Conformational Transformations in the Protein Lattice of Phage P22 Procapsids

Maria L. Galisteo* and Jonathan King
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

ABSTRACT During the packaging of double-stranded DNA by bacterial viruses, the precursor procapsid loses its internal core of scaffolding protein and undergoes a substantial expansion to form the mature virion. Here we show that upon heating, purified P22 procapsids release their scaffolding protein subunits, and the coat protein lattice expands in the absence of any other cellular or viral components. Following these processes by differential scanning calorimetry revealed four different transitions that correlated with structural transitions in the coat protein shells. Exit of scaffolding protein from the procapsid occurred reversibly and just above physiological temperature. Expansion of the procapsid lattice, which was exothermic, occurred after the release of scaffolding protein. Partial denaturation of coat subunits within the intact shell structure was detected prior to the major endothermic event. This major endotherm occurred above 80°C and represents particle breakage and irreversible coat protein denaturation. The results indicate that the coat subunits are designed to form a metastable precursor lattice, which appears to be separated from the mature lattice by a kinetic barrier.

INTRODUCTION

In the assembly of viruses a series of structural transformations occur within highly organized protein complexes, the subunit lattices of the viral capsids. This is particularly clear for the double-stranded DNA viruses, in which a precursor shell is constructed that is empty of DNA, and the DNA is subsequently condensed into it. Associated with the DNA packaging reaction is a transformation of the capsid lattice from precursor to mature form.

For herpesviruses, adenoviruses, and double-stranded DNA bacteriophages the major coat protein does not self-polymerize into the mature virion shell (1–3). Scaffolding molecules, which are not present in the final viral particles, copolymerize with coat protein molecules to form a precursor shell, called the procapsid (4, 5). The transition from the procapsid particle to the mature phage requires exit of the scaffolding molecules, expansion of the shell, and DNA condensation (3, 6).

In T4 and λ phages the scaffolding molecules are degraded, while in others, including P22, they exit intact (3, 7). In P22 the scaffolding molecules released from the procapsid are able to interact with newly synthesized coat protein, thus catalyzing several rounds of procapsid assembly (8). For T4, scaffolding cleavage (9), shell expansion, and DNA packaging (10) can occur independently and in this sequence.

The structures of the procapsids and mature virions of P22 have been analyzed with low-angle x-ray scattering (11, 12) and very recently by cryo-electron microscopy and image processing (13). The procapsid is a double shell with an outer shell radius of 255 Å. The mature shell, which has lost the scaffolding but contains the condensed DNA, has a radius of 285 Å. The expansion results in a volume increase of 30%. The coat lattices in both cases are $T = 7$, but the local subunit-subunit arrangements differ considerably in the two cases (13). Although the scaffolding molecules are lost, there are no covalent modifications of either coat or scaffolding subunits in these processes. Within P22-infected cells, scaffolding release, shell expansion, DNA packaging, and ATP hydrolysis appear tightly coupled (6, 14, 15).

To investigate these reactions in vitro, conditions in which the shell transformations occurred in purified particles were required. Release of P22 scaffolding subunits can be triggered by transfer to denaturing solvents, including treatment with sodium dodecyl sulfate (SDS) (11) and guanidine hydrochloride (GuaHCl) (16). Heat-induced conformational changes in the bacteriophage T4 capsid protein have also been studied (17). In the present study we have examined the response of purified P22 procapsids to increasing temperature and found that this treatment was able to trigger scaffolding release and shell expansion prior to protein denaturation.

P22 procapsids can be purified from *Salmonella typhimurium* cells infected with phage carrying an amber mutation in a DNA packaging gene (16). About 420 molecules of coat protein (47 kDa), which form the outer shell lattice, and approximately 240 molecules of scaffolding protein (33 kDa), which form the protein core, constitute about 90% of the procapsid mass. The remainder is derived from the dodecameric gene 1 portal protein (which forms the DNA packaging vertex of the shell), together with the proteins gp16, gp20, and gp7 (location on the procapsid unknown).

We found (*a*) exit of the scaffolding protein from the procapsid occurred reversibly near physiological temperatures; (*b*) expansion of the procapsid lattice was irreversible and

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Address reprint requests to Dr. Maria L. Galisteo.

**Current address*: Departamento de Quimica Fisica, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain.

Abbreviations used: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; GuaHCl, guanidine hydrochloride; gp, gene product; DSC, differential scanning calorimetry.

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occurred in the 55–65°C temperature range; and (*c*) the coat protein molecules remain associated in a shell until 80–90°C, temperatures that induce irreversible coat protein denaturation. The exit of the scaffolding protein and the expansion of the capsid lattice are likely to correspond to the processes occurring during DNA packaging and shell maturation in vivo.

MATERIALS AND METHODS

Particle purification

P22 procapsids were purified basically as described previously (4), with minor modifications. An initial culture volume of 3 liters yielded approximately 100 mg of purified procapsids. Empty P22 procapsids were obtained by treatment of procapsids with 0.5 M GuaHCl, which induces the release of scaffolding and minor proteins, as described previously (4). The final samples were examined by electron microscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the latter revealing that empty procapsids contain coat protein exclusively (>98%). Purified procapsids and empty procapsids stored at 4°C were stable for months.

Heating of samples

Suspensions of P22 procapsids were stored at a concentration of about 20 mg/ml in 50 mM Tris(hydroxymethyl)aminomethane, 25 mM NaCl, 2 mM EDTA, pH 7.6 at 4°C. Empty procapsids were stored at about 8 mg/ml in the same buffer at 4°C. Prior to heating, samples were diluted to the desired final concentration and dialyzed in 20 mM dipotassium phosphate, 25 mM NaCl, adjusted with HCl to pH 7.6 (experimental buffer).

Phage particle suspensions were turbid at the concentration range used. To measure protein concentrations, aliquots of the samples were mixed with a GuaHCl solution at a final concentration of 3 M. The extinction coefficients used were those calculated by Fuller and King (16), corrected for the molecular weight values as published in (18): $E_{280}^{1\,mg/ml} = 1.04$ for the coat protein and 0.57 for the scaffolding protein. Assuming a procapsid protein content of 67% coat and 23% scaffolding, a final extinction coefficient of 0.83 was used. The extinction coefficients of the minor proteins are not known.

Samples were heated in a Gilford spectrophotometer equipped with a thermoset accessory. About 1 ml of P22 procapsid or empty procapsid suspension was placed in a 1-cm path length cuvette at 25°C. Changes in absorbance at 330 nm with temperature were monitored continuously. After 20–30 min at a particular temperature, an aliquot was put on ice. The temperature was then raised to a higher setting. This procedure was repeated for increasing temperatures. Aliquots taken at different temperatures were kept at 4°C until they were used for subsequent studies.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) experiments were performed in a differential scanning microcalorimeter (model DASM-4; Mashpriborintorg, Moscow region, Russia) as described in (19). An additional constant pressure of 2 atmospheres over the liquids in the cells was used, in order to avoid degassing during the calorimetric experiments. The transitions were corrected for the instrumental base line, the chemical base line, and the effect of the instrument response time, as previously described (20). We determined which transitions were reversible by heating just past the transition of interest, then cooling and reheating.

Electron microscopy

Samples for electron microscopy were negatively stained with 2% uranyl acetate on carbon-coated copper grids. Samples were deposited on grids at room temperature. Grids were viewed in a JEOL 1200 CX II transmission electron microscope.

Agarose gel electrophoresis

Phage particles were electrophoresed through nondenaturing gels as described in (21), with some modifications. Tray (running) buffer was 40 mM Tris(hydroxymethyl)aminomethane base, 5 mM sodium acetate, 1 mM EDTA, pH 8.2. Gels were 1.8% SeaKem (HGT) agarose (obtained from FMC Corporation, Rockland, ME) in tray buffer. Samples were dissolved 1:2 in 20% sucrose, 25 mM phosphate, 1 mM magnesium chloride (pH 7.4), and 0.01% bromphenyl blue, with 3–7 μg of protein loaded per well.

The gels were run in a Minnie Horizontal Agarose Submarine Unit (model HE33) from Hoefer Scientific Instruments (San Francisco, CA), which has a chamber with 50% ethylene glycol and 50% water, designed for maintaining a low temperature during operation. Samples were electrophoresed for 5–6 h at a constant voltage of 50 V, yielding a voltage gradient of 3.9 V/cm. The temperature of the tray buffer while the gels were run did not exceed 27°C.

Gels were stained with 0.05% Coomassie brilliant blue R-250 dissolved in 10% acetic acid for 1.5 h and then destained by diffusion into 10% acetic acid at room temperature for 2–3 days (22).

To examine the protein composition of bands in the agarose gels, excised slices of the gel were put in dialysis tubing with about 200 μl of tray buffer overnight, after which the proteins were eluted in the agarose gel apparatus with tray buffer (50 V for 2 h). Eluted proteins were visualized on a silver-stained SDS gel.

Sucrose gradient sedimentation

Sucrose gradient sedimentation was performed using a SW50.1 rotor in a Beckman L8–55 M ultracentrifuge. Samples containing about 0.2 mg of protein were loaded onto 5–20% sucrose gradients in experimental buffer. Sixty percent sucrose was used as a cushion. Centrifugation was carried out at 35,000 rpm for 35 min at 20°C. Eighteen or nineteen constant volume fractions were collected from a pinhole at the bottom of the tube.

Protein composition of sucrose gradient fractions was analyzed by SDS-PAGE in 10% gels after precipitation of the samples with trichloroacetic acid. Gel bands were visualized with Coomassie blue staining and scanned in a video densitometer (model 620) from Bio-Rad Laboratories, Inc. (Richmond, CA).

Sephacryl S-200 column chromatography

For gel filtration experiments, 150 μl of procapsids in experimental buffer was maintained at the temperature of interest for 30 min prior to being loaded onto a column of Sephacryl S-200 previously equilibrated at the desired temperature. The jacket of the column was connected to a M3Lauda waterbath equipped with a temperature controller. The temperature inside the column was checked by introducing a digital thermometer (MGW Lauda R42/2) after every experiment. Fractions were collected with a flow rate of 15 ml/h, and the elution profile was monitored by recording the absorbance of fractions at 280 nm with a flow cell. The resolution of the column was checked by introducing a sample containing procapsids (MW $\approx$ 3e7), bovine serum albumin (MW = 66,000), carbonic anhydrase (MW = 29000), and cytocrome *c* (MW = 12,400). The scaffolding protein has an anomalous exclusion volume in the column, equal to that of bovine serum albumin, probably resulting from scaffolding protein having an elongated structure (23). Fractions were precipitated with trichloroacetic acid at a final concentration of 10% and were subjected to SDS-PAGE in 10% gels. Gels were stained with Coomassie blue R-250 and scanned with a densitometer LKB 2202 equipped with an LKB 2220 integrator (LKB Pharmacia).

RESULTS

P22 procapsids were purified from infected cells blocked in DNA packaging (14, 15, 23). These particles appeared to be homogeneous on the basis of electron microscopy and sucrose gradient sedimentation, and they contained P22 constituent proteins in their expected ratios.

To help distinguish changes in the organization of the scaffolding subunits from changes in the coat lattice, we also studied, in parallel, procapsids from which the scaffolding and the minor proteins had been removed by treatment with 0.5 M GuaHCl. We refer to these particles as empty procapsids.

Differential scanning calorimetry

Fig. 1 shows the original differential scanning calorimetry thermograms for P22 procapsid and empty procapsid suspensions. The scan of empty procapsids, which contained only coat protein, exhibits three transitions, all of them irreversible: an exotherm centered at 61°C, an endotherm centered at 71°C, and the main transition centered at 87°C (Tables 1 and 2). The procapsid thermogram contains these same three transitions and a small additional transition centered at 48.5°C, which was reversible. If the three irreversible thermal effects are normalized to coat protein content, similar enthalpy values are found for empty procapsids and procapsids (Table 1). The three irreversible thermal effects were strongly scan rate dependent (Table 2), as expected for kinetically controlled processes (20, 24, 25).

The 48.5°C transition is the only one at which equilibrium thermodynamics can be applied. The van't Hoff enthalpy for this transition was calculated from the equation

$$\Delta H_{vH} = 4RT_m^2 C_p^m / \Delta H$$

which assumes a two-state process. ΔH_{vH} is the van't Hoff enthalpy, R is the gas constant, T_m is the temperature of the maximum of the DSC transition, C_p^m is the heat capacity at T_m, and ΔH is the calorimetric enthalpy (26). For this transition, the van't Hoff enthalpy was 1755 ± 29 kJ/mol.

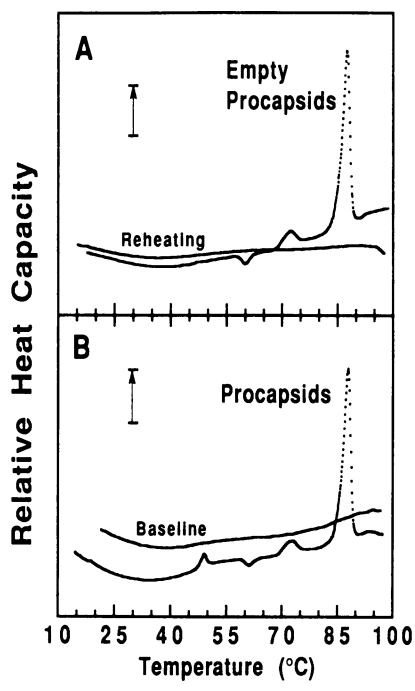

FIGURE 1 Original DSC recording of P22 (*A*) empty procapsids suspension (protein concentration, 0.86 mg/ml; the second heating of the sample is also shown) and (*B*) procapsids suspension (protein concentration, 0.6 mg/ml; a typical baseline is also shown). The experiments were performed in 20 mM phosphate, 25 mM sodium chloride, pH 7.6. The scan rate was 2.0 K/min. The bar corresponds to 200 kJ/K·mol, and the arrow shows the direction of the heat absorption.

TABLE 1 Thermodynamic parameters corresponding to the thermal events detected in P22 procapsids and the empty procapsids by differential scanning calorimetry

Temperature range	Empty procapsids*		Procapsids‡	
	T_m (°C)	ΔH (kJ/mol)	T_m (°C)	ΔH (kJ/mol)
40–50°C	Absent	Absent	48.5 ± 0.2	See text
55–65°C	60.3 ± 0.1	−92 ± 6	61.1 ± 0.3	−86 ± 7
70–78°C	70.9 ± 0.3	204 ± 38	71.4 ± 0.3	209 ± 9
80–90°C	87.4 ± 0.2	1767 ± 112	87.3 ± 0.3	1692 ± 78

Data were obtained with a scan rate of 2.0 K/min. * Data from three independent experiments (0.8–1 mg/ml). ‡ Data from four independent experiments (0.6–3.2 mg/ml). The values have been normalized to coat protein content.

TABLE 2 Summary of the main characteristics of P22 procapsid DSC transitions

Temperature range (°C)	Thermal effect	Scan-rate dependence*	Reversibility
40–50	Endothermic	Small	+
55–65	Exothermic	+	−
70–78	Endothermic	+	−
80–90	Endothermic	+	−

* The scan-rate dependence was checked in the range of 0.25–2.0 K/min. The dependence consisted of a T_m decrease when decreasing the scan rate. Thus, for the main transition, the T_m shifted from 87.4°C at 2 K/min to 85.3°C at 0.25 K/min. For the 40–50°C transition, the T_m decreased 0.8°C from 2 to 0.25 K/min.

Electron microscopy

Procapsids

Procapsid samples previously heated at different temperatures were examined in the electron microscope (Fig. 2). No significant morphological changes were detected until 55–65°C. In this temperature range two kinds of particles were observed: particles that looked like native procapsids and particles that looked like empty procapsids (Fig. 2 *B*). Electron micrographs of procapsids heated in this temperature range show that the number of empty particles increases and the number of native-like particles decreases with increasing temperature.

Particles heated in the range of 70–80°C, in addition to looking empty, appear more irregular and display perforated edges (Fig. 2 *C*). Some small filamentous aggregates are also present.

In the 80–90°C temperature range, where the DSC main transition occurs, broken particles and large filamentous aggregates are present (Fig. 3). The size of the aggregates increases and the number of broken particles decreases with time at 85°C. A pellet/supernatant separation of the heated sample was performed, which revealed that the aggregates were composed mainly of coat protein, while the scaffolding protein remained in solution.

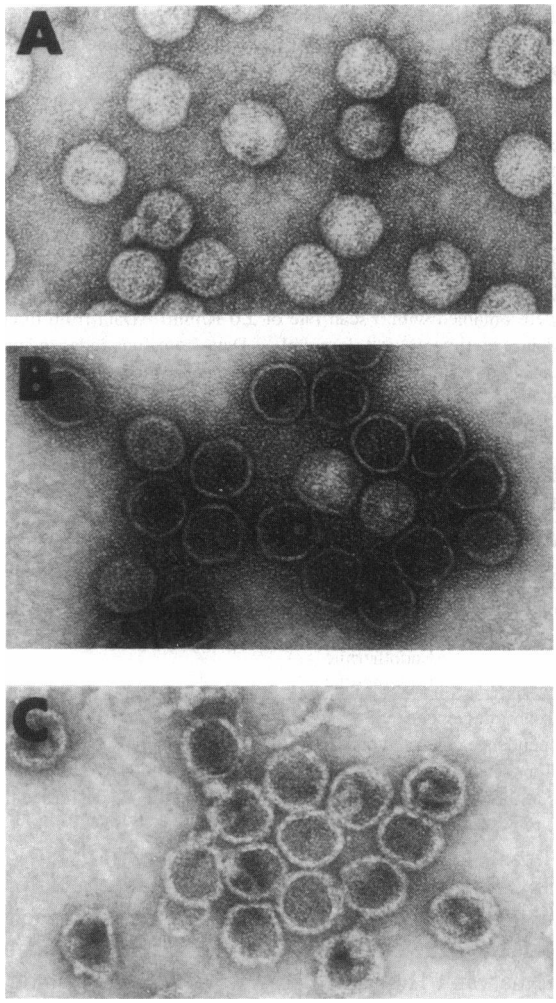

FIGURE 2 Electron micrographs of P22 procapsid suspensions (0.7 mg/ml) that were heated for 20 min at (*A*) 25°C, (*B*) 65°C, and (*C*) 75°C. 150,000 × magnification.

Empty Procapsids

Except for the absence of the filled-to-empty particle transition (since these particles already lack scaffolding), the response of empty procapsids to heat as examined by electron microscopy was very similar to that of procapsids (Fig. 4).

Agarose gel electrophoresis

Mobility in agarose gels has been shown to be extremely sensitive to particle size (21). Since procapsids have the capacity to expand in vivo, we wished to determine whether or not similar expansions were induced by heat in vitro. Agarose gel results are depicted in Fig. 5. For both empty procapsids (Fig. 5 *A*) and procapsids (Fig. 5 *B*) single bands corresponding to the native material were observed at 25°C (Fig. 5, *bands 1*). These same bands were observed until 52°C. In the range 55–65°C a new band with decreased mobility was observed in both samples (Fig. 5, *bands 2*). At 55°C the band corresponding to native particles was more intense. At 65°C the distribution had shifted, and band 2 of slower mobility had become more intense.

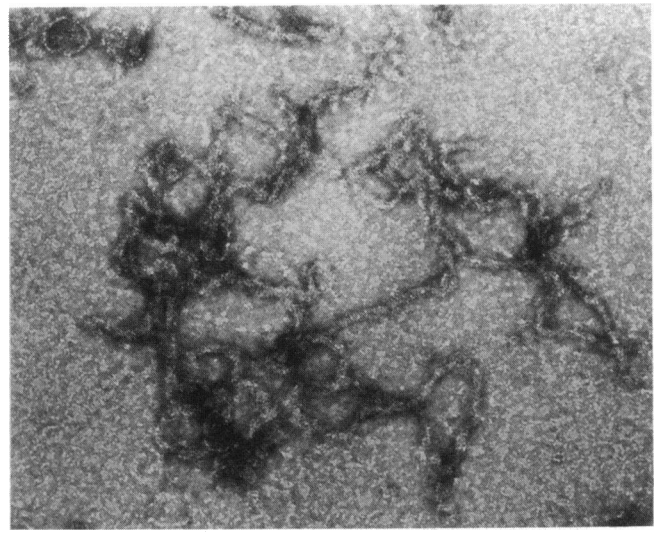

FIGURE 3 Electron micrograph of P22 procapsids (0.7 mg/ml) heated at 85°C for 10 min, where the presence of aggregates is detected. × 120,000 magnification.

At all temperatures examined, the migration and intensity of bands deriving from the procapsid sample were essentially the same as those of bands deriving from the empty procapsid sample. Analysis of the protein composition of the bands in the procapsid sample revealed that the faster-mobility band (band 1) contained both coat and scaffolding proteins, while the slower-mobility band (band 2) contained only coat protein. Since the empty procapsids lack scaffolding protein, the change in mobility between bands 1 and 2 could not be due to scaffolding loss and must reflect properties of the coat lattice. The reduction of mobility is consistent with an increase in particle radius.

On further heating of both procapsids and empty procapsids to 70°C the particles migrated as a single band (Fig. 5, *bands 3*), which ran even more slowly than the upper band detected in the range 55–65°C. Aggregates formed from heating above 85°C could not enter the gel.

Sucrose gradient sedimentation of procapsids

To corroborate results described above and analyze the protein composition of heated species, sucrose gradient centrifugation of previously heated samples was performed. The sucrose gradient fractions were electrophoresed through SDS gels to determine the distribution of procapsid proteins. Fig. 6 shows the sedimentation behavior of procapsids as a function of temperature. When the temperature was raised to 55°C, the sedimentation of the particles decreased, reflecting a loss of scaffolding protein. Since shell expansion results in an increase in frictional coefficient without a change in mass, it may also contribute to the decreased sedimentation. A further increase in temperature to 85°C resulted in a continued decrease in the sedimentation coefficient of the particles. At 90°C coat protein was distributed throughout the gradient, presumably in heterogeneous aggregates.

The asymmetrical distribution of the scaffolding protein with respect to the coat protein peak at 55°C and 65°C sug-

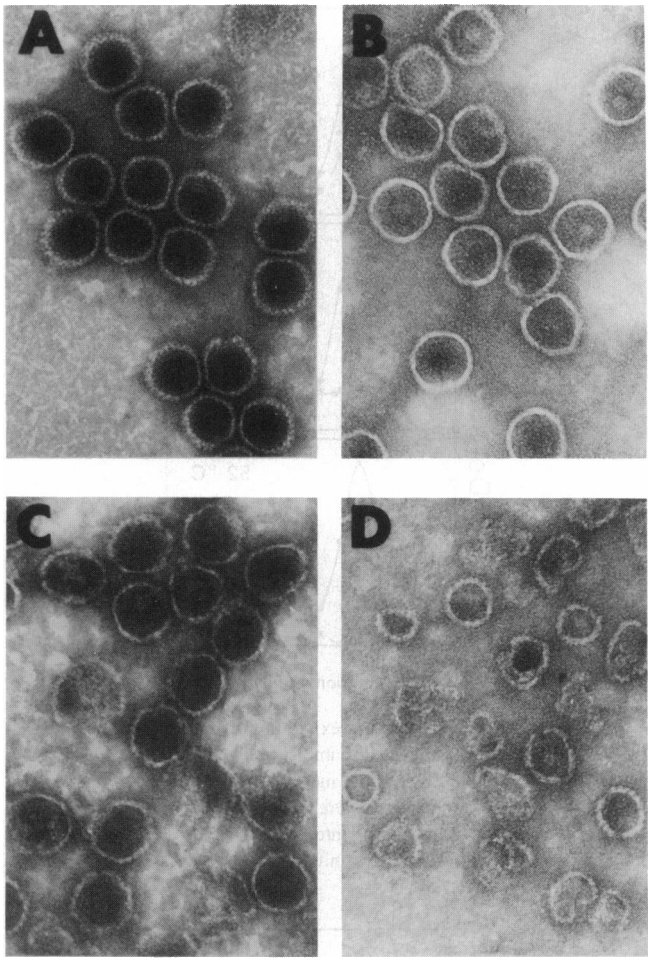

FIGURE 4 Electron micrographs of P22 empty procapsids (0.87 mg/ml) that were heated for 20 min at (*A*) 25°C, (*B*) 65°C, and (*C*) 75°C and (*D*) for 5 min at 85°C. × 150,000 magnification.

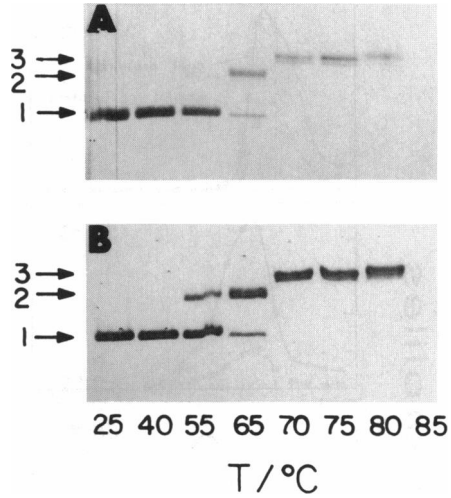

FIGURE 5 Agarose gel of P22 (*B*) procapsids (0.7 mg/ml) and (*A*) empty procapsids (0.87 mg/ml) previously heated as described in Materials and Methods at 25°C for 20 min; 55°C for 20 min; 65°C for 20 min; 70°C for 20 min; 80°C for 20 min; 85°C for 10 min. About 3 μg of protein was loaded onto each well. Particles migrated toward the positive electrode. The reduced intensity of bands arising from empty procapsids heated above 55°C is a staining artifact.

gests that there is more than one population of shells at these temperatures. From the agarose gel results, the faster-migrating population would contain scaffolding protein while the slower-migrating population would be depleted of scaffolding protein and expanded. Raising the temperature to 65°C shifted the distribution further to the slower class of particles deficient in scaffolding subunits. At temperatures in the range of 70–85°C, coat protein particles were still recovered, but the scaffolding protein sedimented as soluble subunits at the top of the gradient.

With regard to the minor proteins, gp20 was found at the top of the gradient at temperatures of 55°C and higher. gp1 and gp16 remained associated with the particles until 80–85°C, at which point these minor proteins were also detected at the top of the gradient (results not shown).

Column chromatography

The agarose gel electrophoresis and sucrose gradient results indicate that scaffolding protein irreversibly exits the procapsid particle between 55 and 65°C. These techniques were performed at ambient temperature on previously heated samples and therefore do not yield information about reversible processes. To determine if scaffolding protein could reversibly exit procapsids at lower temperatures, procapsid samples were loaded onto a thermostated Sephacryl S-200 column at several temperatures. The column effectively resolved scaffolding remaining in the particles from free scaffolding.

By this technique we found that scaffolding exit did occur in the temperature range of 37 to 52°C (Fig. 7). Fig. 8 shows the percentage of scaffolding remaining in procapsids loaded onto columns at different temperatures. In addition, we found that gp20 exits reversibly in the range 43–55°C (results not shown).

The heat-induced release of scaffolding protein from the procapsid was found to be protein concentration dependent, as expected for a dissociation process occurring under equilibrium conditions. As is shown in Fig. 8, at a procapsid concentration of 2.1 mg/ml, no scaffolding remained associated with particles at 51°C. At a procapsid concentration of 10 mg/ml, 35% of the scaffolding molecules remained associated with particles at this temperature.

The reversibility of this process was verified by running preheated procapsid samples in columns at 25°C. Procapsid samples were preheated for 1 h at 46, 48, or 50°C, put at room temperature for about 2 h, and run in a Sephacryl S-200 column at 25°C. About 75–80% of the scaffolding protein was associated with the particle in these experimental conditions. Similar results were found when preheated samples were run in sucrose gradients.

DISCUSSION

Within cells, the transition from procapsid to mature capsid, comprising scaffolding release and shell expansion, is an essential aspect of the machinery of packaging of DNA into

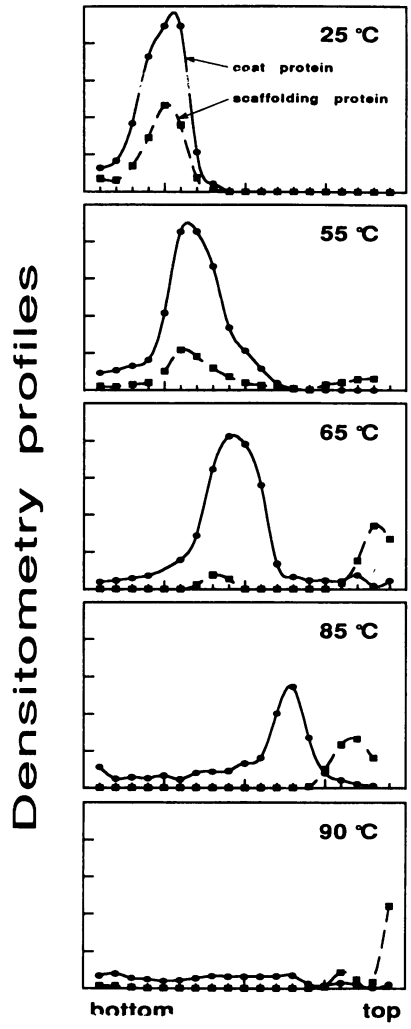

FIGURE 6 Densitometry profiles corresponding to sucrose gradient centrifugation of P22 procapsids previously heated at the indicated temperatures for 20 min (90°C, 10 min). Values on the *y*-axis represent the integrated intensity of Coomassie blue-stained protein bands in arbitrary units.

the capsid. Nonetheless, the ability to undergo this transition appears to be an intrinsic property of the assembled coat and scaffolding lattices. The in vitro study performed here suggests that thermal energy is able to drive physiologically relevant conformational changes in these proteins.

Exit of the scaffolding protein from the procapsid

Exit of scaffolding subunits from the procapsids was detectable at 37°C and took place over a narrow temperature range (midpoint ≈ 44°C, at 2.1 mg/ml) close to physiological temperatures (see Fig. 8). This result is an indication of the low strength of scaffolding-coat and/or scaffolding-scaffolding interactions in the procapsid. A noteworthy feature of this process is that the heat-induced exit of the scaffolding protein from the procapsid does not break the coat protein shell lattice, as is also observed with GuaHCl-induced release (16). The exit presumably occurs through the holes located at the centers of hexamers of the procapsid lattice (13).

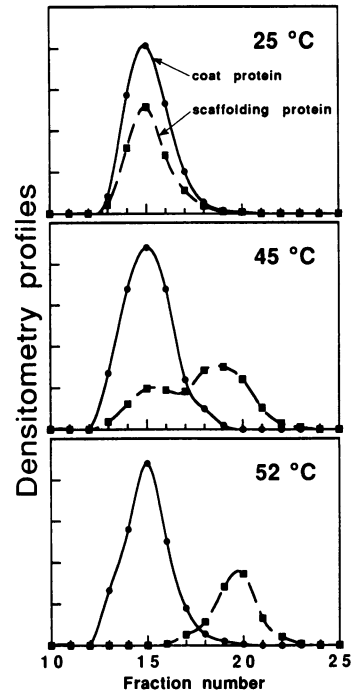

FIGURE 7 Temperature-induced exit of the scaffolding protein from P22 procapsids (2.1 mg/ml). Procapsid samples were chromatographed through an S-200 column maintained at the indicated temperatures as described in Materials and Methods. Fractions were electrophoresed through an SDS gel. Values on the *y*-axis represent the integrated intensity of Coomassie blue-stained protein bands in arbitrary units.

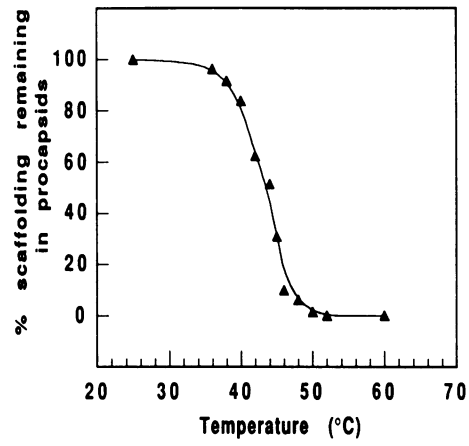

FIGURE 8 Scaffolding protein dissociation from P22 procapsids as a function of temperature (see Fig. 7). The determination of the scaffolding protein content inside and outside the particle for every temperature was calculated as follows. We took the 25°C distribution of scaffolding protein as a function of elution volume as the procapsid distribution and the 60°C distribution of scaffolding protein as a function of elution volume as the monomer distribution. The distributions of scaffolding protein for intermediate temperatures were empirically resolved into sums of the procapsid and monomer distributions.

Chromatographic experiments showed that procapsids no longer contain scaffolding protein above 52°C. On the other hand, the particles that have been heated to 52°C and then cooled contain scaffolding protein, which implies that the

scaffolding exit is reversible up to 52°C. As the temperature was increased further, the amount of scaffolding protein that could reenter the particles upon cooling was reduced as seen by these same techniques. The fraction of scaffolding protein remaining outside the particle after heating to a particular temperature in the range 55–65°C and then cooling correlates well with the fraction of expanded particles, as seen in the agarose gels and by electron microscopy. It thus appears that particle expansion irreversibly precludes scaffolding entry.

The primary difference between the DSC profiles of the procapsids and empty procapsids was the presence of the 48.5°C endotherm in the procapsid sample only (Fig. 1). This result could be due to unfolding of the scaffolding protein. However, the 48.5°C transition has a calorimetric enthalpy of 110 kJ/mol if assigned to scaffolding, and 55 kJ/mol if assigned to coat protein. These low enthalpy values indicate that the transition is unlikely to result from protein unfolding (27). To resolve this point, DSC of purified scaffolding protein was performed at scaffolding concentrations equivalent to that present in the procapsid samples. No thermal events were found in the range 10–110°C, probably indicating a low level of tertiary structure of this protein in solution (M. L. Galisteo, unpublished results).

The ratio $\Delta H_{vH}/\Delta H_{cal}$ provides a measure of the minimum number of independent domains or cooperative units contributing to an endothermic peak (26). The value of this ratio was 16 if the thermal effect was assigned to scaffolding, and 32 if the effect was assigned to coat protein. Thus the process involved in this transition has a high level of intermolecular cooperativity.

What is the nature of this process? Increasing temperature probably induces a cooperative change in the conformation of internal scaffolding molecules. This conformational change would be such that intermolecular interactions that help to maintain the scaffolding protein core intact in procapsids (scaffolding-scaffolding and/or scaffolding-coat) become disrupted. Once these interactions have been disrupted, the exit of free scaffolding subunits through gaps in the coat protein lattice, which are seen in cryo-electron procapsid reconstructions (13), would be a favorable process, especially taking into account the very high local concentration of scaffolding molecules inside procapsids (ca. 200 mg/ml).

Expansion of the coat protein lattice from 55 to 65°C

Irreversible expansion from 55°C to 65°C, detected by agarose gel electrophoresis and sucrose gradient sedimentation, prevents departed scaffolding protein from reentering the particle upon temperature shiftdown. This may reflect the closing of the holes that occurs in the transition from procapsid to mature capsid, as it has been observed by comparison of cryo-electron microscopy of procapsids and phage (13). This expansion appears to be highly cooperative, since no intermediately expanded species are detected in the 55–65°C temperature range (Fig. 5).

The exothermic event found by DSC for both procapsids and empty procapsids (Fig. 1) takes place in the same temperature range as the particle expansion observed by agarose gels (Fig. 5). The fact that this thermal effect appears in both particles with or without scaffolding protein suggests that it is due to processes experienced by the coat protein. This process is obviously a nonequilibrium one and, as expected, was found to be scan-rate dependent (Table 2). These results suggest that the energy release during the exothermic transition is derived from the particle expansion process.

That the expansion may occur with a negative change in enthalpy has been previously suggested to occur in the expansion of T4 polyheads. These are long cylindrical associations of the T4 major coat protein in the uncleaved precursor conformation. Expanded polyheads can be obtained by cleavage of polyheads. Ross et al. (17) compared the DSC scans corresponding to expanded and unexpanded T4 polyheads. They found that the thermal denaturation of the expanded particles occurred at a higher T_m and with a higher enthalpy than the thermal denaturation of unexpanded particles, concluding that a negative enthalpy change takes place upon expansion. These results imply that a kinetic barrier inhibiting the expansion has to be overcome in order for the expansion to occur. In the present study, heat is the factor that triggers the process. It is possible that in vivo, other cellular components may act to decrease the energy of this kinetic barrier, permitting the expansion to occur at physiological temperatures.

Expanded procapsids obtained from treatment with low concentrations of SDS have the same diameter as phage particles (11). We found that expanded particles (Fig. 5, *bands 2*) had approximately the same mobility in agarose gels as SDS-treated procapsids (results not shown), suggesting that the extent of expansion induced by heat, which we have studied in this work, is essentially the same as that experienced by SDS-treated procapsids.

For other phages such as T4, the expansion is characterized by stabilization of the prohead shell, the new structure being much more resistant to denaturing agents (9, 28). P22 coat subunits within the shell are cleaved by trypsin at a limited number of sites. In agreement with the T4 results, coat subunits in P22 procapsids and empty procapsids heated in the range 55–65°C, which induces shell expansion, were significantly less sensitive to trypsin digestion (M. L. Galisteo, unpublished experiments).

Thermal denaturation of the coat protein in the lattice

The main endotherm detected by DSC in the 80–90°C temperature range appears to represent the thermal denaturation of the coat protein, with no contribution from the scaffolding protein. The specific enthalpy of this transition for empty procapsids (only coat protein) is about 35 J/g, in good agreement with values previously reported for thermal denaturation of compact globular proteins with similar melting temperatures (27). In addition, when the enthalpy value obtained

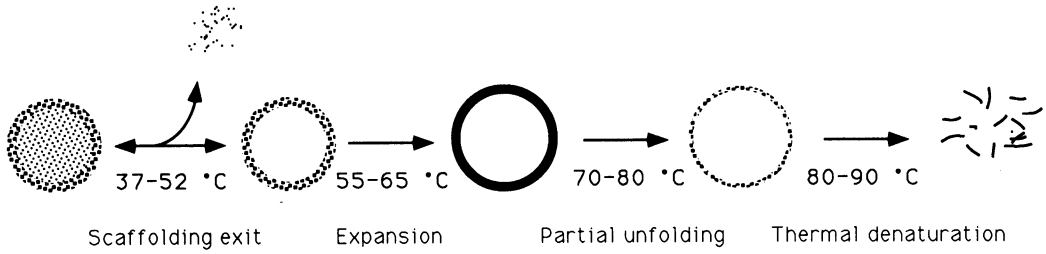

FIGURE 9 Summary of the processes experienced by P22 procapsids in response to increasing temperatures.

for procapsids was normalized to coat protein content, the same value was obtained as in empty procapsids (Table 1).

As the main transition was irreversible, prior to the application of equilibrium thermodynamics, the scan-rate dependence has to be checked (20, 24, 25, 29). The transition was shifted to lower temperatures when the scan rate was decreased (Table 2), indicating that coat protein thermal denaturation is a kinetically controlled process. Therefore, no thermodynamic information other than the total calorimetric enthalpy and the T_m can be obtained from this transition. Coat protein thermal denaturation was accompanied by lattice disruption and the appearance of aggregates. However, this transition was protein concentration independent, suggesting that the rate-limiting step occurred before the disruption of the coat protein lattice and that the irreversible process must be first order.

The main endotherm was preceded by a small endotherm centered at about 71°C. This transition has also been assigned to the coat protein, since it was present not only in procapsids but also in empty procapsids, with the same T_m and enthalpy values when normalized to coat protein content (Table 1). The enthalpy of this transition was ~10% of the main transition enthalpy, and it is probably due to partial denaturation of coat protein subunits in the shell, generating the altered shell morphology detected in the electron microscope (see Figs. 2 *C* and 4 *C*). The lower mobility of these particles in agarose gels (Fig. 5, *bands 3*, as compared to *bands 2*) could be due to exposure of unfolded "arms" of coat protein to the exterior of the particle, rather than to a subsequent expansion of the shell lattice. This transition is irreversible, and no valid thermodynamic information about the unfolding can be obtained, other than the T_m and the calorimetric enthalpy. A pretransition preceding a main transition has also been observed in the DSC thermogram of T4 uncleaved polyheads, where it has been tentatively assigned to the unfolding of the NH_2-terminal domain of the coat protein (17). In Fig. 9 we have summarized the results described in this paper.

Scaffolding exit and procapsid expansion in vivo

Bacteriophage scaffolding proteins have been shown to have an important role in the initiation of procapsid assembly (30–32), to be required for correct assembly of the coat protein shell (8, 30), and probably to prevent the entry of other intracellular proteins into the procapsid (6). Expansion of the shell does not appear to be required for scaffolding exit or cleavage within the cell. For P2, T4, and lambda amber mutants in phage genes have been isolated that result in proheads lacking scaffolding protein but remaining unexpanded (6).

For P22, purified procapsids lose their scaffolding protein core and minor proteins upon treatment with 0.5 M GuaHCl, without alteration of coat shell dimensions (16). The results obtained by heat treatment of purified P22 procapsids discussed in this paper further support models in which scaffolding exit would occur independently and prior to expansion. This is in accord with results obtained in vivo for phages P2, T4, and lambda mentioned above.

Shell expansion in vivo probably reorganizes the coat protein subunits in the shell for a role in DNA packaging (10, 33). However, the nature of that role still remains obscure. It may simply be required to close the scaffolding exit holes and generate a protective shell for the DNA (13) or there may be further functions. Hohn et al. (34) and Serwer (35) proposed a model in which the energy released in capsid expansion would force the DNA into the capsid. However, more recent experiments argue against a model in which shell expansion drives DNA packaging. For example, in T3 and lambda phages, a percentage of the genome is packaged in the prohead before the shell expansion takes place (36, 37), and in T4, both unexpanded and expanded proheads are able to package DNA in vitro (10). It thus appears that for T3 and lambda, packaging triggers expansion, although in T4 expansion and packaging can be uncoupled in vitro.

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