Retroviral integration protein (IN) has been shown to be both necessary and sufficient for the integration of reverse-transcribed retroviral DNA into the host cell DNA. It has been demonstrated that self-assembly of IN is essential for proper function. Analytical ultracentrifugation was used to determine the stoichiometry and free energy of self-association of a full-length IN in various solvents at 23.3 °C. Below 8% glycerol, an association stoichiometry of monomer-dimer-tetramer is observed. At salt concentrations above 500 mM, dimer is the dominant species over a wide range of protein concentrations. However, as physiological salt concentrations are approached, tetramer formation is favored. The addition of glycerol to 500 mM NaCl, 20 mM Tris (pH 8.4), 2 mM β-mercaptoethanol significantly enhances dimer formation with little effect on tetramer formation. Furthermore, as electrostatic shielding is increased by increasing the ionic strength or decreasing the cation size, dimer formation is strengthened while tetramer formation is weakened. Taken together, the data support a model in which dimer formation includes favorable buried surface interactions which are opposed by charge-charge repulsion, while favorable electrostatic interactions contribute significantly to tetramer formation.

A distinguishing characteristic of retroviruses is the integration of their genome into host DNA. Integrase (IN) is the enzyme responsible for many of the processes leading to genomic insertion. Because genomic insertion is an essential step in the life cycle of retroviruses, IN has been the focus of intense study (1, 2).

Both the full-length primary structure (3) and the crystallographic structures for the catalytic portion of IN from the avian sarcoma virus (ASV) and human immunodeficiency virus-I (HIV-I) have been reported (4, 5). Full-length, wild type HIV-I IN exhibits limited solubility in aqueous solvents, making it difficult to conduct solution studies with this protein (6). However, wild type, full-length ASV IN is relatively soluble, making it a more practical protein for studying its aqueous solution behavior (7).

ASV IN has a monomer molecular weight of 31,750 (8). Structural analyses suggest that its catalytic core is similar to that of HIV-I IN (5). Furthermore, ASV IN and HIV-I IN are both members of a superfamily of polynucleotidyl transferases that show similar structure and, presumably, use similar mechanisms of catalysis (3). All studies to date indicate that self-assembly is required for IN activity (3). Studies by Jones, et al. (7) showed that ASV IN formed dimers and tetramers in 50 mM Tris (pH 8.0), 750 mM NaCl, and 2 mM βME. Other studies have demonstrated that enzymatically active fragments of ASV IN and HIV-I IN also self-associate (3) and that assembly is required for activity. In addition, a more soluble, mutant, full-length HIV-I IN has been isolated that exhibits a dimer-tetramer equilibrium when examined in 25 mM HEPES (pH 7.5), 1 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, and 10% (w/v) glycerol (6).

To date, there is no crystal structure available for full-length ASV IN. However, partial proteolysis experiments, deletion mutation results, and sequence similarity with other retroviral integrases, retroposons, and bacterial insertion elements suggest that there are three distinct domains in full-length ASV IN: the N-terminal, central core, and C-terminal domains (3). Structural analysis reveals that both the central core and C-terminal domains form dimers (9, 10). All three domains have been implicated in the self-association of the full-length proteins. The N-terminal domain contains an HCC, Zn2+ finger motif, and there is evidence that Zn2+ promotes self-association and alters the assembly stoichiometry of mutant, full-length HIV-I IN (11). Deletion mutations indicate that the C-terminal domain is necessary for tetramer formation (6). However, there is no convincing evidence for which domain interfaces are responsible for dimer formation and which are responsible for tetramer formation.

The aim of this study is to better characterize the self-association of wild type, full-length ASV IN, and in so doing, to contribute to a better understanding of IN function. Here we report the effects of pH, ionic strength, cation type, and glycerol concentration on full-length, wild type ASV IN dimer and tetramer formation. All of these solvent properties affect the self-association significantly, indicating that care must be taken when comparing published results acquired under different conditions. Furthermore, the results suggest that the electrostatic contributions to dimer and tetramer formation are very different, with charge-charge repulsion opposing dimer formation, and favorable electrostatic interactions (e.g. dipole-dipole) favoring tetramer formation.

EXPERIMENTAL PROCEDURES

Materials—ASV integration protein (IN) was purified as described previously (12). Protein was stored at −20 °C in a buffer of 50 mM Tris (pH 7.5), 0.5 M NaCl, 2 mM dithiothreitol, 0.1 M EDTA, and 40% glycerol. Sterile Centrex cellulose acetate columns for the centrifugal gel exchange were purchased from Schleicher and Schuell, Keene, NH. Sephadex G-25 beads were purchased from Amersham Pharmacia Bio-

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‡ The abbreviations used are: IN, integrase; ASV, avian sarcoma virus; HIV-I, human immunodeficiency virus-I; MES, 2-[N-morpholino]ethanesulfonic acid; βME, β-mercaptoethanol.

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ASV Integrate Self Association

Solubility of ASV IN as a Function of Ionic Strength—The solubility of IN was determined by the "hinge-point" method (19) in solvents containing varying concentrations of either Na⁺ or K⁺. The solubility increases as the ionic strength increases for both cations (Fig. 1). However, at all ionic strengths, ASV IN is more soluble in KCl than in NaCl containing buffers. These results suggest that electrostatic contributions are important to ASV IN aggregation. To explore this possibility further, sedimentation equilibrium experiments were undertaken to assess the effect of varying salt concentration and pH on ASV IN self-association.

The Effect of Salt Concentration on ASV IN Self-association at pH 8.4—Short column sedimentation equilibrium experiments were conducted to determine \( \Delta G_{1\rightarrow2} \) and \( \Delta G_{2\rightarrow3} \), as a function of salt concentration, using either Na⁺ or K⁺-containing solvents (Fig. 2). For both cations, there is an overall strengthening of dimer formation and weakening of tetramer formation as the salt concentration is increased. These effects appear to saturate, with both dissociation energies becoming nearly constant at salt concentrations above 0.5 M for either cation.

The solubility limit of ASV IN precluded analysis of self-association in the ultracentrifuge at physiological salt concentrations. At the lowest ionic strength, however, tetramer formation is the energetically favored species for either cation. These results suggest that tetramer would be the favored species at physiological salt concentrations.

At higher salt concentrations, both dimer and tetramer for-
mM NaCl and varying amounts of glycerol (Fig. 5). The monomer-dimer equilibrium could be observed only at glycerol concentrations of less than 8%. At higher glycerol concentrations, there was insufficient monomer to detect the monomer-dimer equilibrium, and only the dimer-tetramer association was characterized. Remarkably, the dimer-tetramer equilibrium is virtually unaffected by glycerol concentrations as high as 40%. The fact that the monomer-dimer and dimer-tetramer equilibria exhibit such different sensitivities to glycerol strongly suggests that solvation effects are very different for these two assembly steps.

**ASV IN Self-association in Solvents with Different Cations**—Distinctly different effects are observed on $\Delta G_{2\rightarrow1}$ and $\Delta G_{4\rightarrow2}$ when these dissociation energies are determined in solvents containing different cations at 250 mM (Fig. 6). The cation species are arranged in this figure in increasing order of electrostatic shielding potential. Both $\Delta G_{2\rightarrow1}$ and $\Delta G_{4\rightarrow2}$ exhibit clear trends, with dimer formation being strengthened slightly, while tetramer formation is weakened considerably, as the cationic shielding potential increases. These results are in accord with the ionic strength effects (Figs. 2 and 4) and indicate

**The Effect of pH on ASV IN Self-association in 500 mM KCl**—The charge per monomer of ASV IN estimated from its amino acid composition is about +12e at pH 8.4, rising almost linearly to +20e at pH 6.1. Thus, a change in the association behavior might be expected as the pH is varied. To test this, $\Delta G_{2\rightarrow1}$ and $\Delta G_{4\rightarrow2}$ were determined over a pH range of 6.1 to 8.4. The free energy of both dimer and tetramer formation were found to be nearly constant, changing only by a few kJ/mol as the pH is increased from 6.1 to 8.4 (Fig. 3). These results indicate that ionic strengths of 500 mM or greater are sufficient to suppress electrostatic effects.

**The Effect of Salt Concentration on ASV IN Self-association at pH 6.1**—Previous work at ionic strengths well below 500 mM has shown that both the full-length ASV and its catalytic core are essentially enzymatically inactive at pH 6, probably because of a small structural rearrangement (10). To further determine how the self-association of the IN is affected by salt concentration at lower pH, the salt dependence of $\Delta G_{2\rightarrow1}$ and $\Delta G_{4\rightarrow2}$ were determined at pH 6.1 (Fig. 4). In general, the protein is more soluble at lower ionic strengths at this lower pH, possibly because of the higher net charge per IN monomer, allowing studies at lower salt concentrations. While the overall dependence of the self-association on salt concentration (Fig. 4) is similar to that observed at pH 8.4 (Fig. 2), at pH 6.1 tetramer is significantly more stable in KCl than NaCl. Furthermore, at physiological KCl concentrations, tetramer is the clearly the favored oligomer at pH 6.1.

**The Effect of Glycerol Concentration on ASV IN Self-association**—Many studies of both ASV and HIV-I IN include varying amounts of glycerol in the solvent (6, 26–34). To determine the effect glycerol might have on ASV self-association, a series of experiments were conducted at pH 8.4 in buffer containing 500 mM NaCl and varying amounts of glycerol (Fig. 5). The monomer-dimer equilibrium could be observed only at glycerol concentrations of less than 8%. At higher glycerol concentrations, taken together with the solubility results, these data suggest that tetrameric ASV IN is less soluble than either monomer or dimer under these conditions.

**The Effect of pH on ASV IN Self-association in Solvents with Different Cations**—Many studies of both ASV and HIV-I IN include varying amounts of glycerol in the solvent (6, 26–34). To determine the effect glycerol might have on ASV self-association, a series of experiments were conducted at pH 8.4 in buffer containing 500 mM NaCl and varying amounts of glycerol (Fig. 5). The monomer-dimer equilibrium could be observed only at glycerol concentrations of less than 8%. At higher glycerol concentrations, taken together with the solubility results, these data suggest that tetrameric ASV IN is less soluble than either monomer or dimer under these conditions.
that electrostatic interactions contribute measurably and in an opposite manner to IN dimer and tetramer formation. The more pronounced effect of shielding potential on tetramer formation suggests a larger electrostatic contribution to this interaction than to dimer formation.

The Effect of Temperature on ASV IN Self-association—When examined in 500 mM KCl at pH 8.4, there were only small increases in $\Delta G_{2 \rightarrow 4}$ and $\Delta G_{4 \rightarrow 1}$ as the temperature was raised from 4 to 37 °C (Fig. 7). This suggests that either enthalpic contributions to assembly are small or that there is significant enthalpic/entropic compensation in both assembly steps.

DISCUSSION

The self-association of ASV IN has been examined in a variety of solvent conditions. The assembly was described satisfactorily as a freely reversible monomer-dimer-tetramer equilibrium, except in solvents containing >8% v/v glycerol, where only the dimer-tetramer equilibrium was observed. As these data show, there are significant changes in the free energies of both steps of assembly as the solvent conditions are varied. Of particular note is the rather dramatic, and opposite, effect that increasing salt concentration has on $\Delta G_{2 \rightarrow 1}$ and $\Delta G_{4 \rightarrow 2}$ (Figs. 2 and 4), as well as in the different response of $\Delta G_{2 \rightarrow 1}$ and $\Delta G_{4 \rightarrow 2}$ to changes in cation shielding potential (Fig. 6).

Qualitatively, the salt-dependent increase in $\Delta G_{2 \rightarrow 1}$ (Fig. 2 and 4) with increasing salt concentration is in accord with dimer formation being opposed by charge-charge repulsion between monomers. At the same time the decrease in $\Delta G_{4 \rightarrow 2}$ with increased salt concentration suggests that electrostatic interactions favor tetramer formation. However, the shape of these curves, particularly their nearly mirror image nature and the apparent saturation of $\Delta G_{2 \rightarrow 1}$ and $\Delta G_{4 \rightarrow 2}$ at high salt concentrations, suggest that either specific ion binding or a less specific electrostatically dependent rearrangement in the protein structure may occur. In an attempt to distinguish between these two possibilities, $\Delta G_{2 \rightarrow 1}$ and $\Delta G_{4 \rightarrow 2}$ were measured at a fixed ionic strength, but using cations of different size and thus different shielding potentials (Fig. 6). In this figure, the trends in $\Delta G_{2 \rightarrow 1}$ and $\Delta G_{4 \rightarrow 2}$ as the shielding potential is varied are the same as those seen when the ionic strength is varied. This result suggests that the effects are more a consequence of general electrostatic behavior than they are because of specific ion binding.

Varying the shielding potential has a greater effect on $\Delta G_{4 \rightarrow 2}$ than it does on $\Delta G_{2 \rightarrow 1}$, which suggests that there is a greater electrostatic contribution to $\Delta G_{4 \rightarrow 2}$ than there is on $\Delta G_{2 \rightarrow 1}$ (Fig. 6). Consistent with this view is the minimal effect of glycerol on $\Delta G_{4 \rightarrow 2}$ (Fig. 5), as might be expected for an interaction that is driven largely by electrostatics and is less dependent on solvation effects. By contrast, $\Delta G_{2 \rightarrow 1}$ increases substantially with glycerol concentration, as might be expected for an interaction in which solvation effects and buried surface area are important (35).

Jenkins, et al. (6) have described the self-association properties of a mutant HIV-I IN. In accord with our results on the effect of glycerol (Fig. 5), they observed only a dimer-tetramer equilibrium at 4 °C in 10% glycerol, 25 mM HEPES (pH 7.5), 1 mM NaCl, 1 mM dithiothreitol, and 1 mM EDTA. Although none of our solvent conditions exactly match these, comparison with our results is invited. They report a tetramer to dimer dissoci-
ation constant of $2.2 \times 10^{-5}$ M, which yields a tetramer dissociation energy of 24.7 kJ/mol. This is within the confidence interval for what we observe, which is 22.0 kJ/mol (with a 95% confidence interval of 18.6 to 24.9 kJ/mol) for ASV IN in 1 M NaCl (pH 8.4), at 23.3 °C in the absence of glycerol (Fig. 2). That temperature (Fig. 7) (pH) (Fig. 3) and glycerol (Fig. 5) have little effect on tetramer formation in ASV IN suggests that these variables may have similarly weak effects on HIV-I IN assembly.

Nuclear magnetic resonance analysis reveals that the C-terminal domain of HIV-I IN forms dimers (9). Furthermore, Jenkins, et al. (6) have demonstrated that a mutant HIV-I IN lacking the C-terminal domain is unable to form tetramers. Although it is tempting to attribute the energy of tetramer formation to the contact area between the C-terminal domains, our data suggest that, for full-length ASV IN, tetramer formation may be driven largely by favorable electrostatic interactions and so may reflect more global properties of the protein.

Both HIV-I and ASV IN contain an HHCC, Zn$^{2+}$ finger motif near their N terminus (3). Lee, et al. (11) have shown that Zn$^{2+}$ promotes self-association of mutant, full-length HIV-I IN and that the HHCC motif is required for this effect. The addition of 10 μM Zn$^{2+}$ to wild type, full-length ASV IN in 500 mM KCl, 20 mM Tris, 2 mM βME (pH 8.4) resulted in the formation of aggregates of indeterminate stoichiometry that were not in freely reversible equilibrium with lower order oligomers (data not shown). The aggregation precluded accurate analysis of $\Delta G_{2\rightarrow 1}$ and $\Delta G_{4\rightarrow 2}$ in these samples. Analysis of ASV IN H9N, in which a point mutation was made in the HHCC motif, showed a weakening of $\Delta G_{2\rightarrow 1}$ by ~6 kJ/mol and a strengthening of $\Delta G_{4\rightarrow 2}$ by ~5 kJ/mol (25). It is not possible to interpret such observations further since self-association is a function of the entire protein structure and is sensitive to the surroundings of the protein.

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