ).

absence of V<sub>L</sub> peptide afforded no DNP-binding or ester hydrolysis activity.

The binding of  $\epsilon$ -2,4-DNP-L-lysine (DNP Lys) by mutant and wild-type Fv proteins was assessed by titration of the intrinsic fluorescence of MOPC315 Fv (**10**). Wild-type Fv [Fv(315)] and the Phe mutant [Fv(Y34F<sub>L</sub>)] bind DNP Lys with similar affinities at pH 6.8 (dissociation constant  $K_d = 0.27 \pm 0.03 \mu M$ ), whereas the His mutant [Fv(Y34H<sub>L</sub>)] binds this ligand one-sixth as tightly ( $K_d = 1.4 \pm 0.3 \mu M$ ) (Fig. 4A) (values are reported  $\pm$  SE). These data suggest that the Tyr hydroxyl group is not important for recognition of the DNP moiety. DNP-aminoalkyl carboxylic acids of different lengths (**6** through **9**, Fig. 1) (**9**) were used to probe the location of the positively charged imidazole ring with respect to the DNP site in Fv(Y34H<sub>L</sub>) at pH 6.0. The relative affinities of Fv(Y34H<sub>L</sub>) for these ligands (**7**>**8**>**9**>**6**) are different from that of the Fv(315) (**9**>**7**>**8**>**6**). In general, Fv(Y34H<sub>L</sub>) binds **6** through **9** one-half to one-eighth as tightly as Fv(315), consistent with its lower affinity for DNP Lys at pH 6.8. In contrast to Fv(315), Fv(Y34H<sub>L</sub>) binds **7** and **8** most tightly, suggesting a compensating electrostatic interaction between the negatively charged carboxylate of the ligand and the protonated imidazolium side chain.

The ability of wild-type and mutant Fvs to hydrolyze esters **1** through **3** was assayed by measuring the rate of coumarin release fluorimetrically at pH 6.8 (**4**, **5**). Fv(Y34H<sub>L</sub>) efficiently hydrolyzes **1** in a manner consistent with Michaelis-Menten kinetics (Michaelis constant  $K_m = 2.2 \pm 0.2 \mu M$ , rate constant  $k_{cat} = 0.18 \pm 0.03 \text{ min}^{-1}$ , and  $k_{cat}/K_m = 8.2 \times 10^4 M^{-1} \text{ min}^{-1}$ , pH 6.8) (Fig. 4B and Table 1). Under these conditions, Fv(Y34H<sub>L</sub>) turns over at least 11 times and still retains 44% of its activity. The loss of activity probably results from the accumulation of the inhibitory reaction product **8**. The Fv-catalyzed reaction was competitively inhibited with DNP Lys (inhibition constant  $K_i = 3.8 \pm 1.5 \mu M$ ) (**19**). Ester **2** was a poor substrate [ $k_{cat}(2) = 0.015 \text{ min}^{-1}$ ], and the hydrolysis of **3** was not accelerated [ $k_{cat}(3) < 0.005 \text{ min}^{-1}$ ]. Only the hydrolysis of **1** was signifi-



**Fig. 2.** (A) Nucleotide and protein sequence of the synthetic gene for MOPC315 V<sub>L</sub>. The protein sequence of the V<sub>L</sub> peptide [residues 1 to 115 (**10**)] was derived from a cDNA sequence of MOPC315 light chain mRNA (**22**). The protein sequence was converted to a DNA sequence, and restriction enzyme recognition sites were incorporated. Otherwise, the most frequently occurring *E. coli* codons

were used. A gene consisting of these sequences was constructed from four restriction fragments (Eco RI–Bst EII, Bst EII–Ava II, Ava II–Bss HII, and Bss HII–Hind III, denoted by the arrows above the sequence). Complementary synthetic oligonucleotides 78 to 99 bases in length were phosphorylated, annealed, and ligated into a derivative of M13mp18, either singly or as a pair. The cloned segments were sequenced and assembled into the full-length gene in several steps. Cassette and primer mutagenesis (**23**) were used to make subsequent modifications, which resulted in the deletion of the Eco RI site. (B) Expression vector pLcIIFXVL. The synthetic V<sub>L</sub> genes were expressed as a fusion with the cII gene of coliphage  $\lambda$  (**17**). A synthetic duplex encoding the factor Xa recognition and cleavage sequence, Ile-Glu-Gly-Arg, was inserted in-frame at the 5' end of the V<sub>L</sub> gene in M13. The  $\beta$ -globin gene in the expression vector pLcIIFX $\beta$ (nic<sup>-</sup>) (**17**) was excised by digestion with Bam HI and Hind III and replaced by the hybrid FXVL sequence. The Tyr<sup>34</sup> mutations [Tyr<sup>34</sup>(TAT) to His(CAT) and Phe(TTT)] were made in the V<sub>L</sub> gene in M13 by the method of Kunkel (**23**) and then transferred into pLcIIFXVL by substitution of the Bst EII–Hind III fragment.

cantly accelerated by Fv(Y34H<sub>L</sub>), which is consistent with the preference for ligands 7 and 8, and the affinity-labeling data. The initial rate of hydrolysis of 1 by Fv(Y34H<sub>L</sub>) at pH 6.8 is ~45-fold as fast as the reaction with either Fv(Y34F<sub>L</sub>) or Fv(315) (Table 1). At pH 8.7, the reactivity of Fv(Y34H<sub>L</sub>) and Fv(315) both increase, but differentially ( $v[\text{Fv}(\text{Y34H}_L)]/v[\text{Fv}(\text{315})] = 3.3$ , at 10  $\mu\text{M}$  1). Under these conditions, Fv(Y34H<sub>L</sub>) catalytically hydrolyzed 1, whereas Fv(315) reacted stoichiometrically. The increased reactivity of Fv(315) at higher pH may be due to deprotonation of the Tyr hydroxyl and subsequent transesterification to form a relatively stable phenol ester.

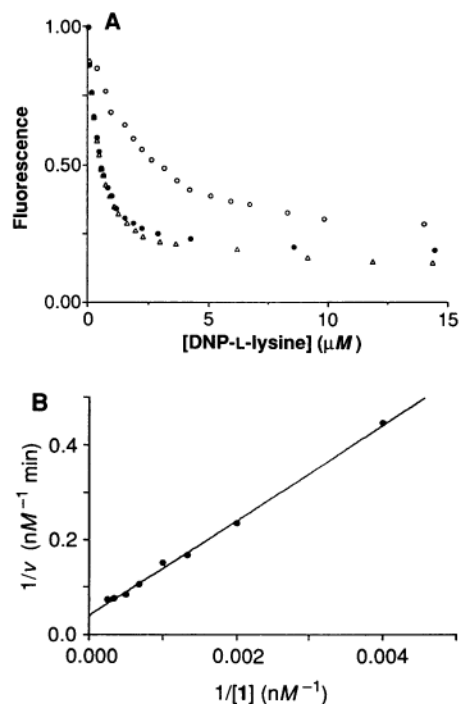
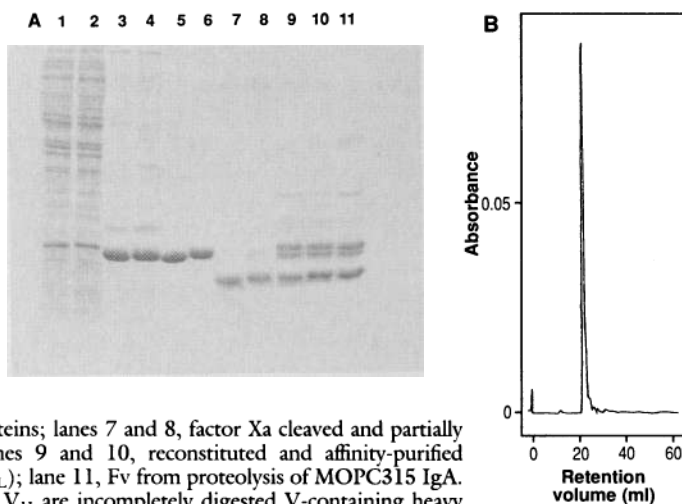
The imidazole-acylating agent diethylpyrrocarbonate rapidly and completely inhibits the hydrolysis of 1 at pH 6.0 (5, 20), supporting a catalytic role for His<sup>34</sup>. The imidazole side chain of His<sup>34</sup> could catalyze

the hydrolysis of ester by three mechanisms: (i) nucleophilic attack on the ester carbonyl to form a labile imidazolide intermediate, which is then rapidly hydrolyzed; (ii) activation of an attacking water molecule; and (iii) electrostatic stabilization of the negatively charged transition-state created by hydroxide ion attack on the ester carbonyl. The maximum velocity  $V_{\text{max}}$  of Fv(Y34H<sub>L</sub>) hydrolysis of 1 increases linearly by only a factor of 3 from pH 5.6 to 8.6. This result suggests that the rate-limiting step of hydrolysis does not involve simple hydroxide ion attack, since such a reaction should be first order in hydroxide ion concentration. Because Fv(Y34H<sub>L</sub>) is also more active at higher pH (where the neutral form of imidazole predominates), it seems unlikely that the observed rate acceleration is simply due to electrostatic stabilization. At present we have no evidence supporting either of the

other mechanisms, although imidazole derivatives hydrolyze active esters primarily by the nucleophilic pathway in aqueous solution (7).

In order to assess the contributions of the binding site to hydrolysis of 1, we compared the Fv(Y34H<sub>L</sub>) catalyzed reaction with the reaction with 4-methylimidazole under the assay conditions (5). The ratio of the bimolecular rate constants ( $k_{\text{cat}}/K_m$ )/ $k_{4\text{-MeIm}}$  is  $9 \pm 3 \times 10^4$  (21). This rate acceleration is similar to that of an antibody that contains an active site carboxylate that acts as a specific base in the catalysis of an elimination reaction (3). The rate of hydrolysis of 1 by Fv(Y34H<sub>L</sub>) can also be compared with that of MOPC315 Fab that has been chemi-

**Fig. 3.** Expression, purification, and cleavage of Y34H and Y34F cII-VL fusion proteins and reconstitution of functional Fv's. (A) SDS-polyacrylamide gel electrophoresis analysis of various stages in the production of mutant Fv proteins: lanes 1 and 2, induced *E. coli* MZ1 [pLcIIFXVL(Y34F) and (Y34H)]; lanes 3 and 4, solubilized inclusion bodies; lanes 5 and 6, purified fusion proteins; lanes 7 and 8, factor Xa cleaved and partially purified V<sub>L</sub> peptides; lanes 9 and 10, reconstituted and affinity-purified Fv(Y34F<sub>L</sub>) and Fv(Y34H<sub>L</sub>); lane 11, Fv from proteolysis of MOPC315 IgA. The bands above V<sub>L</sub> and V<sub>H</sub> are incompletely digested V-containing heavy chain fragments that copurify with V<sub>H</sub>. (B) Gel filtration analysis of Fv(Y34H<sub>L</sub>). Inclusion bodies were obtained from induced *E. coli* MZ1 harboring pLcIIFXVL(Y34H or Y34F) (17), solubilized in 8M urea, reduced with 20 mM dithiothreitol overnight at 4°C, and chromatographed on a 1 cm by 30 cm column of S-sepharose in 8M urea, 20 mM Na-MES, pH 6.0, with a 0 to 200 mM NaCl gradient (yield: 10 to 30 mg of purified protein per liter of induced cells [extinction coefficient at 280 nm  $\epsilon_{280}$  (1 mg/ml) = 1.0 (10)]. The purified fusion proteins were oxidized in air for 30 hours at 4°C in 2.5M urea and 20 mM tris-Cl, pH 8.4, and then exhaustively dialyzed against 20 mM tris-Cl, pH 8.4, at 4°C. Fusion protein (3 mg) in 15 ml of 20 mM tris-Cl, pH 8.4, 1 mM CaCl<sub>2</sub>, and kanamycin sulfate (30  $\mu\text{g}/\text{ml}$ ) was incubated for 30 hours, 37°C with 600  $\mu\text{g}$  of bovine factor X [prepared from the BaSO<sub>4</sub> eluate of bovine plasma (17, 18) (Sigma)] activated in situ with 2.5  $\mu\text{g}$  of crude Russell's viper venom (Sigma). Crude V<sub>L</sub> was made 100 mM in NaCl and passed through a 2-ml bed of Q-sepharose, dialyzed against distilled water, lyophilized, and dissolved in 8M urea and 20 mM potassium phosphate, pH 6.0. Fv(315) was purified from ascites fluid from MOPC315 myeloma grown in BALB/c mice as previously described (6, 10), except that the gel filtration step after pepsin cleavage was omitted. The V<sub>H</sub> peptide was separated from V<sub>L</sub> by ion-exchange chromatography (Pharmacia mono Q 10/10 column) in 20 mM tris-Cl, pH 8.0, and 8M urea, with a 0 to 250 mM NaCl gradient. Cleaved V<sub>L</sub> (0.1 to 1 mg) and equimolar V<sub>H</sub> were rapidly diluted tenfold from 8M urea into 100 mM potassium phosphate, pH 6.0 (final protein concentration = 50  $\mu\text{g}/\text{ml}$ ) and allowed to stand 30 min at 25°C. Active Fv was adsorbed onto DNP-lysine sepharose (10, 24), washed with 100 mM NaCl and 50 mM tris-Cl, pH 8.0 and eluted with a small volume of 30 mM DNP-glycine, pH 8.0. The eluate was dialyzed against 100 mM potassium phosphate, pH 6.8. Overall yield from the fusion protein is 5 to 20%. The yield of reconstitution is 20 to 30% based on V<sub>H</sub>. Residual V<sub>L</sub> dimer or DNP-glycine was removed by gel-filtration fast protein liquid chromatography (FPLC) on a Superose 12 column in 100 mM potassium phosphate, pH 6.8. Hybrid Fv reconstituted in this fashion has the same affinity for DNP-L-lysine as Fv purified from pepsin-treated MOPC315 IgA that was not reconstituted. Because of convenience, myeloma-produced Fv(315) was used in this study. Protein purity was assessed by electrophoresis through 14% polyacrylamide SDS gels (25) followed by Coomassie staining and by gel filtration FPLC analysis with a Superose 12 column (flow rate of 0.5 ml/min, sample size of 100  $\mu\text{g}$ ).



**Fig. 4.** (A) Fv fluorescence titration with DNP-L-lysine. Fv(315) (●), Fv(Y34F<sub>L</sub>) (Δ), or Fv(Y34H<sub>L</sub>) (○) (400  $\mu\text{l}$ , 450 to 500 nM,  $\epsilon_{280} = 37,500\text{M}^{-1}$ ) in 100 mM potassium phosphate, pH 6.8 (buffer A), was titrated with solutions of 10 to 100  $\mu\text{M}$  DNP-L-lysine ( $\epsilon_{360} = 17,500\text{M}^{-1}$ ) in buffer A while monitoring the protein fluorescence (excitation: 282 nm; emission: 343 nm). Fluorescence was corrected for the change in volume. Dissociation constants ( $K_d$ ) were determined by a modified Scatchard analysis (26). (B) Lineweaver-Burk plot of initial rate data obtained for the hydrolysis of 1. Four microliters of a 100-fold concentrated solution of substrate 1 in dimethyl sulfoxide was added to the 400  $\mu\text{l}$  of Fv (115 nM) in buffer A while monitoring 7-hydroxycoumarin fluorescence at 25°C (excitation: 355 nm; emission: 457 nm). A standard of 7-hydroxycoumarin was added at the completion of each assay to assess quenching of coumarin fluorescence by DNP at high substrate concentrations. The buffer background rate was subtracted from the initial rates. Each point represents the average velocity of at least duplicate experiments. Factor X, factor Xa, or Russell's viper venom had no hydrolytic activity towards 1.

cally derivatized with an imidazole at Lys<sup>52H</sup> by a flexible tether seven atoms in length (5). Under similar conditions, the imidazole-derivatized Fab hydrolyzes **1** approximately one-sixteenth as rapidly as Fv(Y34H<sub>L</sub>) ( $k_{\text{cat}} = 0.011 \text{ min}^{-1}$ , pH 7.0). The greater reactivity of Fv(Y34H<sub>L</sub>) is likely due to the fewer degrees of freedom of the His<sup>34</sup> side chain allowed by the protein backbone and surrounding side chains. In addition, the His imidazole may be better aligned for attack on the ester carbonyl of **1**, or for activation of a water molecule.

We have demonstrated the feasibility of using site-directed mutagenesis to introduce a catalytically active residue into an antibody combining site. In the absence of high-resolution structural information, chemical cross-linking data have proven useful in identifying sites for the introduction of catalytic groups. Additional site-directed or random mutagenesis combined with *in situ* screens or selections could be used to generate more efficient catalytic antibodies. Alternatively, Cys residues could be introduced for the subsequent attachment of synthetic catalysts to produce semisynthetic catalytic antibodies (5).

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21. The second-order rate constant for hydrolysis of **1** by 4-methylimidazole ( $k_{4\text{-MeIm}}$ ) was calculated from the pseudo-first-order rate constant, obtained by plotting the initial rate of coumarin release versus [4MeIm], in 400- $\mu\text{l}$  reactions containing 10 to 40 mM 4-methylimidazole, pH 6.8, and 10  $\mu\text{M}$  **1**. The value for  $k_{4\text{-MeIm}}$  was  $0.90 \pm 0.06 \text{ M}^{-1} \text{ min}^{-1}$ .
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## Lineage-Specific Requirement of *c-abl* Function in Normal Hematopoiesis

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**Structural abnormalities of the *c-abl* proto-oncogene are found in hematopoietic cells of more than 90 percent of individuals with chronic myelogenous leukemia. Therefore *c-abl* may be important in normal as well as malignant hematopoiesis. Normal human hematopoietic progenitor cells were exposed to three different *c-abl* sense or antisense oligodeoxynucleotides, and the effects on myeloid and erythroid colony formation were examined. The *c-abl* antisense oligodeoxynucleotides inhibited myeloid, but not erythroid, colony formation. The *c-abl* sense oligodeoxynucleotides and *bcr* sense and antisense oligodeoxynucleotides were not inhibitory in this assay. These data show that *c-abl* is critical in normal myelopoiesis and may explain the relatively selective expansion of leukocytes in patients with chronic myelogenous leukemia.**

**T**HE *c-abl* PROTO-ONCOGENE ENCODES a protein with tyrosine kinase activity. Although the functional significance of the protein is unknown (1, 2), it is known that more than 90% of chronic myelogenous leukemia (CML) patients have *c-abl* structural alterations in their leukocyte DNA (2). These structural alterations are caused by the translocation of *c-abl* from chromosome 9 to the breakpoint cluster region (*bcr*) on chromosome 22, resulting in the formation of *bcr-abl* hybrid genes. The

most characteristic clinical feature of the chronic phase of CML is an increase in mature and immature myeloid elements in bone marrow and peripheral blood (3). Kinetic studies indicate that these abnormal cells do not proliferate or mature faster than the normal counterpart (4). Rather, the basic defect underlying the exuberant granulopoiesis in CML appears to be an expansion of the myeloid progenitor cell pool in bone marrow and peripheral blood (5). Although hematopoiesis in the chronic phase of CML is altered, it retains some normal features. These observations raise the possibility that *c-abl* function is required during normal human hematopoiesis.

Inhibition of specific gene functions by exposing target cells to synthetic antisense oligomers (6) was used to investigate the role of *c-myb* in normal human hematopoiesis (7, 8). We have now used the same technique to show that exposure of early and late hematopoietic progenitors to *c-abl* antisense oligomers inhibits *in vitro* myelopoiesis and spares erythropoiesis. This suggests a lineage-specific requirement of *c-abl* function in normal hematopoiesis.

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