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Title: Studies of the Conformation and Conformational Stability of Nef Protein of HIV 1.

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ABSTRACT

The nef gene is located near the 3' end of the HIV genome, partially overlapping the U3 region of the long terminal repeat sequence. In vivo, Nef is essential for efficient viral replication and progression of AIDS. The critical role of Nef in AIDS pathogenesis was emphasized when some subjects carrying naturally occurring Nef deletions became long-term nonprogressors.

In this study, the full length Nef was characterized structurally and thermodynamically. The thermodynamic parameters of full length Nef were compared with core domain.

We have characterized the structure of full length Nef using CD and absorbance spectroscopy. Near-UV CD established that Nef has slightly more tertiary structure than Core. Since both proteins had nearly similar tertiary structure, it validates the existing hypothesis that the core domain is the only part of Nef that adopts a tertiary fold. Nef and Core were analyzed for secondary structure content. The mean residue ellipticity at 222 nm ($[\theta]_{222}$) was found to be -9370 and -8850 deg cm^2 $dmol^{-1}$ for Nef and Core, respectively at pH 7.4. It may be concluded from secondary structure content that both Nef and the Core possesses high degree of secondary structures; and both the proteins acquire maximum secondary structures at pH 7.4 which happens to be physiological pH.

Since Core contained maximum secondary structure of the protein we may speculate that the N-terminal may be devoid of major secondary structures.

The heat-induced denaturation of protein established that both Nef and Core were stable in the pH range 8.4-5.4. The maximum stability was found to be at physiological pH where the T_m was 53.3 and 50.6 ^{0}C for Nef and Core respectively. Since the T_m of Nef is 2.7 ^{0}C more than the C-terminal, it can be envisaged the N-terminal may also contribute to the stability of the protein. It will be worthwhile to note that T_m is not a proper index of protein stability for the shift in the denaturation equilibrium. It is, in fact, ΔG_D^o that depends not only on T_m but also on ΔH_m and ΔC_p. To investigate further, the ΔG_D^0 upon thermal unfolding was determined which was found to be 7.65 kcal mol^{-1} and 5.08 for Nef and Core respectively at physiological pH. One interesting finding is that there was a difference of 2.57 kcal mol^{-1} of ΔG_D^0 between Nef and Core protein while the reported value of ΔG_D^0 for N-terminal is 2.7. This additional free energy may correspond to the N-terminal. The value of ΔC_p was found to be 1.65 kcal mol^{-1} K^{-1} for FL Nef while 1.09 kcal mol^{-1} K^{-1}for the Core. GdmCl-induced denaturation of full length Nef monitored by two different optical probes i.e. $[\theta]_{222}$ and $\Delta\varepsilon_{292}$ showed that the unfolding is a two-state process, for the normalized transition curves obtained from different probes are coincident with one another. Furthermore, ΔG_D^0, C_m and m values obtained from both transition curves, suggests that unfolding is a two-state process by GdmCl-induced denaturation.

To conclude, we may hypothesize that although most of the structure of Nef is contained in the Core domain, the N-terminal may contribute significantly to the conformational stability of the protein.