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SIMIAN VIRUS 40 ASSEMBLY, STUDIED BY TEMPERATURE-INDUCED CONFORMATIONAL CHANGES IN CAPSID PROTEIN VP1

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In the morphogenesis of simian virus 40 (SV40), the viral DNA is first folded by the cellular core histones into nucleosomes to form the viral chromatin or minichromosome (1). Virion assembly requires the interaction of the capsid proteins VP1, VP2, and VP3 with SV40 chromatin to form a shelled particle with the minichromosome inside (reviewed in references 2 and 3).

In previous studies we combined a genetic approach with biochemical and electron microscopic studies to examine the shell assembly pathway. This involved characterization of nucleoprotein complexes formed in cells infected with conditional lethal mutants of the major capsid protein VP1 (2, 3). These mutants are temperature-sensitive in assembly and have been classified as tsB, tsC, and TsBC by complementation analysis (4, 5). Our studies of tsB mutants have revealed that at the nonpermissive temperature (40°C) the block in virion assembly results in accumulation of semiassembled particles that consist of chromatin attached to a partially assembled shell composed of VP1, VP2, and VP3 (6). We do not yet know the exact relationship between these semiassembled particles formed at 40°C and the actual intermediates that have been hypothesized (7–9) to form transiently during virion assembly. To measure the extent of temperature-induced structural pertubation in VP1 of tsB mutants, we estimated an approximate value for the enthalpy of VP1 renaturation. Conversion of altered VP1 to its native form was followed by determining the types and amounts of nucleoprotein complexes assembled at equilibrium in tsB265-infected cells incubated at various temperatures. The sedimentation profiles of these [^{3}H] thymidine-labeled complexes are shown in Fig. 1. At nonpermissive temperature (40°C), the altered VP1 yielded primarily the semiassembled particles (100–160S), as we have shown previously (2, 3, 6). At slightly lower temperatures, we detected an equilibrium mixture of chromatin (Ch) at 75S, semiassembled particles (SAP), and 220S-sedimenting particles (P). Finally, at permissive temperature (33°C), the profiles revealed predominantly two major classes of complexes: chromatin at 75S and virions at 220S. The data could be analyzed by assuming the following hypothetical assembly pathway:

$$\mathrm{Ch} + n_i\,(\mathrm{Cap}) \overset{K_1}{\rightleftharpoons} \mathrm{P}$$

$$\mathrm{Ch} + n_j\,(\mathrm{Cap}') \overset{K_2}{\rightleftharpoons} \mathrm{SAP}$$

$$\mathrm{Cap}' \overset{K_3}{\rightleftharpoons} \mathrm{Cap}.$$

K_1 corresponds to the equilibrium constant for the assembly of virion-like particles (220S) from chromatin and n_i molecules of native capsid (Cap). K_2 denotes the equilibrium constant for SAP assembly from chromatin and n_j molecules of altered capsid (Cap'). K_3 defines the conformational equilibrium constant for the renaturation of the altered capsid.

Measurements of the relative equilibrium concentrations of the various complexes over the range of temperature used in this study yielded the enthalpy change for the Cap' → Cap transition by way of the Gibbs-Helmholtz equation or its equivalent, the van't Hoff equation: $\Delta H_v = -R\,d(\ln K_3)/d\,(1/T)$, where R is the gas constant and the temperature T is expressed in °K. By solving for Cap and Cap' from the K_1 and K_2 equilibrium expressions

$$[\mathrm{Cap}] = ([\mathrm{P}]/[\mathrm{Ch}])^{1/n_i} \cdot 1/(K_1)^{1/n_i}$$

and

$$[\mathrm{Cap}'] = ([\mathrm{SAP}]/[\mathrm{Ch}])^{1/n_j} \cdot 1/(K_2)^{1/n_j},$$

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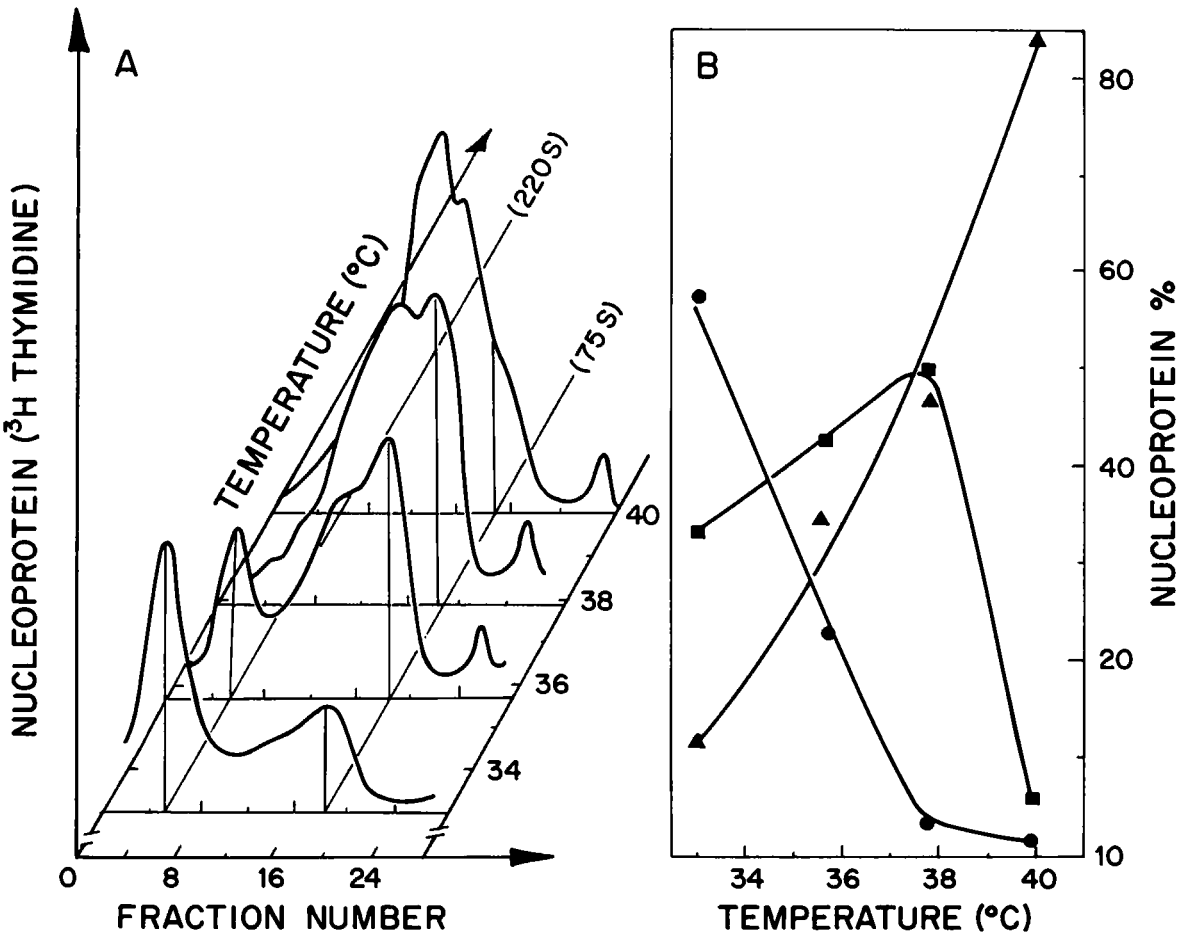

FIGURE 1 Nucleoprotein complexes isolated from tsB-infected BSC-40 cells, labeled with [^{3}H] thymidine at various temperatures, and analyzed by sedimentation in sucrose gradients (*A*). The nucleoprotein percent in *B* was calculated from the relative amounts of complexes present in peaks corresponding to chromatin (■) at 75S, virion-like particles (●) at 220S, and semiassembled particles (▲) from 100–160S.

we were able to rewrite the equation $K_3 = [\text{Cap}]/[\text{Cap}']$ in terms of measureable parameters. An expression for ΔH_v could then be derived by substituting for K_3, and by assuming that K_1 and K_2 do not vary appreciably with temperature. Thus

$$\Delta H_v = -(R/n_i) \cdot [d(\ln [\text{P}]/[\text{Ch}])/d(1/T)] + (R/n_j) \cdot [d(\ln [\text{SAP}]/[\text{Ch}])/d(1/T)].$$

The values for $d(\ln [\text{P}]/[\text{Ch}]/d(1/T)$ and $d(\ln [\text{SAP}]/[\text{Ch}])/d(1/T)$ were calculated from the data in Fig. 1. These gave $\Delta H_v = -(59.6/n_i) - (75/n_j)$ kcal/mol. If the structure of SV40 virion were similar to that of polyoma (10), n_i would correspond to 360, the number of VP1 molecules in a complete shell, which is presumably present in virion-like particles at 220S. Hence, ΔH_v would depend primarily on the degree of (Cap') polymerization. ΔH_v varied from −0.45 kcal/mol (for $n_j = 260$) to −1.7 kcal/mol (for $n_j = 50$). These n_j values were estimated from the sedimentation range of SAP (160–100S), Fig. 1, and from our previous work on the size of polymers dissociated from SAP in 1 M NaCl (6). If the major capsid proteins were totally denatured at the nonpermissive temperature, the ΔH_v of renaturation would be about −328 kcal/mol (11). The surprisingly low ΔH_v values obtained for the Cap' to Cap transition indicate very similar overall structures for VP1 molecules folded in vivo at the nonpermissive and permissive temperatures.

From the above study, we can draw two conclusions: the tsB mutation causes only a subtle change in the VP1 tertiary structure at the nonpermissive temperature, and the VP1 polymerization pathway observed at 40°C must somehow be similar to that occurring at 33°C. The major difference appears to be that the altered VP1 structure does not allow the assembly of complete shells, probably because of incorrect protein-protein contacts. Overall, the data corroborate our previous conclusion that the structure and biochemical characteristics of the tsB semiassembled particles provide clues about the virion assembly pathway (6). The results are more consistent with an SV40 assembly pathway wherein the capsid proteins are polymerized around the chromatin as opposed to a model in which the chromatin is introduced into a performed shell.

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