ecules (124.9° in A and 123.9° in B) despite the different dispositions of the methylbutenyl group. The conformation of this type of diazepine ring (fused to a benzo ring) that is seen in molecule B occurred more frequently (in 111 out of 122 molecular fragments) according to our search of the current Cambridge Data Base.

The differences in the conformation have some effects on the geometry associated with the diazepine ring and the methylbutenyl side chain. The most prominent features are the bond angles at the N3 and C13 atoms. The endocyclic angles of C4-N3-C12 and C7-C13-C12 in A (127.6 (5)° and 123.9 (6)°) are significantly greater than those of B (124.1 (5)° and 119.5 (6)°). In addition, some bond lengths associated with the C5 and N6 atoms and the methylbutenyl group for A/B molecules have variations greater than 5 esd's. The differences associated with the methylbutenyl group are likely due to their large temperature factors described earlier. Other differences may be related to the conformation around nitrogen N6. Both N6 atoms have sp^3 character as reflected in the distance of N6 from the plane of C5, C7, and C15 (0.360 (6) Å for A and 0.368 (7) Å for B). Notice that N6 retains the same chirality (*S*) in both conformers and its lone-pair electrons point away from the center in molecule A, but toward the center in molecule B.

The observation of two distinct conformations in the crystal suggests that at least two conformers are in equilibrium in solution. A series of one-dimensional NMR spectra at different temperatures showed that two molecular species coexist in equilibrium on the NMR time scale up to 20 °C.[15] At -40 °C, these two species are clearly resolved into two populations (90%:10%). Interconversion of the two conformers involves flipping of the methylbutenyl side chain from one side of the aromatic ring plane to the other side, with modest energy barriers. The difference in total energies associated with these two conformers is 1.3 kcal/mol on the basis of the calculation using MOPAC[17] with PM3 force field parameters.[18] Conformation A has lower energy, and this suggests that it is the major species in solution. These two conformers may be considered as atropisomers, reminiscent of an ansamycin antibiotic, streptovaricin C,[19] also an inhibitor for RTase.

The mode of action of R82913 appears to be quite different from that of the dideoxynucleosides.[2,20] Whether the conformations seen in the present crystal structure or the flexibility associated with the diazepine ring is relevant to its biological function as an inhibitor for the RTase of HIV-1 remains to be conclusively answered by solving the structure of the complex between RTase and the inhibitor. However, it is interesting to note that the molecule contains a conjugated ring system not unlike a purine ring. A superposition of molecule A with deoxyadenosine 5′-monophosphate[21] (with adenine in the syn conformation) reveals the overall similarity between them (Figure 3). It is conceivable that the nucleotide triphosphate binding site in RTase has a cavity that can accommodate this inhibitor and bind it extremely tightly. It has been previously noted that nucleoside analogues containing an ethylene moiety such as a cyclopentene ring are excellent inhibitors for enzymes in the nucleoside synthesis pathway[22] and for HIV viruses.[2]

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Supplementary Material Available: A table of the fractional atomic coordinates of the two independent molecules of R82913 of the LT form and a figure showing the packing interactions (3 pages). Ordering information is given on any current masthead page.

(15) NMR spectra were recorded on a GE GN-500 500-MHz spectrometer. The sample was 20 mM R82913 in deuterated methanol. The temperature range was from -60 °C to 40 °C. The smaller population (10%) starts to become evident at 0 °C for some resonances. The population ratio remains unchanged throughout the temperature range. However, the crystallization process selects out the 1:1 ratio of the two conformers to form the crystal lattice, thereby shifting the equilibrium in solution. This phenomenon is quite common in the crystallization of many biological macromolecules. For example, the minor species Z-DNA, instead of the abundant B-DNA, crystallized from the low-salt solution of d(CGCGCG) readily.[16]

(16) Wang, A. H.-J.; Quigley, G. J.; Kolpak, F. J.; Crawford, J. L.; van Boom, J. H.; van der Marel, G. A.; Rich, A. *Nature* (*London*) **1979**, *282*, 680-686.

(17) Stewart, J. J. P. 1987, MOPAC Version 5.0, QCPE No. 455.

(18) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209-215.

(19) Rinehart, K. L., Jr.; Knoll, W. M. J.; Kakinuma, K.; Antosz, F. J.; Paul, I. C.; Wang, A. H.-J.; Reusser, F.; Li, L. H.; Kreuger, W. C. *J. Am. Chem. Soc.* **1975**, *97*, 196-197.

(20) Another non-nucleoside compound with significant anti-HIV activity has recently been synthesized: Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C.-K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. *Science* **1990**, *250*, 1411-1413.

(21) Reddy, B. S.; Viswamitra, M. A. *Acta Crystallogr.* **1975**, *B31*, 19-26.

(22) Moyer, J. D.; Malinowski, N. M.; Treanor, S. P.; Marquez, V. E. *Cancer Res.* **1986**, *46*, 3325-3329.

Iron Chelate Mediated Proteolysis: Protein Structure Dependence

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Cleavage of DNA or RNA by metal chelates is an important new approach to characterizing important structural features of nucleic acids and their complexes in solution.[1] Naturally occurring proteases and selective peptide cleaving agents are useful for dissecting protein structure and function, but more choices are desirable.[2] Artificial proteolytic reagents which are directed by *proximity* rather than by residue type represent a new approach which could be employed for sequence analysis, functional analysis of structural domains, or determining the spatial arrangement of subunits within a supramolecular structure. They could also be incorporated into new, targeted therapeutic agents.

Recently, there has been considerable interest in the cleavage of proteins by metal ions or chelates bound at particular sites.[3,4] In our laboratories, site-specific cleavage of proteins is achieved by introducing a metal-binding site at one position in a polypeptide chain. Recently, we found that an iron chelate attached to cysteine-34 of the protein bovine serum albumin hydrolyzed the protein backbone at two sites—between Ala-150 and Pro-151, and between Ser-190 and Ser-191—without excessive decomposition of amino acid side chains.[4a]

This approach could permit mapping a site of interest by determining which individual peptide bonds are adjacent to it. However, because no crystal structure is available for bovine serum albumin, no detailed information concerning the steric require-

(1) (a) Moser, H. A.; Dervan, P. B. *Science* (*Washington, D.C.*) **1987**, *238*, 645-650. (b) Chen, C. B.; Sigman, D. S. *Science* (*Washington, D.C.*) **1987**, *237*, 1197-1201. (c) Tullius, T. D.; Dombroski, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5469-5473. (d) Cech, T. R.; Latham, J. A. *Science* (*Washington, D.C.*) **1989**, *245*, 276-282. (e) Mei, H. Y.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1339-1343.

(2) (a) Stark, G. *Adv. Protein Chem.* **1970**, *24*, 261. (b) Spande, T. F.; Witcop, B.; Degani, Y.; Patchornik, A. *Adv. Protein Chem.* **1970**, *24*, 98.

(3) (a) Rush, J. D.; Koppenol, W. H. *J. Biol. Chem.* **1986**, *261*, 6730-6733; *J. Am. Chem. Soc.* **1988**, *110*, 4957-4963. (b) Brodie, B. B.; Axelrod, J.; Shore, P. A.; Udenfriend, S. *J. Biol. Chem.* **1954**, *208*, 741-750. (c) Kim, K.; Rhee, S. G.; Stadtman, E. R. *J. Biol. Chem.* **1985**, *260*, 15394-15397. (d) Tabor, S.; Richardson, C. C. *J. Biol. Chem.* **1987**, *262*, 15330-15333. (e) Chiou, S.-H. *J. Biochem.* **1983**, *94*, 1259-1267.

(4) (a) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1990**, *112*, 2457-2458. (b) Schepartz, A.; Cuenoud, B. *J. Am. Chem. Soc.* **1990**, *112*, 3247-3249. (c) Hoyer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249-3250.

0002-7863/91/1513-1859$02.50/0

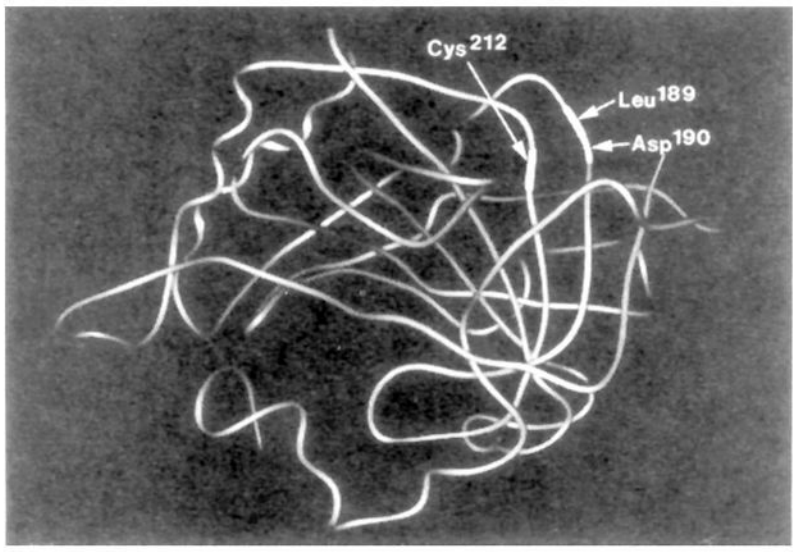

Figure 1. Human carbonic anhydrase I showing Cys-212, Leu-189, and Asp-190 residues in the polypeptide backbone. Cys-212 was modified with Fe–BABE, and the peptide bond between Leu-189 and Asp-190 was hydrolyzed. The sulfur of Cys-212 is 5.3 Å from the carbonyl carbon of Leu-189.

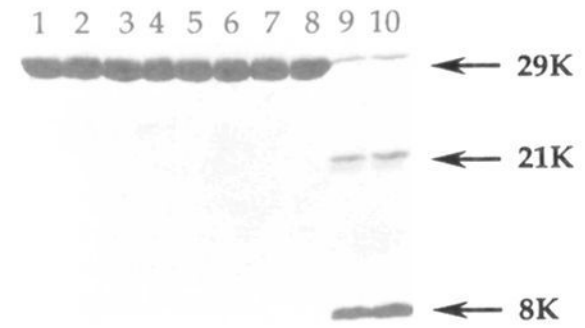

Figure 2. Separation of cleavage products by $NaDodSO_4$–polyacrylamide gel electrophoresis: unmodified HCAI (lane 1); HCAI with 10 mM ascorbate, 100 μM H_2O_2, and 1 mM $FeSO_4$ (lane 2) or with 10 mM ascorbate, 100 μM H_2O_2, and 1 mM 1-(*p*-nitrobenzyl)-EDTA–Fe (lane 3). Chelate-conjugated HCAI in the absence of ascorbate/H_2O_2 (lane 4); with 100 μM H_2O_2 but in the absence of ascorbate (lane 5); with 10 mM ascorbate but in the absence of H_2O_2 (lane 6); with 0.5% $NaDodSO_4$, 10 mM ascorbate, and 100 μM H_2O_2 (lane 7); with 5 M guanidine hydrochloride, 10 mM ascorbate, and 100 μM H_2O_2 (lane 8). Cleavage of chelate-conjugated HCAI with 10 mM ascorbate and 100 μM H_2O_2 at 25 °C for 10 s (lane 9) or with 10 mM ascorbate and 1 atm of O_2 at 37 °C for 30 h (lane 10).

ments or selectivity of the cleavage reaction was available. To address this question, we attached the iron chelate to cysteine-212 of human carbonic anhydrase I (HCAI), whose structure has been determined at high resolution[5] (Figure 1).

Alkylation of cysteine-212 of the enzyme with 1-[*p*-(bromoacetamido)benzyl]-EDTA–Fe (Fe–BABE)[6] was carried out according to the methods of Carlsson et al.[7,8] with minor modifications. The loss of SH was monitored with Ellmans's reagent,[9] and it matched the gain in chelating groups, measured by ^{59}Fe labeling. The modified enzyme was reactivated by dilution,[8] and recovered enzymatic activity was determined to be 97% by measuring CO_2 hydration activity.[10] The cleavage of the enzyme was performed by mixing modified carbonic anhydrase I to a final concentration of 100 μM at 25 °C with 50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonate (HEPES) buffer, pH 7.0, containing 10 mM ascorbate and 100 μM hydrogen peroxide for 10 s. The reaction was stopped by adding 35 μL of $NaDodSO_4$ sample application buffer. Denatured samples were analyzed on 7.5–20% gradient $NaDodSO_4$–polyacrylamide slab gel electrophoresis.[11]

The Cys-212 iron chelate cleaved HCAI at one site, producing two discrete cleavage fragments. These products migrate at positions corresponding to MW $\approx$ 21K and 8K (Figure 2). Control experiments demonstrated the specificity of the observed cleavage and its dependence on protein tertiary structure. In contrast to cleavage by the Cys-212 conjugated chelate, free Fe^{2+} in the reaction medium did not afford any cleavage (lane 2), nor did unattached chelate [1 mM 1-(*p*-nitrobenzyl)-EDTA–Fe, 10 mM ascorbate, 100 μM H_2O_2, lane 3] or chelate-conjugated unfolded protein (0.5% $NaDodSO_4$ or 5 M guanidinium chloride, lanes 7 and 8). The cleavage by attached Fe–BABE was dependent on the presence of ascorbate and H_2O_2 (10 s at 25 °C, lane 9). In the presence of oxygen and ascorbate at 25 °C for 10 s, no cleavage of the protein–chelate conjugate was observed (lane 6); however, incubation at 37 °C for 30 h gave the same cleavage products observed with H_2O_2 (lane 10).

The cleaved peptides were identified by their N- and C-terminal sequences.[12] The native enzyme has a blocked N-terminus. The N-terminus of the cleaved 21K peptide is also blocked. The C-terminal sequence of the 21K peptide is -Leu-Pro-Ser-Ser-Leu, which is uniquely located at residues 185–189 of HCAI. Thus, scission occurred after residue Leu-189, producing a 189 amino acid fragment having M_r 20 726 and containing the N-terminus of HCAI.

The N-terminal sequence of the 8K peptide, Asp-Phe-Trp-Thr-, is uniquely located at residues 190–193 of HCAI. The C-terminus of the 8K fragment, -Val-Arg-Ala-Ser-Phe, is identical with the C-terminal sequence of native HCAI. Thus, cleavage occurred between residues Leu-189 and Asp-190, giving a 71 amino acid fragment having M_r 8035 and containing the C-terminus of HCAI.

The cleavage was very efficient; with a concentration of H_2O_2 equal to the protein concentration, all of the HCAI molecules bearing chelates were cleaved in 10 s. As with bovine serum albumin, the amino acids at the site of cleavage were detected unaltered; the reaction has the same result as cleavage by a proteolytic enzyme.[4a] However, for the 8K fragment the yield of the N-terminal amino acid in the first cycle of Edman degradation was consistent with yields of the following residues, implying that side reactions were insignificant.

How does this result compare to expectations from the crystal structure[5] of HCAI? Cys-212 lies between two polypeptide loops, near residues Gly-145 and Leu-189. The sulfur of Cys-212 is 5.9 Å from the carbonyl carbon of Ser-188, 5.3 Å from the carbonyl carbon of Leu-189, and 5.7 Å from the carbonyl carbon of Asp-190; thus the 189–190 peptide bond is the closest on that loop to Cys-212. However, for the other loop the Cys-212 sulfur is 6.1 Å from the carbonyl carbon of Ile-144, 5.1 Å from the carbonyl carbon of Gly-145, and 6.5 Å from the carbonyl carbon of Val-146.

Although no crystal structure is available for chelate-conjugated HCAI, Cys-212 of HCAI has been modified previously with paramagnetic and fluorescent probes by the same methods.[7] The modified and reactivated enzymes have similar kinetic properties, inhibitor-binding constants, circular dichroism spectra, and stabilities toward guanidine hydrochloride as the native enzyme.[7]

What is the mechanism of the cleavage reaction? In studies of cytochrome P-450, several authors have proposed nucleophilic attack of iron-coordinated peroxo groups at carboxyl carbon.[13]

(5) (a) Kannan, K. K.; Ramanadham, M.; Jones, T. A. *Ann. N. Y. Acad. Sci.* **1984**, *429*, 49–60. (b) Kannan, K. K.; Notstrand, B.; Fridborg, K.; Ohlsson, A.; Petef, M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 51–55.

(6) DeRiemer, L. H.; Meares, C. F.; Goodwin, D. A.; Diamanti, C. I. *J. Labelled Compd. Radiopharm.* **1981**, *18*, 1517–1534.

(7) Carlsson, U.; Aasa, R.; Henderson, L. E.; Jonsson, B.-H.; Lindskog, S. *Eur. J. Biochem.* **1975**, *52*, 25–36.

(8) Carlsson, U.; Henderson, L. E.; Lindskog, S. *Biochem. Biophys. Acta* **1973**, *310*, 376–387.

(9) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.

(10) Rickli, E. E.; Ghazanfar, S. A. S.; Gibbons, B. H.; Edsall, J. T. *J. Biol. Chem.* **1964**, *239*, 1065–1078.

(11) (a) Laemmli, U. K. *Nature* **1970**, *227*, 680–685. (b) Morrissey, J. H. *Anal. Biochem.* **1981**, *117*, 307–310.

(12) (a) The cleaved fragments were electroblotted on PVDF membranes for analyses (Matsudaira, P. *J. Biol. Chem.* **1987**, *261*, 10035–10038). (b) N-Terminal sequence analyses of the cleaved fragments were performed by automated Edman degradation using an Applied Biosystems 470A gas-phase sequencer. Carboxyl termini of the fragments were identified by carboxypeptidase Y digestion (Kitabatake, N.; Indo, K.; Doi, E. *J. Agric. Food Chem.* **1988**, *36*, 417–420).

(13) (a) Hamilton, G. A. In *Molecular Mechanism of Oxygen Activation*; Hayaishi, O., Ed.; Academic Press: New York, 1974; pp 438–440. (b) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315–356. (c) Sligar, S. J.; Kennedy, K. A.; Pearson, D. C. In *Oxidases and Related Redox Systems*; King, T. E., Mason, H. S., Morrison, M., Eds.; Pergamon Press: New York, 1982; pp 837–856.

Transfer of labeled oxygen from the oxidant to the carboxyl group of the product has been demonstrated by Sligar et al.[13c] Using $^{18}O_2$, we have found that ^{18}O is transferred to the carboxyl group of Leu-189 with ≈90% efficiency. In the crystal structure of HCAI, the 189-190 peptide bond is oriented parallel to Cys-212, while the 145-146 bond is directed away from it. The direct attack of coordinated oxygen on carboxyl carbon could be very sensitive to orientation, providing a plausible explanation for the observed selective cleavage. This suggests a cautionary note similar to what is observed in protein cross-linking: observation of the effect is good evidence for proximity, but lack of an effect may be due to unfavorable orientation rather than lack of proximity.

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Registry No. HCAI, 9001-03-0; Cys, 52-90-4; H_2O_2, 7722-84-1; ascorbate, 50-81-7.

Redirecting the Immune Response: Ligand-Mediated Immunogenicity

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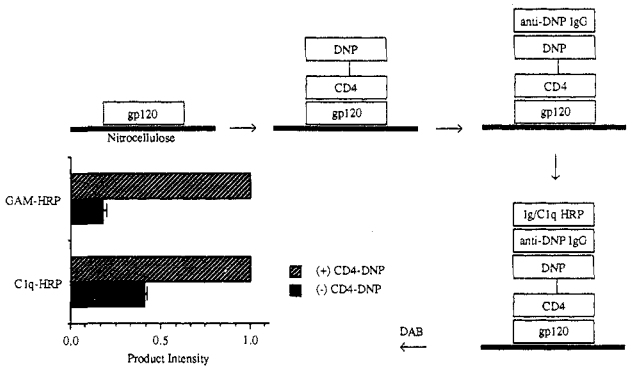
The immune system is remarkable in its ability to protect vertebrates from microorganisms. However, viruses and other pathogens have evolved a number of strategies for successfully evading neutralization by the immune system. For example, viral coat proteins can have high mutation rates,[1] conserved canyon-like motifs inaccessible to antibody combining sites,[2] self-like glycosylation patterns,[3] and only transient exposure of highly antigenic conserved sites involved in viral uncoating.[4] We now describe a new approach toward immunotherapy that may allow one to redirect the immune system's powerful memory response against substances not normally recognized as foreign. Immunological recognition of the target is achieved via a two-step process. Highly immunogenic antigens are tethered to ligands that bind selectively to conserved sites on a viral or cellular surface. Noncovalent binding interactions result in the introduction of this new antigen onto the target's surface, thereby tagging that microorganism or cell for recognition and neutralization by the immune system (Figure 1). This strategy suggests that it may be possible to direct a neutralizing antibody (ideally antibodies already present in sera[5-7]) against any cellular or viral surface for which a selective ligand is known. Such ligands might come from screens of recombinant libraries, collections, or fermentation broths.

This approach has been demonstrated in vitro by using the nonpeptidyl and peptidyl ligands, biotin and CD4, respectively, and the antigen, dinitrobenzene (DNP), which is recognized by approximately 1-2% of naturally occurring antibodies.[5] The CD4-DNP conjugate directs a monoclonal anti-DNP antibody to gp120, the envelope protein of the human immunodeficiency virus (HIV), via its functionally conserved CD4 binding domain.[8] The binding of soluble CD4 (sCD4) to gp120 has been extensively characterized (K_D = 10^{-9} M) and has been exploited in other anti-AIDS therapeutic strategies.[9-13] In the second example biotin directs the same anti-DNP antibody to the tetrameric protein streptavidin (K_D = 10^{-15} M). The antigen, DNP, elicits antibodies with 10^3-10^4-fold higher K_A's (2×10^8 M^{-1}) than do antigens of similar size.[14] In addition, natural anti-DNP antibodies account for 1% of all antibodies of the IgM subclass, and 0.8% of the IgG subclass with K_A's ≈ 10^4-10^6 M^{-1}.[15,16] In both examples the anti-DNP antibody is recognized by the first component of the complement cascade, C1q,[17] demonstrating the viability of ligand-mediated immunogenicity in generating active immunity against the target of interest, such as HIV or HIV-infected cells. In both cases the antigenic determinant only becomes multimeric and hence able to activate complement when presented in the context of the naturally occurring target protein.

Scheme I



Dinitrobenzene was derivatized with a water-soluble tetraethylene glycol spacer (16 Å) to ensure that the DNP group would be accessible for antibody binding. Monofunctionalization of tetraethylene glycol diamine with 2,4-dinitrofluorobenzene, followed by formation of the isothiocyanate with thiophosgene, afforded the acylating reagent which was used to introduce the DNP group onto the ε-amino groups on the surface of sCD4. Limited derivatization of the ε-amino groups of surface lysines on sCD4 resulted in a DNP conjugate that retained gp120 binding activity. The sCD4-DNP conjugate comigrated with unmodified sCD4 when analyzed by polyacrylamide gel electrophoresis with silver staining. The ultraviolet-visible spectrum of sCD4-DNP was consistent with 1 mol of DNP/mol of sCD4.

To test the ability of sCD4-DNP to target anti-DNP antibody to the HIV envelope protein gp120, an enzyme-linked immunosorbant assay (ELISA) experiment was performed (Scheme I). Recombinant gp120 was blotted onto nitrocellulose, and the remaining protein binding sites were blocked with bovine serum albumin (BSA). CD4-DNP was then added to the dot blot chamber and incubated for 30 min, and the excess CD4-DNP was removed by washing with PBS. Anti-DNP antibody AN09[18] was then added, followed by the same washing procedure. A second antibody, goat anti-mouse-Ig antibody-horseradish peroxidase (GAM-Ig-HRP), was used to detect the amount of anti-DNP antibody that bound gp120. Formation of the antibody complex was assayed by the addition of 3,3'-diaminobenzidine

* Author to whom correspondence should be addressed.

(1) Preston, B. D.; Poiesz, B. J.; Loeb, L. A. *Science* **1988**, *242*, 1168-1171.
(2) Rossman, M. G. *J. Biol. Chem.* **1989**, *264*, 14587-14590.
(3) Geyer, H.; Holschbach, C.; Hunsmann, G.; Schneider, J. *J. Biol. Chem.* **1988**, *263*, 11760-11767.
(4) Yarchoan, R.; Mitsuya, H.; Broder, S. *Sci. Am.* **1988**, 110-119.
(5) Farah, F. S. *Immunology* **1973**, *25*, 217-226.
(6) Galili, U.; Mandrell, R. E.; Hamadeh, R. M.; Shohet, R. B.; Griffiss, J. M. *Infect. Immun.* **1988**, *56*, 1730-1737.
(7) Rodman, T. C.; Pruslin, F. H.; Chauhan, Y.; To, S. E.; Winston, R. *J. Exp. Med.* **1988**, *167*, 1228-1246.
(8) Weiss, R.; Clapham, P. R.; Wever, J. N.; Galgleish, A. G.; Lasky, L. A.; Berman, P. W. *Nature (London)* **1986**, *324*, 572-575.
(9) Brodsky, M. H.; Warton, M.; Myers, R. M.; Littman, D. R. *J. Immunol.* **1990**, *144*, 3078-3086.
(10) Till, M. A.; Ghetie, V.; Gregory, T.; Patzer, E. J.; Porter, J. P.; Uhr, J. W.; Capon, D. J.; Vitetta, E. S. *Science* **1988**, *242*, 1166-1168.
(11) Chaudhary, V. K.; Mizukami, T.; Fuerst, T. R.; FitzGerald, D. J.; Moss, B.; Pastan, I. *Nature (London)* **1989**, *335*, 369-372.
(12) Capon, D. J.; Chamow, S. M.; Mordenti, J.; Marsters, S. A.; Gregory, T.; Mitsuya, H.; Byrn, R. A.; Lucas, C.; Wurm, F. M.; Groopman, J. E.; Broder, S.; Smith, D. H. *Nature (London)* **1989**, *337*, 525-531.
(13) Traunecker, A.; Schneider, J.; Kiefer, H.; Karjalainen, K. *Nature (London)* **1989**, *339*, 68-70.
(14) Karush, F. *Adv. Immunol.* **1962**, *2*, 1-40.
(15) Karjalainen, K.; Makela, O. *Eur. J. Immunol.* **1976**, *6*, 88-93.
(16) Ortega, E.; Kostovetzky, M.; Larralde, C. *Mol. Immunol.* **1984**, *6*, 88-93.
(17) Muller-Eberhard, H. J. *Annu. Rev. Biochem.* **1988**, *57*, 321-347.
(18) Leahy, D. J.; Rule, G. S.; Whittaker, M. M.; McConnell, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3661-3665.

0002-7863/91/1513-1861$02.50/0