A Helical Epitope in the C4 Domain of HIV Glycoprotein 120*

(Received for publication, July 12, 1995, and in revised form, August 24, 1995)

Frank A. Robey, Tracy Kelson-Harrist, Peter P. Roller, and Marjorie Robert-Guroff

From the *Peptide and Immunochemistry Unit, Laboratory of Cellular Development and Oncology, NIDR, †Laboratory of Tumor Cell Biology, NCI, and ‡Laboratory of Medicinal Chemistry, Division of Cancer Treatment, NCI, National Institutes of Health, Bethesda, Maryland 20892.

The fourth conserved domain of the human immunodeficiency virus type 1 (HIV-1) envelope, the C4 region of glycoprotein 120 (gp120), is an amphipathic stretch of amino acids that, based on mutational analyses, constitutes a major component of the CD4 binding region in gp120. In the absence of crystallographic and NMR data on C4 in intact gp120, we sought to gain insight into C4's conformation and accessibility in gp120 by taking an immunochemical approach. In this study, a peptomer composed of a repeat peptide of C4 amino acids 419–436 from gp120 of HIV-1MN was synthesized for use as a conformationally constrained immunogen. Circular dichroism studies disclosed that the polymerized peptide, peptomer-(419–436), in 0.01 M Na2HPO4 buffer, pH 7.4, at 25 °C contained a dominant α-helical structure (53 ± 1%) compared with 2 ± 4% α-helical content for the monomeric peptide-(419–436). The peptomer in Ribi's adjuvant induced the production of rabbit antibodies that recognized recombinant and native gp120 but, consistent with the literature, the C4 peptide having no conformational constraints did not. The experimental results show that only those antibodies formed against a helical immunogen from C4 will recognize recombinant and native gp120 and, therefore, the results support the notion that C4 is an α-helix in gp120.

Glycoprotein 120 (gp120) of human immunodeficiency virus type 1 (HIV-1) is the surface glycoprotein that binds to the CD4 receptor on cells in the first step of a cascade of molecular events leading to HIV infection (1–3). The primary site on gp120 that binds to CD4 resides in the fourth conserved (C4) domain (4), and it has been shown that a change in C4 at a single tryptophan residue could abrogate gp120 binding to CD4 (5). The virus carrying a mutation at this position in gp120 is noninfectious (5). Although amino acid changes outside C4 also may interfere with the CD4 binding ability of gp120 (6), Pollard et al. (7) found that 41% of the gp120 residues were not necessary for CD4 binding, and evidence was presented that suggested the existence of a minimum CD4 binding region in gp120.

Despite the volumes of additional published information that describe various aspects of the gp120-CD4 interaction, the exact role of C4 in binding CD4 is not understood, partly because a synthetic C4 peptide has no biological activity. However, answers to key questions about HIV vaccines and therapeutics hinge on understanding the molecular nature of the C4-CD4 interaction.

Because there are no available physical data to describe precisely the conformation of C4 in the intact gp120 we have followed the lead of others (8) and relied on a theoretical treatment of a sequence of amino acids from C4 to assess what the conformation might be. Since most short synthetic peptides have very little, if any, conformation, it was clear that attempts to construct an immunologically active synthetic peptide from C4 having defined conformational constraints was warranted. Antibodies made to defined conformationally constrained immunogens could be used to probe conformationally constrained epitopes that reside in the parent gp120.

It is well known in the field of polymer physical chemistry that increasing chain lengths of certain polymers often result in an increase in α-helicity (9). For the work described here, we investigated the influence that head-to-tail polymerization would have on the conformation of the amphipathic peptide from C4. A single method to enhance conformations of peptides from the C4 region to mimic those conformations found in the native gp120 could, in principle, be applied to diagnostics, therapeutics, and vaccine development. We also studied the immunological activities of the new construct to see if they were compatible with the intact gp120.

As far as we know, the following is the first description of a construct made of a head-to-tail polymerized peptide, which has a defined conformation and immunogenicity/antigenicity that are considerably different than those of the monomeric peptide. As such, the defined construct may present the peptide in an average conformation that is close to that found for the peptide in the parent protein. This could be an advantage over using monomeric peptides alone or peptide monomers conjugated to carriers since most peptides have very little, if any, conformation. The approach described here can be extended beyond HIV to study many other biological systems that possess important helical conformations within a parent protein's overall conformation.

Because a single tryptophan in C4 appears to be central to the CD4 binding ability of gp120 (4, 5), we concentrated our synthetic efforts on constructing conformationally constrained peptides from the amphipathic C4 sequence of gp120, which contain Trp-427 at the central position.

* Financial support (to F. A. R.) from the Director's Discretionary Fund of the Office of AIDS Research, NIH, made continuation of this work possible. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Bldg. 30, Rm. 211, NIH, Bethesda, MD 20892. Tel.: 301-496-4779; Fax: 301-402-0823; E-mail: FAR3@yoda.nidr.nih.gov.

‡ The abbreviations used are: gp, glycoprotein; HIV-1, human immunodeficiency virus type 1; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; TBS, Tris-buffered saline.

MATERIALS AND METHODS

The syntheses of the peptides were performed on an Applied Biosystems, Inc. (Foster City, CA) model 430A automated peptide synthesizer. All chemicals used in the peptide synthesis were from Applied Biosystems, Inc. Recombinant gp1201-416 was a gift from Genentech, South San Francisco, CA. gp1201-416 from supernatant media of virus-producing cultures was prepared as described (10).

Peptomer Syntheses—The general methods for synthesizing peptomers have been described in detail elsewhere (11–15). In brief, for
FIG. 1. Schematic of the synthesis of peptomer-(419–436) derived from the C4 of gp120 MN isolate. The syntheses of peptomers beginning with haloacetyl, cysteine-containing peptide starting materials have been described in detail previously (11–15). Boxed inset: lane A, sodium dodecyl sulfate-polyacrylamide gel Coomassie Blue-stained peptomer-(419–436); lane B, molecular weight standards.

RESULTS AND DISCUSSION

Peptomers are polymers of specifically cross-linked synthetic peptides and are formed from cysteine-containing peptides that first are bromoacetylated or chloroacetylated at the N terminus (11–15). Fig. 1 shows the schematic for the synthesis of peptomer-(419–436) starting with the chloroacetyl-, cysteine-derivatized monomer. Peptomer-(419–436) displays a distribution of molecular weights as seen with SDS-PAGE and staining with Coomassie Blue (Fig. 1, inset).

When the peptide sequence is placed into the helical wheel described by Schiffer and Edmondson (16), it becomes clear that there are two sides to the resulting conformation, a hydrophobic side and a hydrophilic side (not shown). This theoretical treatment indicates that under the appropriate constraints, such as those found in an intact protein, the peptide could be in an α-helical conformation. By equating the Trp at residue 427 as being equal to ε-helical, the formula of n, n + 3, and n + 4 for placing hydrophobic residues at appropriate positions for making an α-helix (16) can be demonstrated here. For the example shown, if Trp-427 is equal to n, then n = 3 is Ile, n = 4 is Ile, n + 3 is Val, and n + 4 is Gly. With the exception of Gly at the n + 4 position, all amino acids in the formula n, n ± 3, n ± 4 are hydrophobic.
indicating that in the protein residues 419–436 contain sufficient spacing of hydrophobic amino acids to support an α-helix.

A study then was performed to compare the circular dichroism (CD) spectra of a peptide consisting of amino acids 419–436 from the MN isolate of HIV-1 to the peptomer-(419–436). Fig. 2 demonstrates that, although the sequences of the constitutive peptides are identical, polymerizing-peptide-(419–436) from the N terminus to the C terminus results in the formation of a material having a dominant α-helical structure that is considerably greater than that of the free peptide. From the CD spectra shown, we calculate that peptide-(419–436) in water contains 2 ± 4% α-helical content, whereas the peptomer-(419–436)-containing solution contains 53 ± 1% α-helical content. Secondary structure percentages were calculated for each construct.

Fig. 2. Conformational studies of the C4 peptide constructs. CD spectra of N-Ac-dimer-(419–436) (--), peptomer-(419–436) (—), and N-Ac-peptide-(419–436) (- - -). The percent conformation, α-helix, β-sheet, and unassigned (random) are listed in the table inset for each construct.

The effect of polymerization on the conformation of a scrambled version of peptide-(419–436) and the influence of Trp-(427) on the peptomer conformation were studied. The CD spectrum of the scrambled peptomer demonstrates an increase in α-helical structure compared with peptide-(419–436), but this increase in conformation was not as pronounced as it was with peptomer-(419–436) (data not shown). The percent helix was calculated for the scrambled peptomer, but it appeared to be approximately 15% helix when judged in comparison with the other peptomers’ CD spectra.

Tryptophan-containing synthetic peptides often are synthesized using a formyl group on the tryptophan residue to protect the tryptophan from oxidation. The conformation as judged by the CD spectrum of peptomer-(419–436) containing a formyl protecting group on the central Trp ([CHO-Trp-427] in place of Trp-427) resembles the peptide-(419–436) CD spectrum. These results point to Trp-427 as having an important function in the maintenance of the α-helix of the peptomer in aqueous solutions that are devoid of molecules known to stabilize helices such as trifluoroethane or certain detergents.

The influence of Ribi’s adjuvant on the conformation of an immunogen is unknown. However, we found that when the helical C4 construct is used to immunize rabbits in Ribi’s adjuvant, an antisera that recognized native (Fig. 3, A1 and A2) and recombinant gp120 (not shown) was produced. The antisera made by immunizing rabbits with the C4 peptide-(419–436) did not recognize gp120 (Fig. 3, C1 and C2), and we were unable to detect any α-C4 antibodies in ELISAs with the anti-peptide antisera.

A single rabbit was immunized with the CHO-Trp-427-containing peptomer-(419–436) in Ribi’s adjuvant. The antisera obtained from this rabbit reacted strongly with the peptide in the ELISA; however, it did not react with native or recombinant gp120 in the dot blot assay or ELISAs (data not shown). Because the CHO-Trp-427-containing peptomer-(419–436) contained no detectable α-helix (see above), we interpret this to indicate that this antisera was against only a linear portion of the peptomer.

Thus, with Trp at position 427 in the peptomer-(419–436), the conformation of the peptomer is helical, and anti-peptomer antisera binds gp120, recombinant and native. When the Trp at this position is protected with a CHO group, there is no helical conformation in the peptomer, and the anti-peptomer antisera does not bind recombinant or native gp120. This strongly suggests that gp120 contains a helical epitope comprised of the highly conserved C4 amino acid sequence.

If it is found that the C4 helix is highly conserved among all the HIV-1 strains, this discovery will provide an important common link between conformations shared among all the HIV-1 strains. Because Trp at this position is important for supporting a helical conformation and, as others have shown, the Trp at this position is critical for HIV infection (4, 5), it may be that a Trp-containing helical conformation in C4 is needed for infection. Aside from amino acid sequence homologies, there have been very few, if any, descriptions of common conformational properties shared by the various strains of HIV-1.

Attempts to perform competition experiments with the monomeric peptide in ELISA-type formats were uninterpretable for showing conformational specificity; whereas the peptide in non-detergent-containing buffers displayed no helical structure as judged by the CD spectrum, the monomeric peptide did have as much as 20% helical structure in the 0.03% Brij 35-containing buffer that we use in the ELISA. In addition, the peptide itself, when immobilized to the ELISA plate, strongly reacted with the antisera made against peptomer-(419–436) in the ELISA, and, because of this, it was clear that a large amount of the anti-peptomer antisera was against nonconformationally constrained peptide. An alternative explanation for the anti-
sera recognizing the peptide would be that the antisera could be forcing the peptide into a helical conformation upon recognition and binding to the peptide by the antisera. We presently are not able to measure the conformation of a peptide or protein immobilized to polystyrene, so this explanation remains a possibility.

A polymer was synthesized previously using disulfide-linked monomers to form random head-to-tail and tail-to-head polymers (18). In Freund's adjuvant, this construct was found to produce antibodies in mice that could not recognize the envelope protein gp160 (18). Here, we show that rabbits immunized with peptomer-(419–436) in Freund's also did not produce antibodies that recognized gp120 (Fig. 3, B1 and B2). Freund's adjuvant may be acting to unfold the helix because the paraffin oil and mannide monooleate components of the Freund's may interfere with the contributions of hydrophobic amino acids that are necessary for holding the helix together. Based on these results, we would recommend not using Freund's as an adjuvant for producing antibodies that recognize certain conformationally constrained epitopes.

The dimer of peptide-(419–436) may be a more defined immunogen than the total peptomer mixture because the dimer is purified and is a single molecular entity. We did not include the dimer in the immunization tests because of the possibility, like the monomer, of it being nonimmunogenic due to its low molecular weight. The polystyrene-immobilized dimer did bind the anti-peptomer antisera. However, the antigenic properties of the dimer compared with the peptide or peptomer do not provide any clues about the possible immunogenicity of the dimer.

The literature that addresses the influence of a particular adjuvant on the conformation of an immunogen appears to be very scarce. A paper by Kenney and co-workers (19) evaluated the influence of adjuvants on the quantity, affinity, isotype, and epitope specificity of murine antibodies and, to this end, the immunogens were not characterized from a viewpoint that would address conformation. However, these authors were able to assess several noted differences in the immune responses in mice when various adjuvants were used. These results, showing that adjuvants selectively and independently enhance different qualities of the antibody response, may be based in part on the conformation of the immunogen as presented to the immune system.

With the capabilities that are available today for constructing conformationally constrained peptides as we have demonstrated in this report, the potential for targeting the immune response to conformationally defined epitopes within a parent protein will become routine as additional examples are reported and the ease with which this is done becomes more obvious. By defining an antigenic material that is used to probe the structure of a parent protein, we should be able to gain useful structural information about complex proteins that could not be obtained through other well established methods such as NMR and crystallography.

Acknowledgments—We acknowledge Dr. Tim Gregory and the other scientists at Genentech in South San Francisco, CA for the generous gifts of recombinant gp120.

REFERENCES
1. Dalgleisch, A. G., Beverly, P. C. L., Clapman, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984) Nature 312, 763–767
2. Klatzmann, D., Champagne, E., Chamaret, S., Gruett, J., Guetard, D., Her-cend, T., Gluckman, J. C., and Montagnier, L. (1984) Nature 312, 767–768
3. McDougal, J., Kennedy, M. S., Sligh, J. M., Cort, S. P., Mawle, A., and Nicholson, J. K. A. (1986) Science 231, 382–385
4. Lasley, L., Nakamura, N., Smith, D. H., Fennie, C., Shimazos, C., Patzer, E., Redman, P., Gregory, T., and Clapman, P. R. (1987) Cell 50, 975–985
5. Cardonnier, A., Montagnier, L., and Emerman, M. (1989) Nature 340, 317–314
6. Kowalski, M. L., Potts, J., Basiliopoul, L., Dohman, T., Goh, W. C., Terwilliger, E., Dayton, A., Rosen, G., Hasettine, W., and Sodrowski, J. (1987) Science 237, 1351–1353
7. Pollard, S. R., Rosa, M. D., Rosa, J. J. and Wiley, D. C. (1992) EMBO J. 11, 585–591
8. Cese, K. B., Margalit, H., Cornette, J., Putney, S. D., Robey, W. G., Ouyang, C., Streicher, H. Z., Fischinger, P. J., Gallo, R. C., Delisi, C., and Berzofsky, J. A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4249–4253
9. Poland, D., and Scheraga, H. A. (eds) (1970) in Theory of Helix-Coil Transitions in Biopolymers. Statistical Mechanical Theory of Order-Disorder Transitions in Biological Macromolecules, Academic Press, New York
10. Kalyanaraman, V. S., Rodriguez, V., Veronese, F., Rahman, R., Lusso, P., D'Elvico, A. L., Copeland, T., Orozlan, S., Gallo, R. C., and Sarrngadharan, M. G. (1990) AIDS Res. Hum. Retroviruses 6, 371–380
11. Lindner, W., and Robey, F. A. (1987) Int. J. Pept. Protein Res. 30, 794–800
12. Robey, F. A., and Fields, R. L. (1989) Anal. Biochem. 177, 373–377
13. Robey, F. A., Harris, T. A., Heegaard, N. H. H., Nguyen, A. K., and Batini, D. (1992) Chim. Oggi 27–31
14. Robey, F. A. (1992) in Current Protocols in Immunology (Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevack, E. M., and Strober, W., eds) pp. 9.5.1–9.5.12, Greene Publishing Associates, Inc., Brooklyn, NY
15. Robey, F. A. (1994) in Methods in Molecular Biology (Pennington, M., and Dunn, B., eds) Vol. 35, pp. 73–91, Humana Press, Inc., Totowa, NJ
16. Schiffer, M., and Edmonson, A. B. (1967) Biophys. J. 7, 121–135
17. Provencher, S. W., and Gloeckne, J. (1981) Biochemistry 20, 33–37
18. Saitry, K. J., and Arlinghaus, R. B. (1991) AIDS & 699–707
19. Kenney, J. S., Hughes, B. W., Masada, M. P., and Allison, A. C. (1989) J. Immunol. Methods 121, 157–166

A Helical Epitope in C4 of gp120