Circular Dichroism Studies Suggest That TAR RNA Changes Conformation upon Specific Binding of Arginine or Guanidine†

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ABSTRACT: Short basic peptides from the HIV Tat protein bind specifically to a bulge region in TAR RNA, with a single arginine residue providing the only sequence-specific contact. The free amino acid arginine also binds specifically to TAR. Previous circular dichroism (CD) experiments suggested that peptide binding induces a conformational change in TAR. Here we confirm this observation using single arginine-containing peptides and show that arginine or guanidine binding also induces a conformational change in TAR. A peptide containing a single arginine within a stretch of histidines (CYHHHRHHHHHA) shows pH-dependent binding and a corresponding change in TAR conformation, as detected by a decrease in the CD signal at 265 nm. Arginine and guanidine, which bind to TAR with apparent K_d's of ~1.5 mM, induce similar CD changes. In contrast, lysine, which does not bind specifically to TAR, has no effect. Mutants of TAR that abolish specific binding (a U→C substitution in the three-nucleotide bulge, a deletion of the bulge, or an A-U to U-A base pair change above the bulge) show no change in the CD signal upon binding of peptides, arginine, or guanidine. The results suggest that binding of a single guandinium group to a specific site in TAR induces a change in RNA conformation.

The Tat protein from human immunodeficiency virus (HIV) is a potent activator of HIV transcription and is essential for viral replication. The precise mechanism by which Tat acts is still under investigation, but it appears that Tat works, at least in part, by increasing the efficiency of transcriptional elongation [see review by Frankel (1992) and references cited therein]. Transactivation is dependent on the specific interaction of Tat with the trans-acting responsive (TAR) element, an RNA hairpin located at the 5′ end of the viral mRNAs (Roy et al., 1990; Dingwall et al., 1990; Cordingley et al., 1990; Weeks et al., 1990; Calnan et al., 1991a).

Studies with peptides have shown that the TAR RNA-binding domain of Tat is contained within a short region of basic amino acids, having six arginines and two lysines within nine residues (Weeks et al., 1990; Cordingley et al., 1990; Calnan et al., 1991a). Further studies have shown that a single arginine residue provides the only sequence-specific contact with the RNA (Calnan et al., 1991b) and that the arginine must be surrounded by at least three basic acids on each side to provide nonspecific electrostatic contacts and increase the RNA-binding affinity (Calnan et al., 1991b; J. Tao and A. D. Frankel, submitted for pulication). The free amino acid arginine binds specifically to the same site in TAR as does the Tat peptide (Tao & Frankel, 1992). The observations that the Tat peptides are unstructured (Calnan et al., 1991a) and that free arginine can bind specifically to TAR have led to the conclusion that the precise three-dimensional structure of TAR must be a major determinant of Tat/TAR recognition. This is consistent with many studies of tRNA synthetases and bacteriophage R17 coat protein which show the essential role of RNA structure in RNA–protein recognition [see Rould et al. (1989, 1991), Ruff et al. (1991), and Witherall et al. (1991) for examples].

A considerable amount is known about TAR RNA structure and the requirements for Tat and arginine binding. Nuclease mapping experiments first showed that TAR forms a stable stem–loop structure (Muesing et al., 1987). Subsequent deletion analysis showed that the upper part of the stem–loop (nucleotides +19 to +42 relative to the start of HIV transcription) was sufficient for the Tat response in vivo (Jakobovits et al., 1988). The TAR hairpin contains a six-nucleotide loop and a three-nucleotide bulge (see Figure 1) that are both essential for Tat activity. Tat binding appears to be limited to the region around the bulge (Roy et al., 1990; Dingwall et al., 1990; Cordingley et al., 1990; Weeks et al., 1990; Calnan et al., 1991a,b) while cellular factors may bind to the loop (Marciniak et al., 1990; Wu et al., 1991; Sheline et al., 1991). Although the size of the bulge is not so critical for Tat binding (it must contain at least two nucleotides), there is an important requirement for uridine at the 5′-most position of the bulge (+23) (Weeks & Crothers, 1991; Sumner-Smith et al., 1991). The identity of several base pairs surrounding the bulge is also important; in particular, the two base pairs immediately above the bulge, G26–C39 and A27–U38, are essential (Weeks & Crothers, 1991; Berkhout & Jeang, 1991). In addition to these possible base contacts, it was found that ethylation of two phosphates, located at the junction of the double-stranded stem and bulge, strongly interfered with peptide or arginine binding (Calnan et al., 1991b; Tao & Frankel, 1992). This led to a model in which part or all of the specificity may result from a set of hydrogen bonds between the arginine guanidinium group and two highly oriented phosphates, an interaction termed the "arginine fork" (Calnan et al., 1991b). Another model, based on chemical modification studies of TAR (Weeks & Crothers, 1991), suggested that Tat peptides might interact with specific groups in the RNA major groove. Recent NMR studies suggest that arginine donates hydrogen bonds to the two phosphates and to G26 in the major groove and that the complex is stabilized by a base triple interaction between U23 and A27–U38 (Puglisi et al., 1992).

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