more, this serodeme of *T. vivax* is said to lack minichromosomes, which carry telomeric VSG genes in *T. brucei* (*21*), and may therefore have alternative or simpler mechanisms for switching VSGs. The small size and hydrophobicity of the ILDat 1.2 VSG and the sensitivity of the membrane of this organism to aerolysin (*3*) suggest that studies on the packing of VSGs of *T. vivax*, their association with other cell surface molecules, and their mechanisms of release will be important in the elucidation of the evolution and function of variant antigens in trypanosomes.

REFERENCES AND NOTES

1. A. R. Gray and A. G. Luckins, in *Biology of the Kinetoplastida*, W. H. R. Lumsden and D. A. Evans, Eds. (Academic Press, London, 1976), vol. 1, pp. 493–542.
2. K. Vickerman and T. M. Preston, *ibid.*, pp. 35–130.
3. T. W. Pearson, L. E. Saya, S. P. Howard, J. T. Buckley, *Acta Trop.* **39**, 73 (1982). In contrast, trypanosomes of the West African stock of *T. vivax* and red blood cells were lysed in 5 to 10 minutes when infected whole blood was mixed with an equal quantity of aerolysin (10 μg/ml) in phosphate-buffered saline.
4. J. D. Barry and H. Gathuo, *Parasitology* **89**, 49 (1984); P. R. Gardiner, R. Thatthi, H. Gathuo, R. Nelson, S. K. Moloo, *ibid.* **92**, 581 (1986).
5. P. Leeflang, J. Buys, C. Blotkamp, *Int. J. Parasitol.* **6**, 413 (1976).
6. G. A. M. Cross, *Parasitology* **71**, 393 (1975).
7. N. G. Anderson and N. L. Anderson, *Anal. Biochem.* **85**, 331 (1978); N. L. Anderson and N. G. Anderson, *ibid.*, p. 341.
8. W. L. Hurley, E. Finkelstein, B. D. Holst, *J. Immunol. Methods* **85**, 195 (1985).
9. A. F. Barbet *et al.*, *Parasitology* **83**, 623 (1981); S. Z. Shapiro, *Exp. Parasitol.* **61**, 432 (1986).
10. A. F. Barbet and T. C. McGuire, *Parasitology* **85**, 511 (1983); D. J. Bowles and H. P. Voorheis, *FEBS Lett.* **139**, 17 (1982); P. Rautenberg, E. Reinwald, H-J. Risse, *Mol. Biochem. Parasitol.* **4**, 129 (1981).
11. Antipain, Chymostatin, and Leupeptin (Cambridge Research Biochemicals, England) and L-*trans*-expoxysuccinyl leucylamido(4-guanidino) butane (Sigma) were used in the buffer at final concentrations of 20 μg/ml. Phenylmethylsulfonylfluoride (Sigma) was also present in the Triton buffer for immunoprecipitation at a final concentration of 1.6 m*M*; J. D. Lonsdale-Eccles and G. W. N. Mpimbaza, *Eur. J. Biochem.* **155**, 469 (1986).
12. M. W. Clarke, R. W. Olafson, T. W. Pearson, *Anal. Biochem.* **142**, 360 (1984); *Mol. Biochem. Parasitol.* **17**, 19 (1985).
13. T. Baltz *et al.*, *FEBS Lett.* **158**, 174 (1983); M. A. J. Ferguson and G. A. M. Cross, *J. Biol. Chem.* **259**, 3011 (1984); D. G. Jackson and H. P. Voorheis, *ibid.* **260**, 5179 (1985).
14. M. A. J. Ferguson, K. Haldar, G. A. M. Cross, *J. Biol. Chem.* **260**, 4963 (1985).
15. M. W. Clarke, A. F. Barbet, R. W. Olafson, T. W. Pearson, in *Molecular Strategies of Parasitic Invasion*, N. Agabian, H. Goodman, N. Noguiera, Eds. (Liss, New York, 1986, in press).
16. M. L. Cardoso de Almeida, L. M. Allan, M. J. Turner, *J. Protozool.* **31**, 53 (1984).
17. A. L. W. de Gee, thesis, University of Utrecht, Utrecht, Netherlands (1980), pp. 64–72.
18. P. R. Gardiner, J. F. Finerty, D. M. Dwyer, *J. Immunol.* **131**, 454 (1983).
19. M. L. Cardoso de Almeida and M. J. Turner, *Nature (London)* **302**, 349 (1983).
20. C. A. Hoare, in *The Trypanosomes of Mammals* (Blackwell, Oxford, England, 1972), pp. 401–429; C. A. Hoare, in *The African Trypanosomiases*, H. W. Mulligan, Ed. (Allen and Unwin/Ministry of Overseas Development, London, 1970), pp. 3–23.
21. L. H. T. van der Ploeg, A. W. C. A. Cornelissen, J. D. Barry, P. Borst, *EMBO J.* **3**, 3109 (1984).

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Synthesis of a Site-Specific DNA-Binding Peptide

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The Hin recombinase binds to specific sites on DNA and mediates a recombination event that results in DNA inversion. In order to define the DNA-binding domain of the Hin protein two peptides 31 and 52 amino acids long were synthesized. Even though the 31mer encompassed the sequence encoding the putative helix-coil-helix–binding domain, it was not sufficient for binding to the 26–base pair DNA crossover site. However, the 52mer specifically interacted with the site and also effectively inhibited the Hin-mediated recombination reaction. The 52mer bound effectively to both the 26–base pair complete site and to a 14–base pair "half site." Nuclease and chemical protection studies with the 52mer helped to define the DNA base pairs that contributed to the specificity of binding. The synthetic peptide provides opportunities for new approaches to the study of the nature of protein-DNA interaction.

MANY SEQUENCE-SPECIFIC DNA-binding proteins have been found to consist of two distinct structural and functional domains. One domain is responsible for recognizing and binding a DNA sequence, whereas the other may perform a second function such as binding to other proteins or catalyzing a specific reaction. The structural domains of some proteins, such as the *lac* repressor (*1, 2*), the λ repressor (*3, 4*), and the catabolite activator–binding protein (CAP) (*5*), have been physically separated by limited proteolytic digestion and subsequent purification of the products. It is difficult to prepare the large amounts of homogeneous peptide necessary for a variety of physical techniques including nuclear magnetic resonance (NMR) spectroscopy and x-ray crystallography, and it is difficult to modify the peptide with appropriate "reporter" groups, since it is derived biologically and contains only the 20 common amino acids. Using solid-phase peptide synthesis, we have prepared milligram quantities of a 52–amino acid peptide (52mer) that constitutes the DNA-binding domain of the Hin site–specific recombinase protein. This synthetic peptide has been used as a probe of the DNA-binding site, and its properties are compared to the DNA-binding properties of the intact Hin protein.

The Hin site–specific recombinase inverts a segment of DNA in order to change the expression of the flagellin genes of *Salmonella typhimurium* (*6*). Recombination occurs between two crossover sites designated *hix*L and *hix*R, in inverted repeat configuration, when they are on a supercoiled substrate (*7*). Each *hix* site is 26 bp long, has near twofold symmetry, and shares a 14-bp sequence with the other (see Fig. 1b). Hin is a member of a family of recombinases that complement one another and that includes Gin from phage Mu, Cin from phage P1, and Pin from the *e14* element of *Escherichia coli*. By comparing the crossover sites for all of these recombinases, the consensus sequence given in Fig. 1b has been determined (*8–10*).

The Hin family of recombinases is also related to the resolvase recombinase of transposon γδ. Hin and resolvase share 35% homology of amino acid sequence throughout their length (*11*). Resolvase has a two-domain structure (*12*); digestion with chymotrypsin cleaves it into two peptides. The COOH-terminal peptide binds to the resolvase DNA recognition site, and the NH_2-terminus mediates protein-protein interactions and presumably the recombination activities. The sequence homology implies that Hin should also fold into two domains, and its COOH-terminal domain should bind the *hix* site. We tested this hypothesis by synthesizing two peptides that correspond to the last 52 and 31 amino acids of Hin. The 52mer is homologous with the COOH-terminal fragment released from resolvase by chymotrypsin (Fig. 1a). The 31mer contains 21 residues that are homologous to the helix-turn-helix DNA–binding motif of known repressors and of resolvase. We show in this report that

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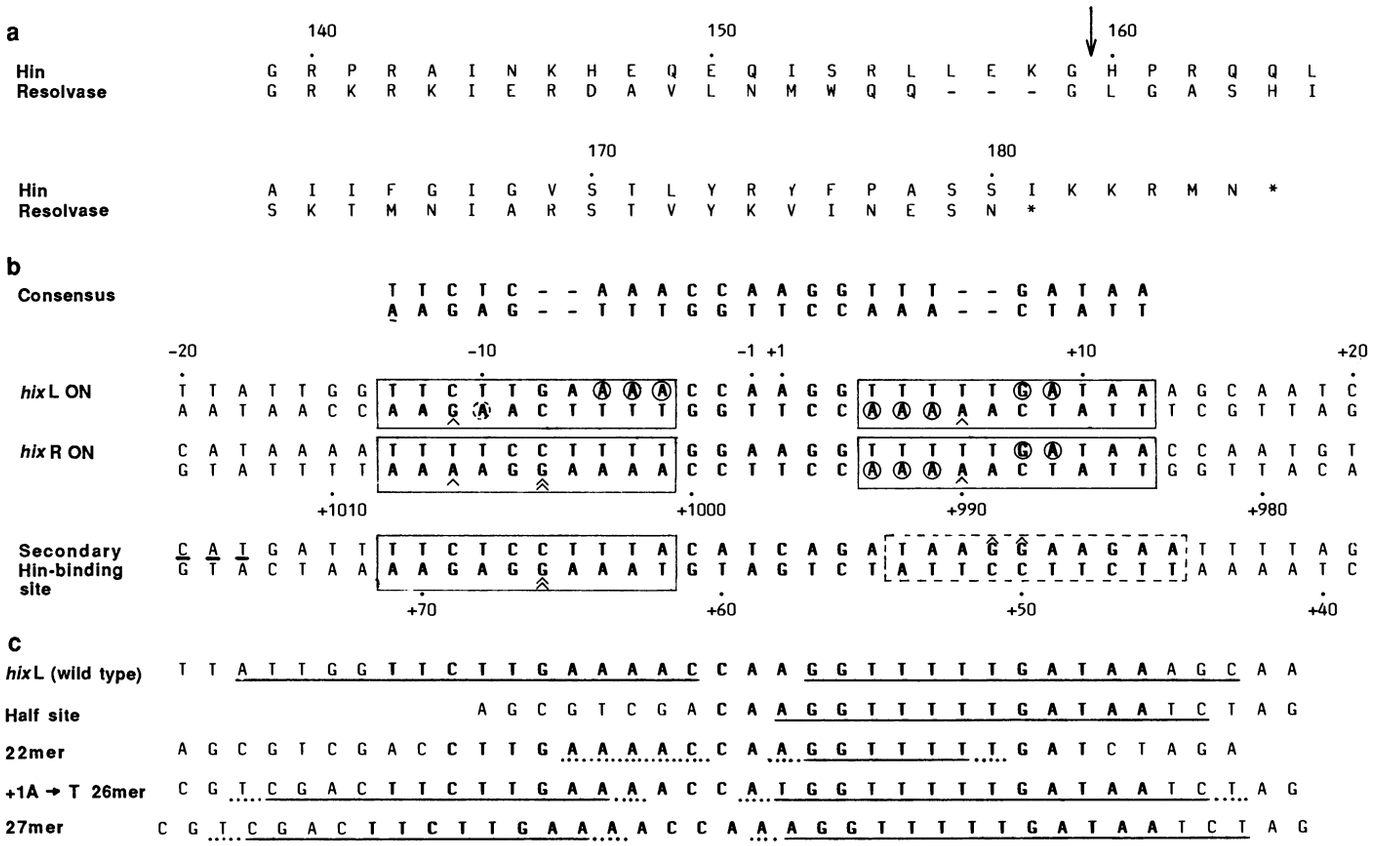

Fig. 1. (**a**) The amino acid sequence of the Hin 52mer peptide and the corresponding homologous sequence of the γδ resolvase (*18, 19*). The 52mer sequence is that of amino acids 139 to 190 of the Hin protein and is homologous to the COOH-terminal fragment released from resolvase by chymotrypsin digestion. The arrow indicates the start of the 31mer peptide. The asterisks indicate translation stops. (**b**) The consensus sequence for the Hin family of recombinases (*10*) and the sequence of *hix*L, *hix*R, and the secondary Hin-binding site with surrounding bases for the *H2* flagellin gene "ON" configuration are shown (*18*). The sequences that correspond to the consensus sequence are in bold letters. Purines protected from DMS methylation by Hin and the 52mer are circled. Dashed circles indicate incomplete protection. Methylation enhancements are indicated by a caret (^); especially strong effects are indicated with a double caret (^^). The probable recognition sequences for the 52mer deduced from all of the protection studies have been boxed. The *hin* start codon near the secondary Hin site is marked with a dashed line between the bases. (**c**) The nucleic acid sequence of *hix*L and four modified sites (*16*). Bases in the bottom strand that are protected from DNase I cleavage by the 52mer peptide are underlined. Dotted lines indicate only partial protection. The experimental details are provided in (*20*).

whereas the 31mer has no activity the 52mer specifically binds to half-*hix* sites and inhibits its inversion by Hin.

The peptides were synthesized from the COOH-terminus to the NH_2-terminus by the solid-phase method of Roise *et al.* (*13, 14*). The 52mer showed DNA-binding activity (see below) without the need for any additional purification. Figure 2 shows a polyacrylamide gel of both peptides. The 31mer appears homogeneous, and the 52mer is approximately 95% pure, with 5% running as a lower molecular weight peptide (*14, 15*).

The ability of the 52mer to bind *hix*L sites is demonstrated in Fig. 3. The deoxyribonuclease (DNase) I cleavage protection patterns given by Hin and the 52mer on a *hix*L-containing fragment of DNA may be compared (Fig. 3a). Hin protects bases −17 through +16 inclusive (on the bottom strand indicated in Fig. 1b and Fig. 3a, lane 1) and the 52mer protects the same bases except for the three central bases from −2 through +1. The protection patterns of Hin and the 52mer on *hix*L given by dimethyl sulfate (DMS) may be compared in Fig. 3b. The two give identical patterns, indicating that contacts with the same bases are made by both. Additional DMS protection data are summarized in Fig. 1b. The DNase I protection pattern for the other crossover site, *hix*R, is shown in Fig. 3c. In this case the protection patterns for Hin and the 52mer differ significantly. As for *hix*L, Hin protects the entire *hix*R site; however, the 52mer protects only the inside half of *hix*R at concentrations that protect both halves of *hix*L. Clear protection of the outside half of the *hix*R requires a threefold higher concentration of the 52mer. This protection probably occurs because the outside half of *hix*R does not conform to the consensus sequence.

Hin and the 52mer also have the same recognition properties with respect to modified *hix* sites. The binding of the 52mer to several altered *hix*L sites (*16*) is summarized in Fig. 1c. The 52mer recognizes the isolated 14-bp inverted repeat (bp −1 to +13)

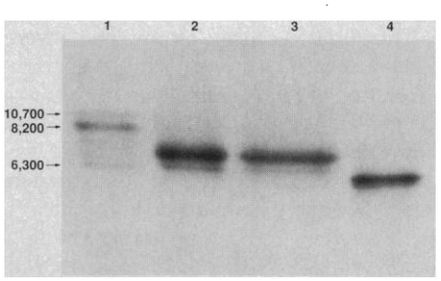

Fig. 2. Polyacrylamide gel of the peptides. An 11 to 22% exponential gradient polyacrylamide SDS gel was run (*21*) and stained with Coomassie blue. Lane 1 contains cyanogen bromide fragments of myoglobin with the indicated molecular weights. Lane 2 contains 3 μg of 52mer eluted from a heptyl-agarose column, lane 3 contains 3 μg of 52mer untreated after lyophilization, and lane 4 contains 3 μg of untreated 31mer.

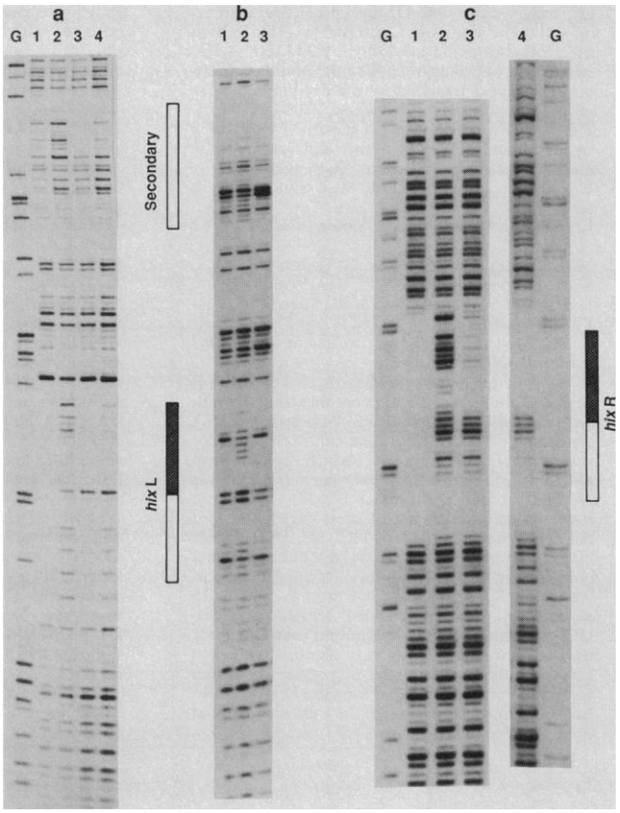

Fig. 3. Binding of the 52mer to *hix*-containing DNA. (**a**) The DNase I cleavage protection patterns (*22, 23*) given by Hin (lane 1; 150 n*M*) and the 52mer (lanes 3 and 4; 11 μ*M*, and 3.8 μ*M*, respectively) on *hix*L-containing DNA are compared. Lane 2 provides the control with no protein or peptide. The sequence is indicated by the DMS guanine reaction of Maxam and Gilbert (*24*) in lane G. (**b**) The DMS protection and enhancement pattern for the same region of DNA. DNA is cleaved at guanines with N7 methylated (accessed from the major groove) and at adenines with N3 methylated (accessed from the minor groove) (*25*). Lane 1 contains 100 n*M* Hin, lane 3 contains 11 μ*M* 52mer, and lane 2 is the control with no additions. (**c**) The DNase I cleavage protection pattern at *hix*R for Hin (lane 1; 150 n*M*) and the 52mer (lanes 3 and 4; 11 μ*M* and 30 μ*M*) is shown. Lanes 2 and G indicate the control and the sequence. The locations of *hix*L and *hix*R are indicated by the boxes and the 14-bp inverted repeat is cross-hatched. For all three panels the DNA was labeled such that the bottom strands in Fig. 1b are observed.

with the same relative affinity as the intact *hix*L site, and no sequences adjacent to this half site are protected. When the outer two base pairs on each side of *hix*L were deleted (Fig. 1c, bp −13, −12, +12, and +13), binding of the 52mer was reduced by more than a factor of five. Thus at least one of the outer two bases is crucial for the recognition of a *hix* site by the 52mer. Sites that have altered bases in the central portion of *hix*L [the central AA (bp −1, +1) changed to AT or AAA] do not change their ability to bind the 52mer. The intact Hin protein shows similar binding patterns (*17*). There is an additional site in the inversion region at bp +44 through +70, which resembles *hix*R, that is partially protected by both Hin and the 52mer from DNase I and DMS (see Fig. 1b). This secondary binding site is not essential for inversion; that is, it is not present on reconstructed substrates with wild-type inversion efficiencies (*16*). However, the secondary site is just upstream of the initiation codon (bp +75 through +77) of the *hin* gene and might be involved in autoregulation of Hin transcription or translation (*17*).

A dissociation constant of approximately 2 μ*M* for the 52mer at the inside *hix*L half site in 100 m*M* NaCl was estimated by measuring the fraction of DNA within the protected *hix*L site relative to a nonprotected region of DNA as the concentration of 52mer was varied. A value of 40 n*M* was obtained for Hin under similar conditions (*16*). In making these determinations, we assumed that all of the peptide or protein present was capable of binding. The dissociation constants for resolvase and its COOH-terminal fragment were reported as <0.2 n*M* and 0.5 to 2.0 μ*M*, respectively (*12*). These values indicate that in both instances the intact protein makes an important contribution to binding. Two possible roles for the rest of the protein are readily envisioned. Binding to the DNA sites may be cooperative and protein-protein interactions at the NH_2-terminus may mediate this cooperativity. Alternatively, the small peptide fragments may be structurally unstable and need the rest of the protein to maintain a correctly folded structure. The 31mer does not protect any DNA sequences from DNase I cleavage at 15 μ*M*. Above 50 μ*M* both the 31mer and the 52mer give nonspecific binding, which is probably a reflection of the basic nature of the peptides. The 31mer may fail to recognize *hix* sites because of instability of the recognition structure, or some necessary recognition element may exist in the first 21 amino acid residues of the 52mer. Experiments to study the binding properties of peptides of intermediate length are currently under way. Thus far, neither a 36–amino acid nor a 45–amino acid peptide was found to be sufficient for specific DNA binding.

Since binding of Hin to the *hix* sites is a prerequisite to inversion, binding of the peptides to the *hix* sites should inhibit Hin inversion (Fig. 4). Concentrations of the 52mer, which protect *hix*L from DNase I cleavage, inhibit switching by Hin. This inhibition is overcome by increasing the Hin concentration. Quantitative initial rate experiments (*15*) showed that the presence of 1.7 μ*M* 52mer required twice as much Hin for half-maximal switching rates. Thus, the apparent inhibition constant for switching and the DNA-binding constant are about the same. The 31mer gives no inhibition of switching at 25 μ*M*.

In summary, we have shown by synthesizing a 52–amino acid peptide whose sequence is homologous to resolvase and identical to the COOH-terminus of the Hin protein that this section of the protein recognizes and binds *hix* sites on DNA. The sequence recognition properties of Hin and the 52mer are similar as evidenced by the DMS protections and enhancements (Fig. 3b), and the protein and peptide show similar binding patterns with the altered *hix* sites (Fig. 1c). Our use of the peptide allows us to study binding in the absence of cooperative interactions between Hin molecules. Furthermore, the peptide binding studies indicate that the center of a *hix* site is not protected by the 52mer. This may allow the NH_2-terminal domain of the recombinase access to the central region so that it can mediate strand exchange reactions. Finally, the putative helix-turn-helix motif, which is contained within the COOH-terminal 31

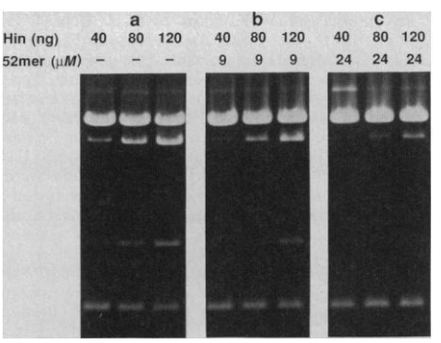

Fig. 4. Inhibition of Hin switching by the 52mer peptide. Recombination by Hin alters the Cla I restriction pattern of the substrate plasmid pMS504 to generate the two intermediate-sized fragments (*7*). Panel **a** shows the switching resulting after a 30-minute incubation with 40, 80, and 120 ng of partially pure Hin (*7*) with the standard assay conditions (*13*). In panels **b** and **c** the same concentrations of Hin are used with 9 μ*M* and 24 μ*M* 52mer added.

amino residues of the Hin peptide, is not sufficient to elicit specific binding to DNA. There may be additional nucleotide recognition elements within the COOH-terminal 52–amino acid residue sequence.

These experiments demonstrate the usefulness of solid matrix peptide synthesis for studying structure-function relations in proteins. It is especially noteworthy that the 52mer binds DNA without the need for purification after synthesis. Peptides, such as the 52mer and analogs that incorporate specific reporter groups, will provide powerful tools for further dissecting precise details of DNA protein interactions.

REFERENCES AND NOTES

1. T. Platt, J. G. Files, K. Weber, *J. Biol. Chem.* **248**, 110 (1973).
2. N. Giesler and K. Weber, *Biochemistry* **16**, 938 (1977).
3. C. O. Pabo and R. T. Sauer, *Annu. Rev. Biochem.* **53**, 293 (1984).
4. ———, J. M. Sturtevant, M. A. Ptashne, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1608 (1979).
5. H. Aiba and J. S. Krakow, *Biochemistry* **20**, 4774 (1981).
6. J. Zieg, M. Silverman, M. Hilmen, M. Simon, *Science* **196**, 170 (1977).
7. R. C. Johnson, M. F. Bruist, M. B. Glaccum, M. I. Simon, *Cold Spring Harbor Symp. Quant. Biol.* **49**, 751 (1984).
8. R. Hiesrand-Never and S. Iida, *EMBO J.* **2**, 1733 (1983).
9. R. H. A. Plasterk and P. van de Putte, *Biochim. Biophys. Acta* **782**, 111 (1984).
10. M. I. Simon and M. Silverman, in *Gene Expression in Procaryotes*, J. Beckwith, J. Davies, J. A. Gallant, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983), pp. 211–227.
11. M. I. Simon, J. Zieg, M. Silverman, G. Mandel, R. Doolittle, *Science* **209**, 1370 (1980).
12. S. S. Abdel-Meguid, N. D. F. Grindley, N. S. Templeton, T. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2001 (1984).
13. D. Roise *et al.*, in preparation.
14. The peptides were synthesized by the optimized manual stepwise solid-phase method of Roise *et al.* (*13*) with phenylaceticamidomethyl (PAM)-resin solid support substituted with resin butoxycarbonyl (BOC)-asparagine (0.82 mmol/g) (Applied Biosystems) and BOC amino acids (Peninsula Laboratories). During the cycle, at each amino acid addition, coupling was repeated until it was at least 99.8% complete as monitored by quantitative ninhydrin analysis [V. K. Sarin, S. B. H. Kent, J. P. Tam, R. B. Merrifield, *Anal. Biochem.* **117**, 147 (1981)]. After 31 amino acids, one-quarter of the resin was removed for generating the 31mer. Peptides were removed from the resin and deblocked by standard HF cleavage and then lyophilized [J. M. Stewart and J. D. Young, Eds., *Solid-Phase Peptide Synthesis* (Pierce Chemical Co., Rockford, IL, 1984), pp. 85–89]. The purity of the peptides were checked in several ways. Their sequences were confirmed by Edman degradation by means of an Applied Biosystems gas-liquid solid-phase protein sequenator [R. M. Hewick, M. W. Hunkapiller, L. E. Hood, W. J. Dreyer, *J. Biol. Chem.* **256**, 7990 (1981)], and a quantitative amino acid composition analysis [J.-Y. Chang, R. Knecht, D. G. Braun, *Methods Enzymol.* **92**, 41 (1983)] indicated that the solid was 100% peptide within the error of the determination. Electrophoresis on an exponential gradient polyacrylamide SDS gel (see Fig. 2) and a polyacrylamide urea gel [B. Kadenbach, J. Jarausch, R. Hartmann, P. Merle, *Anal. Biochem.* **129**, 517 (1983)] revealed the 31mer to be homogeneous and the 52mer to contain only a minor amount (<5%) of a lower molecular weight peptide. The 52mer was also chromatographed on heptyl-agarose, and one broad peak was eluted with a reverse salt gradient in 2*M* urea (*15*). Peptides from fractions across the peak had the same 280/260 nm absorbance ratio, migrated the same on the polyacrylamide gradient gel, and gave the same DNase I protection patterns on *hix*L. The studies in this report were done with the peptide eluted in a 0.95*M* to 0.3*M* $(NH_4)_2SO_4$ step from a heptyl-agarose column. Peptide concentrations were determined by the dye-binding assay of M. M. Bradford [*Anal. Biochem.* **72**, 248 (1976)]. The standard was based on the dry weight of a vacuum-baked sample of 52mer and confirmed by quantitative amino acid composition analysis.
15. R. C. Johnson, M. F. Bruist, M. I. Simon, *Cell*, in press.
16. R. C. Johnson and M. I. Simon, *ibid.* **41**, 781 (1985).
17. M. F. Bruist, A. C. Glasgow, R. C. Johnson, M. I. Simon, in preparation.
18. J. Zieg and M. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4196 (1980).
19. R. R. Reed, G. I. Shibuya, J. A. Steitz, *Nature (London)* **300**, 381 (1982).
20. These experiments were done with a ^{35}S or ^{32}P end-labeled DNA restriction fragment that contains both *hix*L and *hix*R separated by a 299-bp piece of DNA containing the recombinational enhancer. The construction of this fragment, which comes from pMFB36, will be described elsewhere (*17*). Hin was prepared as described earlier (*7*, *15*). Protection reactions were in a volume of 50 μl and the standard buffer contained 20 m*M* tris-HCl, *p*H 7.6, 100 m*M* NaCl, 5 m*M* $MgCl_2$, 1 m*M* dithiothreitol, and polycytidylic acid (100 μg/ml). DNase I was incubated for 30 seconds at 0.4 μg/ml. For DMS experiments sodium cacodylate replaced the tris-HCl and dithiothreitol was omitted, and DMS was used at 85 m*M* for 30 seconds. Samples with high concentrations of either the 52mer or the 31mer were often poorly soluble in formamide. This problem was minimized when samples were first digested with proteinase K at *p*H 9 in 2*M* $NH_4CH_3CO_2$, extracted with phenol:chloroform:isoamyl alcohol (25:24:1), ethanol precipitated, and then reprecipitated. Samples were run on 6% acrylamide urea gels (60 cm long) with a gradient from 1× to 2× buffer [M. D. Biggin, T. J. Gibson, G. F. Hong, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3963 (1983)]. Gels were fixed and dried before exposing. Binding constants were estimated by cutting out and determining the Čerenkov radiation from sections of a dried gel containing DNA fragments from binding and nonbinding regions of the same lane.
21. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
22. D. J. Galas and A. Schmitz, *Nucleic Acids Res.* **5**, 3157 (1978).
23. M. F. Bruist, R. C. Johnson, M. B. Glacum, M. I. Simon, in *Genome Rearrangement*, M. Simon and I. Herskowitz, Eds., vol. 20 of *UCLA Symposium on Molecular and Cellular Biology* (Liss, New York, 1984), pp. 63–75.
24. A. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
25. W. Gilbert, A. Maxam, A. Mirzabekov, in *Control of Ribosome Synthesis*, N. O. Kjelgaard and O. Maaloe, Eds. (Munksgaard, Copenhagen, Denmark, 1976), pp. 139–148.

26. We thank I. Clark-Lewis for the quantitative amino acid composition analysis. We also thank R. Johnson, A. Glasgow, J. Sluka, P. Dervan, and J. Tomich for helpful discussions and critical reading of the manuscript. This work was supported by a grant from the National Science Foundation (NSF DMB82-09295). M.F.B. was supported by fellowship GM-09534-02 from the National Institutes of Health.

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Epitope Mapping by Chemical Modification of Free and Antibody-Bound Protein Antigen

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A monoclonal antibody bound to a protein antigen slows the rate of chemical modification of amino acid residues located at the epitope. By comparing the degree of acetylation of 18 lysine and 7 threonine residues in free and antibody-bound horse cytochrome c, a discontiguous, conformational epitope was characterized on this protein antigen. The new approach is particularly suitable to probe discontiguous and conformational epitopes, which are difficult to analyze by other procedures.

EPITOPES OF PROTEIN ANTIGENS have been classified as contiguous and discontiguous (*1*, *2*). Contiguous epitopes are composed of residues that are close to each other in the polypeptide sequence, whereas discontiguous epitopes consist of residues that are distant in the polypeptide sequence but adjacent on the protein surface. The term "conformational" designates the dependence of antibody binding on the spatial conformation of the epitope. The classification of epitopes is operational, as it depends on the method used for their detection. In one way or another existing methods are based on the competition for the antibody-combining site between the parent antigen used to mount the immune response and species-related proteins, chemically modified proteins, or peptides (*2*). Here we present a novel approach that complements existing methods for probing epitopes on protein surfaces. The rationale is to compare the relative rate of chemical modification of residues of a protein antigen in the presence or absence of a specific monoclonal antibody and to deduce the location of the epitope from the differential chemical reactivity of amino acid side chains. This type of differential chemical modification was applied successfully to map electron-transfer interaction domains for phys-

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