Circular dichroism study on the secondary structural change induced by complex formation of a peptide derived from a CD4 binding site of HIV-1 envelope glycoprotein gp120 and a peptide from the N-terminal domain of CD4

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SUMMARY

Circular dichroism spectroscopy was used to study the conformational change of a peptide containing a CD4 binding region of HIV-1 envelope glycoprotein gp120 complexed with a CD4 fragment. In free solution the gp120 peptide exists primarily as β-sheet and random coil. Upon association with the peptide, encompassing a critical gp120 binding site on the extracellular domain 1 of CD4, the α-helical content of the complex relative to that of the two component peptides increases significantly, at the expense of random coil and turn. An increase in the helix structure for the gp120 peptide, but not the CD4 peptide, was observed in 30% trifluoroethanol (TFE)/H2O (v:v) solution. The conformational change in the gp120 C4 peptide when complexing with CD4 is proposed as part of the process that facilitates the membrane fusion between the virion and its target cell.

INTRODUCTION

It is well known that binding of HIV-1 envelope glycoprotein gp120 to T-cell receptor CD4 of the immune system is the first step of the viral infection and spreading in AIDS patients [1–4]. Thus, understanding the mechanism of interaction between these two proteins is essential to the treatment of AIDS and to the development of a vaccine for HIV-1. To date, the binding sites on gp120 were determined by gp120-specific monoclonal antibodies (mAb) that are found to block the gp120–CD4 interaction [5–7] or by mutagenesis and insertion/deletion, in which amino acid residues on gp120 were mutated, inserted or deleted and the effect on binding was measured.

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Abbreviations: CD, circular dichroism; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; Fmoc, 9-fluorenylmethoxycarbonyl; mAb, monoclonal antibody; gp, glycoprotein; TFE, trifluoroethanol; HPLC, high-performance liquid chromatography.

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Similar approaches have been applied to gp120 binding sites on CD4 [12–14]. Both mAb and mutagenesis approaches suffer from the uncertainty that the effect may be due to a conformational change that indirectly influences the binding behavior. In the former case, the conformational change could be elicited by the antibody on the CD4 binding site, which is, however, not an epitope for the antibody. Similarly, the effect observed in the point mutagenesis experiments may arise from a conformational alteration due to the mutation in the CD4 binding region of gp120, which, however, may not encompass the point of mutation. Thus, both methods do not necessarily provide direct evidence on the binding sites for the protein–protein interaction. The question regarding the method of using antibody against CD4 to identify the HIV-1 gp120 binding region has been addressed by Davis and co-workers [15].

It was also inferred from enzymatic cleavage enhancement and antibody epitope modulation experiments [16,17] that the conformation of gp120 is changed upon its binding to CD4 on the virion surface or soluble CD4. Dissociation of the noncovalently associated gp120–gp41 complex [18,19], probably stimulated by this conformational change, then results in exposure of the highly hydrophobic N-terminus of gp41, which is subsequently inserted into the membrane of the target cell and activates membrane fusion [10]. From the results of circular dichroism experiments, Reed and Kinzel [20] reported that a conformational switch from β-sheet to α-helix took place as the pentadecapeptide in the C4 region of gp120 centered around Trp411 (the amino acid numbering follows that of the LAV strain of HIV-1) was titrated against trifluoroethanol. They suggested the propensity of the peptide to change conformation in solvents of different polarity as a model for CD4-induced conformational change in gp120. Peptide mimetics have been used to block the interaction between CD4 and gp120 as a potential therapeutic approach to inhibiting HIV infection [21–23]. This approach has several advantages. First, it is easier to deliver a small nontoxic peptide mimic to the desired location than a larger protein, such as soluble CD4. Second, the proteins may interfere with normal functions of the targets. For example, soluble CD4 may compete with the CD4 receptor for binding to class II MHC molecules, whereas a small fragment of CD4 (or its analogues) may bind to the critical region of gp120 but may not hamper the immunological function of CD4.

Several studies have implicated the extracellular domain 1 of CD4 to be a primary gp120 binding...