BACKBONE DYNAMICS OF THE EIAV-Tat PROTEIN FROM $^{15}$N RELAXATION STUDIES

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INTRODUCTION

Lentiviruses, such as the equine infectious anemia virus (EIAV) and the human immunodeficiency virus type 1 (HIV-1), cause slow, progressive diseases in their hosts. The gene expression of these viruses is controlled by a potent transactivator (Tat). The EIAV-Tat is a close homologue of the HIV-Tat and simian immunodeficiency virus (SIV) Tat protein. Tat is essential for replication of Lentiviruses. Upon binding to its RNA target, TAR (transactivation responsive element), it is positively regulating the expression of all viral genes. Thus, it is of utmost importance to understand the mechanism of transcriptional activation by Tat in atomic detail in order to be able to design Tat protein inhibiting drugs. In addition, because of the size of 75 amino acids, EIAV-Tat may well be suited to serve as a model compound for general biophysical studies of protein - RNA interactions.

An understanding of protein function requires a detailed knowledge of both time-averaged conformations and conformational fluctuations. Since it has been claimed for EIAV-Tat to adopt a partially flexible structure in aqueous solution as well as in solutions containing variable amounts of trifluorethanol (TFE) [1], the knowledge of an internal mobility of EIAV-Tat protein seems to be especially important. 2D NMR experiments based on heteronuclear correlation provide a powerful approach for studying protein dynamics in solution. Methods based on single-quantum coherence (HSQC) spectra have been recently developed for measuring $^{15}$N or $^{13}$C relaxation parameters ($T_1$, $T_2$ and heteronuclear NOE values)[2]. In the present study we investigated the mobility of the peptide chain by measuring the relaxation parameters of the $^{15}$N amide nitrogens of the peptide backbone. Uniform $^{15}$N isotopically labelled protein differing from the DNA deduced sequence by one amino acid (alanine 2 replaced by glutamic acid) and showing full biological activity was used for this purpose.

ESSENTIAL THEORY

The relaxation data may be interpreted in terms of model-free spectral density functions, yielding information on the amplitudes (via order parameters) and timescale (via correlation times) of internal motions [3]. This work is an attempt to interpret $T_1(^{15}$N) spin-lattice relaxation times and $\{^1$H$\}^{15}$N steady state NOE values, measured at two magnetic fields.
strengths for most of the backbone NH groups of the EIAV-Tat protein, within the framework of the "extended" model-free approach.

Dipolar and CSA contribution to the $T_1(^{15}N)$ relaxation time and $\{^{1}H\}{^{15}N}$ NOE are expressed in terms of the spectral densities, $J(\omega_i)$:

$$1/T_1 = d^2[J(\omega_H+\omega_N) + 3J(\omega_N) + 6J(\omega_H+\omega_N)] + c^2\omega_N^2J(\omega_N),$$

$$f_{NOE} = T_1(y_H/y_N)d^2[6J(\omega_H+\omega_N) - J(\omega_H-\omega_N)].$$

In these expressions, $\omega_H$ and $\omega_N$ are Larmor frequencies for $^{1}H$ and $^{15}N$, $y_H$ and $y_N$ are $^{1}H$ and $^{15}N$ magnetogyratic ratios ($y_H/y_N=-9.869$), $d^2=(\mu_0^2/2\gamma_H^2\gamma_N^2h^2/640\pi^4)\tau_{NH}^{-3}a^2$ and $c^2=(2/15)\Delta \omega^2$.

Using the values of 1.02 Å for the length of the NH amide bond and -160 ppm for the anisotropy of the $^{15}N$ chemical shift tensor one obtains: $d^2=5.19 \times 10^8$ s$^{-2}$ and $c^2=3.41 \times 10^{-9}$.

The spectral density function from the model-free formalism for an isotropically tumbling molecule undergoing internal motions characterized by fast and slow components is:

$$J(\omega) = S_s^2S_f^2\tau_R/[1 + (\omega\tau_R)^2] + S_f^2(1 - S_s^2)\tau_S/[1 + (\omega\tau_S)^2].$$

In this expression $S_s^2$ and $S_f^2$ are the generalized order parameters for fast and slow motions, respectively, $\tau_R$ is the overall rotational correlation time of the molecule and $\tau_S=\tau_R/\tau_{es}/(\tau_S+\tau_{es})$, where $\tau_{es}$ is an effective correlation time describing the slow internal motions. The expression is based on the assumption that the effective correlation time for the fast internal motions is at least two orders of magnitude shorter than $\tau_R$.

**METHOD**

Owing to the relatively high internal mobility of EIAV-Tat protein, its 2D $^{1}H/^{15}N$ spectra show strong overlap of cross-peaks. A successful retrieving of the relaxation data for over 60% of the correlations was possible after applying a powerful deconvolution algorithm allowing to calculate volume integrals for heavily superposed cross-peaks. The algorithm developed in our laboratory relies on generating 3D Lorenzian line-shapes which are subtracted from the experimental spectrum making possible an efficient baseline correction being applied to the remaining part of a spectrum. An SCF approach was used to fit parameters for one extracted peak at a time till parameters for all peaks were calculated and the whole procedure was repeated until a good agreement between the experimental spectrum and calculated one was obtained. Besides the accurate volume integrals the algorithm yielded exact chemical shifts of $^{15}N$ and NH signals and the values of NH-H$_\alpha$ scalar couplings.

Our approach to the analysis of the relaxation parameters was to fit $T_1(^{15}N)$ and $\{^{1}H\}{^{15}N}$ NOE data for each residue simultaneously, optimizing a single value for the overall rotational correlation time $\tau_R$ as well as separate values of the order parameters $S_s^2$ and $S_f^2$ and the correlation time for internal motion $\tau_{es}$ for each residue.
RESULTS AND DISCUSSION

T₁(¹⁵N) relaxation time and ¹H/¹⁵N NOE values were measured at two magnetic field strengths (9.4 T and 14.1 T) at 291 K. Of the 63 ¹H/¹⁵N correlations identified in the 3D heteronuclear Hα/¹⁵N/NH correlation spectrum of EIAV-Tat only 40 are sufficiently resolved to allow accurate peak volume integrals to be calculated by the deconvolution procedure (Fig. 1). An overall rotational correlation time τᵣ of 3.9 ns has been determined. It is shorter than the values obtained for staphylococcal nuclease (9.1 ns) and interleukin-1β (8.3 ns) reflecting a smaller size of EIAV-Tat (75 vs 149 and 153 amino acid residues).

Figure 1. Part of the ¹H/¹⁵N correlation spectrum (600.13/60.81 MHz) of EIAV-Tat protein showing correlations of amide groups.
Figure 2. Experimental and fitted $T_1(^{15}N)$ values (a), $^{1}H$ $^{15}N$ NOE values (b). Calculated model-free approach order parameters (c) and correlation times (d).
Three-dimensional structures of EIAV-Tat based on experimental NOE constraints show possibility of helical conformation in the regions 10-13, 36-44 and 49-61. Unfortunately, the unresolved correlations correspond mostly to the region 49-61 precluding from analysis the most extended helical sequence. A diagram displaying the model-free approach parameters, $S_s^2$, $S_f^2$ and $\tau_{es}$, for all characterized residues is shown in Fig. 2. Correlation times for slow internal motions with values falling between 0.12 ns (His 36) and 1.3 ns (47) show no correlation with an NOE-determined structure. Values of the overall order parameter $S^2 = S_s^2 - S_f^2$ are relatively small (0.23-0.62) reflecting high internal mobility of EIAV-Tat, as expected. The striking feature of the fast and slow motion order parameters, however, is a concerted change of their values in the region 36-44 corresponding to a helical conformation; the $S_s^2$ is larger, whereas the $S_f^2$ smaller than corresponding values in the remaining assigned residues. The distribution of the slow motion order parameter, $S_s^2$, can be interpreted in terms of significantly larger rigidity of a helical region. On the other hand, a hindrance of the slow, large scale motions seems to be compensated by an increased amplitude of fast, local motions. Such behaviour, not typical for rigid, well structured proteins, may reflect a high degree of flexibility of EIAV-Tat protein.