Structural rearrangements on HIV-1 Tat (32–72) TAR complex formation

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Abstract Expression of the early genes of the human immunodeficiency virus type-1 (HIV-1) genome is under the control of a *trans*-activator (Tat) protein. HIV-1 Tat action requires binding to TAR (*trans*-activation responsive element), an RNA sequence located at the 5′-end of all lentiviral mRNAs. We used various spectroscopic methods to investigate conformational changes on HIV-1 TAR binding to the HIV-1 (32–72) Tat peptide BP1. It comprises the RNA binding region and binds specifically to TAR. We conclude from our experiments that the regular A-form of the TAR RNA is slightly distorted towards the B-form when bound to BP1. Thus, the major groove is widened and the binding of BP1 facilitated. BP1 presumably adopts an extended conformation when binding to TAR and may fit well into the TAR major groove.

Key words: (HIV-1); Tat-TAR interaction; Fourier transform infrared spectroscopy; Circular dichroism; UV melting

1. Introduction

The Tat (*trans*-activating) protein activates and enhances DNA transcription by binding to a particular sequence of nascent mRNA, the TAR (*trans*-activation response element) element. Tat is a key regulatory protein as it enhances the expression of all viral genes, and the virus is not able to replicate in the absence of Tat. Tat binds to the uridine-rich bulge of TAR [1–4] and the core and the basic region of Tat are the key elements for specific recognition of TAR [5–10]. Structural studies of the Tat TAR interaction have shown conformational changes in TAR on binding of various Tat peptides [11–14]. The extent to which the recognition region of HIV-1 Tat undergoes conformational changes when bound to TAR RNA is still unknown. For the bovine immunodeficiency virus (BIV) conformational changes occur in both the TAR RNA and the Tat peptide when they form a complex [15,16]. To gain more insight into the nature of conformational changes of HIV-1 Tat and TAR on complex formation we investigated a model of the complex by circular dichroism (CD), Fourier transform infrared (FTIR) and UV-melting studies. We used a 29-mer HIV-1 TAR RNA and the HIV-1 Tat peptide fragment BP1 which comprises the whole wild-type core and basic sequence regions of Tat, amino acids 32 to 72, that are sufficient for strong and specific binding to TAR [6].

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Abbreviations: BIV, bovine immunodeficiency virus; BP1, peptide fragment 32–72 comprising the core and basic sequence regions of HIV-1 Tat protein; CD, circular dichroism; EIAV, equine infectious anemia virus; FTIR, Fourier transform infrared; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; MALDI, matrix assisted laser desorption ionisation; Tat, *trans*-activator; TAR, *trans*-activation response element.

2. Materials and methods

2.1. Sample preparation

The 29-nucleotide HIV-1 TAR RNA was synthesized by in vitro transcription using T7 RNA polymerase and synthetic DNA templates (MWG-BioTech, Ebersberg) [17]. TAR RNA was ethanol precipitated and purified by 20% polyacrylamide gel eletrophoresis under denaturing conditions (8.3 M urea). TAR RNA product was eluted from the gel by using a Schleicher and Schuell electroelution apparatus and subsequent ethanol precipitation. The RNA was dialysed to remove multivalent ions and other low molecular weight impurities against a high EDTA buffer (10 mM phosphate, 5 mM EDTA, 100 mM NaCl, pH 6.4), a low EDTA buffer (10 mM phosphate, 0.1 mM EDTA, 100 mM NaCl, pH 6.4), and finally against water. TAR RNA was then lyophilized and stored at −20°C. Several reactions on a 5 ml scale yielded more than 20 mg of purified RNA.

BP1 was synthesized on an automated continuous-flow peptide synthesizer on polystyrene-polyethylene glycol copolymer (Tentagel) using 9-fluorenylmethyloxycarbonyl chemistry and (benzotriazo-1-yl)-*N*-oxytripyrrolidinophosphonium hexafluorophosphate as activator [18]. The product was purified by reversed-phase high-performance liquid chromatography (HPLC) to more than 95% purity based on mass spectroscopic analysis (Shimadzu Compact MALDI). The synthetic peptide was freeze-dried and stored at −20°C.

2.2. Thermodynamic analysis

Equilibrium melting curves were collected on a Hewlett-Packard 8452A diode array spectrophotometer equipped with a Peltier element and a temperature sensor inserted into the 1 cm cell. Before each measurement the RNA component (2 μM) was renatured by heating to 90°C for 3 min followed by slow cooling to room temperature in 10 mM potassium phosphate, 50 mM NaCl, pH 6.4. The molar ratio of BP1 HIV-1 and TAR was 1:1, and of argininamide to HIV-1 TAR RNA, 6:1. Samples were heated at a rate of 0.4°C per min. Spectra from 235 nm to 350 nm were recorded at intervals of 1°C. Reversibility of the melting process was examined by cooling to the initial temperature. Thermal denaturation curves were followed by the increase of absorbance at 260 nm. The midpoints of the melting transitions, T_m, were determined as described [19].

2.3. FTIR spectroscopy

FTIR spectra were obtained with a Bruker IFS 66v Fourier transform IR spectrometer. 200 scans were collected and Fourier transformed to obtain spectra with 2 cm^{-1} resolution. All measurements were performed in a CaF_2 cell with 25 μM pathlength at room temperature. Spectra were obtained for the HIV-1 TAR alone (0.3 mM) and for the BP1-HIV-1 TAR complex at 1:1 molar ratio (0.3 mM) in a solution containing 10 mM potassium phosphate, 50 mM NaCl at pH 6.4.

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2.4. CD spectroscopy

CD spectra were measured with a Jasco J600A spectropolarimeter in thermostatically controlled cells at 25°C. The buffer was 10 mM potassium phosphate, 50 mM NaCl at pH 6.4. Concentrations of the HIV-1 TAR and of the 1:1 BP1-HIV-1 TAR complex were 30 μM in a 0.05 cm cell. The reference sample contained buffer without RNA and/or peptide, and spectra were measured three times at a scan rate of 20 nm/min, averaged and corrected for contributions of the respective solvent.

2.5. Secondary-structure prediction

Secondary-structure predictions were performed by the methods of Gibrat et al. [20], Levin et al. [21] and Deleage and Roux [22] as supplied by the mail server of the Institute de Biologie et Chimie des Proteines (Lyon) and by the PHD neural network structure prediction method [23] as obtained via the European Molecular Biology Laboratory (EMBL) mail server.

3. Results

3.1. UV melting

To determine the conformational stability of the hairpin structure of HIV-1 TAR thermal melting experiments were carried out with and without specific and unspecific ligands. Binding of BP1 to TAR enhanced significantly the thermostability of HIV-1 TAR. In the presence of the peptide, the midpoint of the thermal transition (T_m) of the RNA increased from 65°C for the unbound HIV-1 TAR to 71°C (Fig. 1). To confirm that this is a specific effect of the BP1 peptide, in a control experiment argininamide was added at a 6-fold molar excess. Argininamide binds to HIV-1 TAR in a rather non-specific manner [6] and left the thermal unfolding transition of TAR virtually unchanged (Fig. 2). Although the free peptide is unstructured in solution (as indicated by CD experiments, see below) the increased thermostability suggests the formation of a specific RNA peptide complex with a high stability over a wide temperature range. Stabilizing effects on their nucleic acid target were previously reported for some polypeptides [24,25].

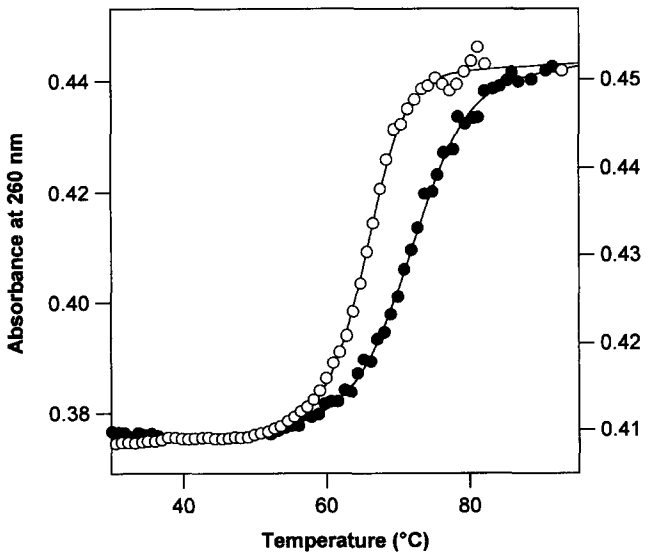

Fig. 1. Melting curves of HIV-1 TAR (○, right vertical scale) and the BP1-TAR RNA complex at 1:1 molar ratio (●, left vertical scale) as monitored by the absorbance at 260 nm. Measurements were made in 10 mM potassium phosphate, 50 mM NaCl (pH 6.4). The BP1 and the TAR RNA concentrations were 2 μM.

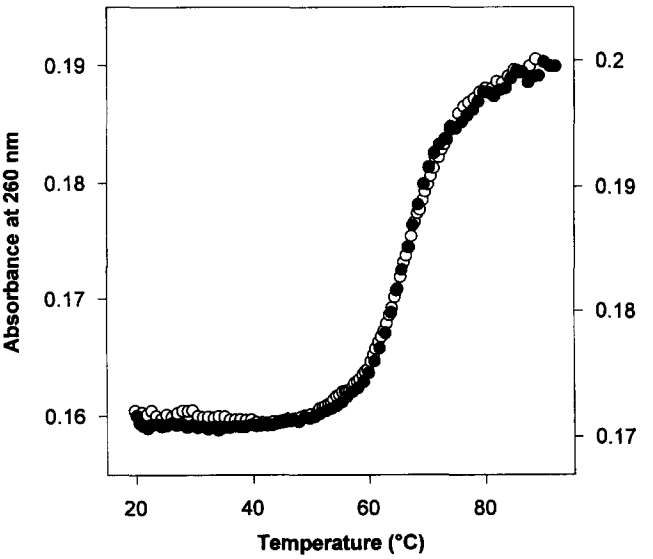

Fig. 2. UV absorbance melting curves of HIV-1 TAR (○, left vertical scale) and the argininamide-TAR RNA complex at 6:1 stoichiometric ratio (●, right vertical scale). Measurements were made in 10 mM potassium phosphate, 50 mM NaCl (pH 6.4). The TAR RNA and the argininamide concentrations were 2 and 12 μM, respectively.

3.2. FTIR spectroscopy

For free TAR an absorbance maximum at 1243 cm^{-1} was observed, characteristic of the antisymmetric O-P-O phosphate stretching vibration in the sugar phosphate backbone of RNA in the regular A-form helix. This band shifted to 1223 cm^{-1} on addition of BP1 (Fig. 3), indicating a conformational change in the RNA backbone and involvement of the phosphates in the interaction with the BP1 peptide [26,27]. No major peptide signals contribute significantly to intensity in this spectral region [44]. The change of the positions of the absorption maxima suggests a distortion of the A-helix (1243 cm^{-1}) towards a B-helix (1223 cm^{-1}) [27] as well as the formation of hydrogen bonds or ionic contacts between phosphates and peptide. This could imply that the narrow major groove as found in a regular A-helix and in free TAR may be widened on peptide binding which is consistent with the results of recent NMR studies by Varani and co-workers on HIV-1 TAR complexed by a similar peptide [14]. They showed that peptide binding induced a conformational alteration in TAR which enhanced the accessibility of functionally important groups in the major groove near the RNA bulge.

3.3. CD spectroscopy

The CD spectrum of TAR alone is characteristic of an A-form RNA with a long-wavelength band centred around 265 nm and a fairly intense negative band around 210 nm (Fig. 4). The strong RNA signals dominate the CD spectrum of the BP1 TAR complex. The shapes of both CD spectra are very similar suggesting that the overall conformation of TAR is in the A-form in the complex with BP1. An increase in the intensity of the 240-nm band, a decrease as well as a red shift of the 265-nm band, and a red shift of the crossover from 239 nm to 241 nm indicate a marginal distortion of the A-form helix towards the B-form [13]. Between 240 and 300 nm no contribution of the peptide CD spectrum is expected, ensuring

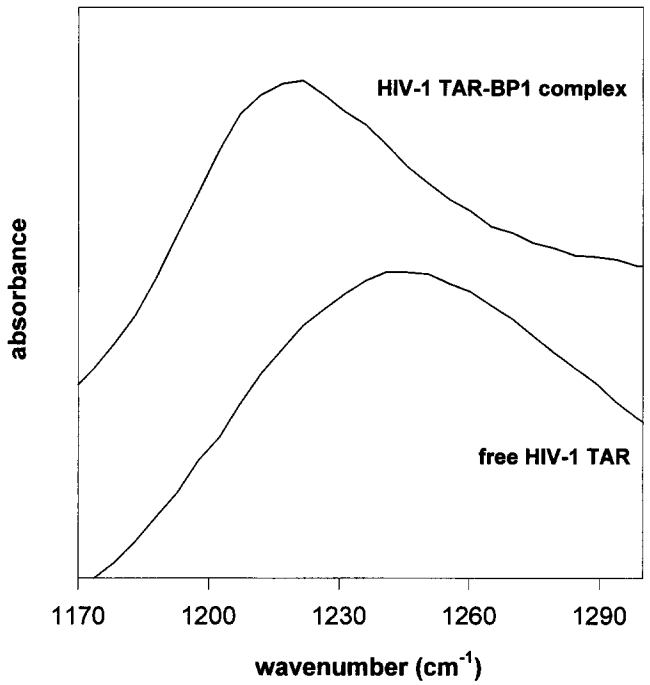

Fig. 3. Direct FTIR solution spectra of HIV-1 TAR and the BP1-TAR RNA complex at 1:1 molar ratio in 10 mM potassium phosphate, 50 mM NaCl (pH 6.4) at 25°C. The concentrations of the RNA and peptide were 0.3 mM.

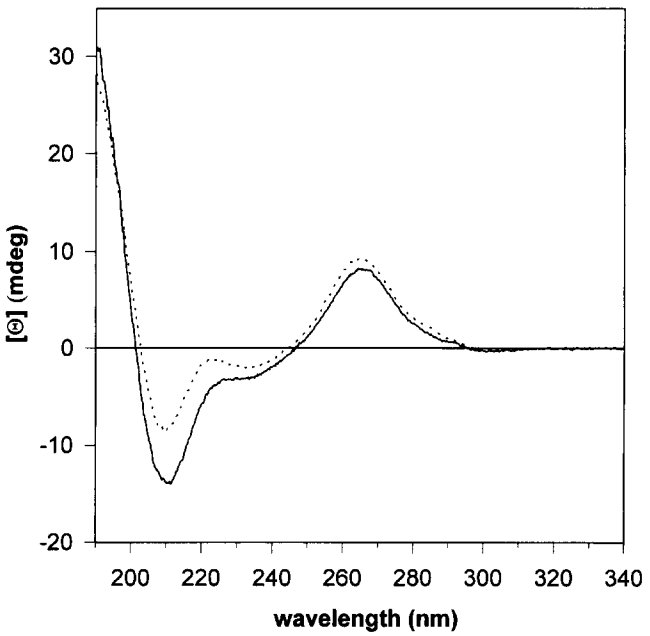

Fig. 4. UV-CD spectra of HIV-1 TAR (broken line) and the BP1-TAR RNA complex at 1:1 molar ratio (solid line) in 10 mM potassium phosphate, 50 mM NaCl (pH 6.4) at 25°C. The concentrations of the RNA and peptide were 30 μM in 0.05 cm cells. The MRW normalisation of the ellipticity is not possible in this case, since one of the samples is heterogeneous.

that the observed changes originate exclusively from the RNA [28]. TAR was previously suggested to adopt B-helix character on binding of smaller Tat-related peptides [13], in agreement with FTIR results. The CD spectrum of free BP1 (Fig. 5) with its minimum near 200 nm shows that BP1 is in an unfolded conformation, consistent with NMR data (Bayer et al., unpublished data). As the TAR spectrum was only marginally distorted on TAR binding to Tat, the difference-CD spectrum derived from the TAR-BP1 complex and free TAR (Fig. 5) should provide a rough guess of the CD spectrum of the bound peptide, and previous studies show that difference-CD spectra are well suited to predict the secondary structures correctly [15,16,29]. According to this procedure, the minimum of molar ellipticity is shifted to 217 nm, an effect typical of polypeptides adopting extended conformations, such as β-strands [28]. As TAR may also contribute to this far-UV region, the extended conformation of bound BP1 cannot be determined safely from this approach. Summarizing, FTIR and CD results would be in agreement with the notion of BP1 adopting an extended conformation on binding in the widened major groove of TAR.

3.4. Secondary structure prediction

To investigate the conformation preference of the amino acid sequence we performed secondary structure predictions using standard protocols. Fig. 6 shows clearly that all these protocols predict a β-type conformation for the core sequence of BP1, in agreement with BP1 adopting an extended conformation in this region, as observed by the CD studies on the BP1-TAR complex.

4. Discussion

The present results suggest that the interaction of BP1 peptide with HIV-1 TAR RNA involves noticeable conformational changes of both components, as observed earlier for other protein-nucleic acid interactions [30–32]. BP1 comprises the highly flexible basic region and the adjacent compact core sequence region of the full-length HIV-1 Tat protein [33]. In

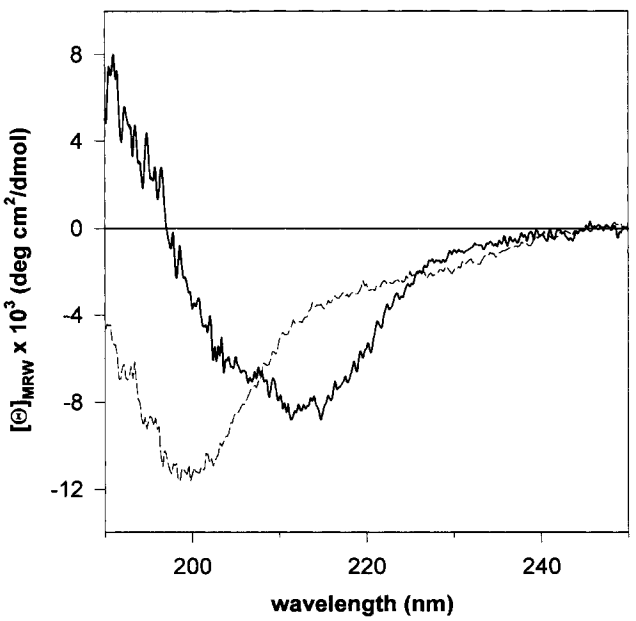

Fig. 5. Far-UV CD spectrum of the BP1 peptide (broken line) and the difference CD spectrum of the BP1-TAR RNA complex minus HIV-1 TAR (solid line) in 10 mM potassium phosphate, 50 mM NaCl (pH 6.4) at 25°C.

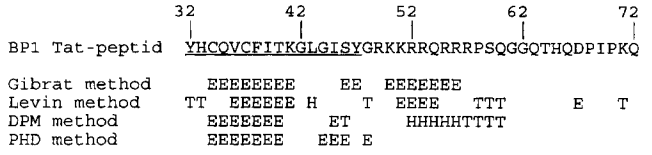

Fig. 6. Secondary structure predictions of BP1 peptide. H, α-helix; E sheet; T, turn. Core sequence region is underlined.

the free form it is in an unfolded conformation. When bound to TAR, the peptide, however, exhibits a CD spectrum suggesting an extended conformation as in β-sheets. This is in good agreement with the results of several sequence based secondary structure predictions, but in contrast to previous structural studies on various Tat peptide-TAR systems that suggested helical tendencies of the basic sequence region alone or on RNA binding [13,34]. In an NMR study of an EIAV-HIV-1 Tat chimeric peptide comprising the core sequence region of EIAV Tat and the basic region of HIV-1 Tat, a high α-helix content in the basic region of the chimeric peptide was observed [34]. The EIAV core sequence region, however, has some intrinsic potential for helix formation [35,36]. Thus, the chimeric peptide may exhibit a higher tendency to form an α-helix than a peptide with the HIV-1 Tat wild type sequence.

Tat and TAR interaction occurs in the deep A-form major groove of the RNA near the bulge [6,7]. Even a slightly widened major groove of the HIV-1 TAR, however, would be too narrow to accommodate an α-helical peptide [37,38].

Extended conformations are found at the recognition sites of both DNA minor groove binding proteins (TATA-box binding protein) [39,40] and RNA binding proteins [37,41,42] when steric requirements render the formation of α-helices disadvantageous. The DNA minor groove has a geometry comparable to that of the A-form RNA major groove.

CD and FTIR measurements suggest a minimal transition from the A to B form of TAR on peptide binding as well as an involvement of backbone phosphates in the interaction with BP1 as originally proposed [6,7,43]. In agreement with this observation, Varani and co-workers [14] showed that free TAR contains a large degree of helical discontinuity, leading to a more accessible major groove that forms the contact area with the peptide. The structure of the BIV TAR Tat peptide complex shows that the peptide containing the basic sequence region of the BIV Tat protein adopts a β-turn conformation and also binds to the widened major groove of the BIV TAR RNA [15,16]. The experimental results on Tat-TAR interactions for BIV and HIV-1 thus seem to become more consistent, and a fuller picture emerges.

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