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Circular Dichroism Studies of the HIV-1 Rev Protein and Its Specific RNA Binding Site†

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ABSTRACT: The circular dichroism (CD) spectrum of the Rev protein from HIV-1 indicates that Rev contains about 50% α helix and 25% β sheet at 5 °C in potassium phosphate buffer, pH 3, and 300 mM KF. The spectrum is independent of protein concentration over a 20-fold range. At neutral pH, Rev is relatively insoluble but can be brought into solution by binding to its specific RNA binding site, the Rev-responsive element (RRE), at a Rev:RNA ratio of about 3:1. Nonspecific binding to tRNA does not solubilize Rev. As judged by difference CD spectra, the conformation of Rev when bound to the RRE at neutral pH is similar to the conformation of unbound Rev at pH 3, although changes in the RNA may also contribute to the difference spectrum. Indeed, some difference is observed near 260 nm, consistent with a conformational change of the RRE upon Rev binding. Rev alone at pH 3 shows irreversible aggregation as the temperature is raised, while Rev bound to the RRE at neutral pH shows a reversible transition with a T_m of 68 °C.

Human immunodeficiency virus (HIV) encodes several regulatory proteins in addition to the Gag, Pol, and Env gene products encoded by most retroviruses. At least two of these regulatory proteins, Tat and Rev, are essential for viral replication (Fisher et al., 1986; Dayton et al., 1986; Sodroski et al., 1986; Terwilliger et al., 1988) and therefore are potential targets for anti-HIV drugs. Tat is a transactivator that increases expression of all viral genes and seems to function at both the transcriptional and posttranscriptional levels [see Cullen and Greene (1989) for a review]. Rev, a small protein of 13 000 daltons, appears to function posttranscriptionally (Malim et al., 1989; Hammarskjold et al., 1989) and is localized to the nucleus and nucleolus (Cullen et al., 1988; Perkins et al., 1989). In the absence of Rev, viral transcripts are fully spliced, resulting in production of both Rev and Tat proteins (Knight et al., 1987). Rev then increases the levels of incompletely spliced mRNAs that are transported to the cytoplasm, shifting viral protein synthesis toward production of the structural proteins (Sodroski et al., 1986; Feinberg et al., 1986; Knight et al., 1987; Sadaie et al., 1988; Malim et al., 1988; Hammarskjold et al., 1989). It has not yet been determined whether Rev acts by suppressing splicing (Feinberg et al., 1986; Emerman et al., 1989), by causing release of mRNAs from the spliceosome (Chang & Sharp, 1989), or by facilitating mRNA transport (Malim et al., 1989a; Felber et al., 1989; Hammarskjold et al., 1989).

The site of action for Rev is a highly conserved sequence located within the *env* gene, called the Rev-responsive element (RRE) (Malim et al., 1989a; Hadzopoulou-Cladaras et al., 1989; Rosen et al., 1988). This region forms a highly structured RNA element which must be maintained in the proper orientation to remain Rev-responsive (Dayton et al., 1989; Olsen et al., 1990; Malim et al., 1989a). Deletions within the RRE have defined at least one domain, the "hammerhead", as being necessary for Rev function (Dayton et al., 1989; Olson et al., 1990; Malim et al., 1990). Recently, several studies have shown that purified Rev binds with high affinity and

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