Vol. 143, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1987 Pages 248-251

A STRONG HOMOLOGY EXISTS BETWEEN THE ACTIVE T-CELL BINDING gp120 OCTAPEPTIDE OF HUMAN IMMUNODEFICIENCY VIRUS AND THE SUBTILISIN CLEAVAGE PEPTIDE OF BOVINE RIBONUCLEASE A

Matthew R. Pincus[1], Robert P. Carty[2], James Chen[3], and Randall B. Murphy[4]

[1]Department of Pathology, New York University Medical Center, 550 First Ave., New York, NY 10016,

[2]Department of Biochemistry, SUNY Health Science Center at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203,

[3]Department of Chemistry, New York University, New York, NY 10003,

[4]Department of Psychiatry, E.W. Bourne Laboratory, New York Hospital, Cornell University Medical College, Westchester Division, 21 Bloomingdale Road, White Plains, NY 10605

Received January 13, 1987

SUMMARY A homology has been found between an octapeptide involved in attachment of the human immunodeficiency virus to helper/inducer T cells and an octapeptide segment of bovine pancreatic ribonuclease A. This segment (residues 19 - 26) contains the sites for subtilisin cleavage of this enzyme into the S-peptide and S-protein. From the X-ray crystal structure of ribonuclease, this sequence is known to be exposed to solvent and interacts little with the rest of the protein. A structure for the human immunodeficiency virus attachment peptide can be deduced from this homology, as a well-defined structure has been determined for this sequence in ribonuclease. This can be readily accomplished using previously developed computer methods based upon conformational energy calculations. The calculated structure for human immunodeficiency virus peptide is identical to the ribonuclease segment (19 - 26) in backbone conformation. It is stabilized by internal interactions of nonpolar residues, and by exposure of polar hydroxyl groups. The results suggest that the T-cell human immunodeficiency virus receptor may be hydrophilic in nature and that conservation of the sequence in two presumably functionally unrelated proteins is related to the need for conservation of exposed structure.

[1]Author to whom all communications and requests for reprints should be addressed.

Abbreviations: gp120, 120,000 MW envelope glycoprotein of human immunodeficiency virus; HIV, human immunodeficiency virus; RNase A, bovine pancreatic ribonuclease A; HTLV-IIIb, human T-cell leukemia virus; LAV, lymphoadenopathy associated virus; VIP, vasoactive intestinal peptide.

0006-291X/87 $1.50

A recent report (1) has revealed that an octapeptide from the gp120 protein of human immunodeficiency virus (HIV) is involved in attachment of the virus to the OKT4 antigen of T4 helper/inducer cells. Synthetic peptides identical or very similar in sequence to this octapeptide strongly inhibit attachment of HIV to the antigen receptor (1). Computer-assisted searches have demonstrated homology to a peptide found in the envelope region of the Epstein-Barr virus (1). In addition, strong homologies exist between the HIV octapeptide and peptides which occur in Human Lymphoadenopathy Virus (LAV) and in Human T-Cell Leukemia Virus (HTLV-IIIb) isolates (1).

We report here an additional apparent homology between the HIV peptide and a sequence comprising residues 19 - 26 of bovine pancreatic ribonuclease A (RNase A). This sequence contains the exposed subtilisin cleavage sites of RNase A between residues 20 and 21, and 21 and 22 (2,3). The sequence alignment is shown in Table 1. Also shown in this table are homologous sequences (1) from HTLV-IIIb, LAV, and vasoactive intestinal peptide (VIP). Substitution of serine for threonine in RNase A is generally regarded as conservative, although three contiguous substitutions may conceivably lead to an altered structure. Assuming these substitutions to be conservative, the homology between the (19 - 26) segment of RNase A and the HIV peptide can be of value in postulating the active structure for the latter. The three-dimensional structure of RNase A has been determined at high resolution (4,5). From this structure, the (19 - 26) segment is known to be highly exposed to solvent and interacts little with the remainder of the polypeptide chain, with the exception of a hydrogen bond formed between the hydroxyl of Tyr-25 and the carboxylate of Asp-14 (4,5). The isolated peptide may therefore be hypothesized to retain significant "native" structure.

If it is assumed that the homologous segments are structurally similar, a model for the active structure of the inhibitory peptide may be constructed from the known structure of the 19 - 26 segment of RNase A.

METHODS The structure of the HIV octapeptide was constructed from the known structure of bovine pancreatic ribonuclease A using methods described previously (6,7). The backbone of the HIV peptide was generated to fit as closely as possible to that of the corresponding 19 - 26 residue segment of RNase A. The sidechain atoms of identical residues (Ala, Asn, and Tyr, Table 1) were likewise generated. The sidechain atoms of the conservatively substituted residues (such as threonine for serine, Table

1) were generated in conformations as similar as possible to those of the corresponding atoms of RNase A. The resulting HIV peptide was then subjected to energy minimization (6,7) in which all the dihedral angles of the octapeptide were allowed to vary.

RESULTS AND DISCUSSION The structure of the gp120 peptide is virtually identical to that of the (19 - 26) peptide of RNase A. The proposed structure is shown in Figure 1. In this figure, the methyl groups of the sidechains of the 3 conservative threonine residues tend to form a hydrophobic "cluster" on the "inside" of the peptide while the hydroxyl groups of these threonine residues are exposed and may form part of the binding determinant of the peptide to its T-cell surface receptor.

This proposed structure is predicated upon the assumption that homology implies conservation of structure. The validity of this assumption has been questioned (8), especially for short, homologous segments of five or fewer amino acid residues. In the present case, the homology involves eight residues, a chain length which is unlikely to adopt grossly different conformations in different proteins especially if it is exposed and essentially non-interactive with the remainder of the protein chain.

The homology between the RNase A and HIV segments suggests that a synthetic RNase A segment could be employed as an inhibitory peptide. Furthermore, it is possible that the entire RNase A molecule may inhibit T-cell association with HIV. The homology further suggests that subtilisin may cleave the gp120 protein specifically in the attchment peptide region, and could thus inactivate the virus. Such an inactive virus could serve as a source for a vaccine.

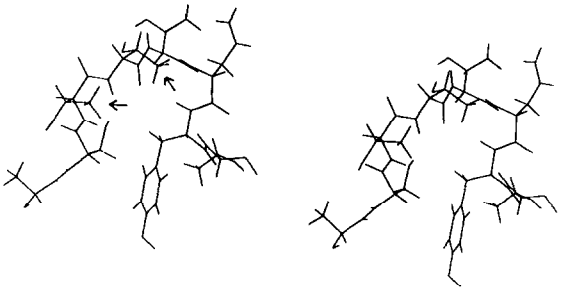

Figure 1. Proposed structure for the T-cell binding octapeptide from HIV gp120 protein, based on its homology to the 19 - 26 peptide segment of RNase A. The two arrows indicate the methyl groups of the first two threonine residues of the HIV sequence which form a hydrophobic cluster with the tyrosine residue (lower right part of figure).

Table 1

Alignment of the Octapeptide Sequences of HIV and RNase A

Peptide	Sequence
HIV	Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr
RNase A (19-26)	Ala-Ala-Ser-Ser-Ser-Asn-Tyr-Cys
HTLV-IIIb & LAV	Thr-Thr-Ser-Tyr-Thr
VIP	Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr

Despite the homology shown in Table 1, there is no apparent relationship between any known function of RNase A and that of the HIV binding peptide. This situation is reminiscent of the much more extensive homology between RNase A and angiogenin (9), a protein involved primarily in stimulation of angiogenesis. The homology between the RNase A and HIV segments may imply the need for conservation of exposed structure which enables attachment of HIV to its T-cell receptor. As is true of the 19 - 26 segment in RNase A, the attachment peptide of the gp120 protein may be an exposed segment which connects two major conformational domains.

REFERENCES

1. Pert, C.B., Hill, J.M., Ruff, M.R., Berman, R.M., Robey, W.G., Arthur, L.O., Ruscetti, F.W., and Farrar, W.L. (1986) Proc. Nat. Acad. Sci. U.S.A. 83, 9254-9258.
2. Richards, F.M. and Vithayathil, P.J. (1959) J. Biol. Chem. 234, 1459-1465.
3. Doscher, M.S. and Hirs C.H.W. (1967) Biochemistry 6, 304-309.
4. Borkakoti, N., Moss, D.S., and Palmer, R.A. (1982) Acta Crystallogr., Sect B: Struct. Crystallogr. Cryst. Chem. B38, 2210-2217.
5. Wlodawer, A., Miller, M., and Sjolin, C. (1982) J. Biol. Chem. 257, 1325-1332.
6. Warme, P.K., Momany, F.A., Rumball, S.V., Tuttle, R.W., and Scheraga, H.A. (1974) Biochemistry 13, 768-782.
7. Pincus, M.R., Gerewitz, F., Wako, H., and Scheraga, H.A. (1983) J. Protein Chem. 2, 131-146.
8. Kabsch, W., and Sander, C. (1984) Proc. Nat. Acad Sci. U.S.A. 81, 1075-1078.
9. Strydom, D.J., Fett, J.W., Lobb, R.R., Alderman, E.M., Bethune, J.L., Riordan, J.F., and Vallee, B.L. (1985) Biochemistry 24 5486-5494.