Role of Entropic Interactions in Viral Capsids: Single Amino Acid Substitutions in P22 Bacteriophage Coat Protein Resulting in Loss of Capsid Stability[†,‡]

Debora Foguel,[§] Carolyn M. Teschke,[∥,⊥] Peter E. Prevelige Jr.,[*,▽] and Jerson L. Silva[*,§]

Departamento de Bioquimica, Instituto de Ciencias Biomedicas, Universidade Federal do Rio de Janeiro; Rio de Janeiro, RJ 21941 Brazil, Department of Biology, Massachussetts Institute of Technology, Cambridge, Massachussetts 02139, and Boston Biomedical Research Institute, 20 Staniford Street, Boston, Massachussetts 02114

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ABSTRACT: Bacteriophage P22 is a double-stranded DNA containing phage. Its morphogenetic pathway requires the formation of a precursor procapsid that subsequently matures to the capsid. The stability of bacteriophage P22 coat protein in both monomeric and polymeric forms under hydrostatic pressure has been examined previously [Prevelige, P. E., King, J., & Silva, J. L. (1994) *Biophys. J. 66*, 1631−1641]. The monomeric protein is very unstable to pressure and undergoes denaturation at pressures below 1.5 kbar, whereas the procapsid shell is very stable to applied pressure and does not dissociate with pressure to 2.5 kbar. However, under applied pressure the procapsid shells are cold labile, suggesting they are entropically stabilized. We have analyzed the pressure stability of mutant procapsid shells having either of two single amino acid substitutions in the coat protein (G232D and W48Q) using light-scattering and fluorescence emission methods. While the wild-type shells were stable under 2.2 kbar of pressure at room temperature (22 °C), the G232D mutant shells showed time-dependent dissociation under these conditions. Decreasing the temperature to 1 °C dramatically accelerated the dissociation of G232D mutant under applied pressure. On the other hand, the W48Q mutant shells could be dissociated easily by pressure at room temperature and displayed little dependence on temperature, suggesting a smaller entropic contribution to the stability of this mutant. The unpolymerized mutant subunits displayed a pressure stability similar to that of the wild type. These data indicate that the single-site substitutions markedly affect the stability of the assembled shell and yet have little effect on the stability of the coat protein subunit itself, suggesting that the substitutions are marking residues involved in inter-subunit interactions, either directly or through local conformational changes. The replacement of a single nonpolar amino acid (Trp48) by a polar residue (Gln) results in loss of the entropic stabilization, suggesting the importance of burial of Trp48 in a nonpolar core to stabilize entropically the icosahedral shells. Our results with the single-mutation shells dissect the protein interactions important for assembly at the level of "protein cavities" (related to volume) and "internal motion" (related to entropy).

A feature common to the life cycle of all viruses is the assembly of the viral capsid from its constituent protein subunits. Although the assembly pathways differ in detail, in all cases the growing capsid must pass through a series of less stable intermediates in various degrees of polymerization en route to the topologically closed shell. The topologically closed shell is in general more stable than either the constituent subunits or the assembly intermediates, and this stability necessarily arises from the inter-subunit contacts. These subunit interactions must be delicately balanced between the ability to form a stable capsid and the capacity for dissociation, either as an editing mechanism during assembly or to release the viral nucleic acid following uptake by the host cell (Rossman & Johnson, 1989).

In general, the polymerization of supramolecular structures such as viruses is driven by entropic interactions and is accompanied by an increase in system volume. Hydrostatic pressure induces the dissociation and denaturation of proteins because the processes of folding and association are generally accompanied by an increase in volume (Weber, 1992; Silva & Weber, 1993; Silva, 1993). This positive volume change is due to the combined effects of the formation of solvent-excluding cavities in protein interiors or at the inter-subunit interfaces and the release of bound solvent (Silva & Weber, 1993). The increase in volume is mostly due to the replacement of dipole-induced dipole bonds (water−amino acid interactions) with London dispersion forces between the nonpolar amino acids that become buried in the interior of the protein. Formation of salt linkages in the protein interior or at the subunit interface also contributes to the increase in volume.

Although several studies have described the cold denaturation of proteins [Lauffer *et al.*, 1958; Brandts, 1964; Sturtevant, 1977; Privalov *et al.*, 1986; Griko *et al.*, 1988;

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[§] Universidade Federal do Rio de Janeiro.

[∥] Massachussetts Institute of Technology.

[⊥] Present address: Department of Molecular and Cell Biology, University of Connecticut, 75 North Eagleville Rd., Storrs, CT 06269.

[▽] Boston Biomedical Research Institute.

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Chen & Schellman, 1989; reviewed in Privalov (1990)], cold denaturation, unlike thermal denaturation, is not well understood. The dissociation of several oligomeric proteins is also favored by lowering the temperature (Weber, 1993). Weber (1993) attributes the entropy-driven character of protein associations to the large number of weak protein bonds that results in a broad distribution of protein conformations. The combination of high pressure and low temperature is an important tool to tackle this problem, especially because pressures in the range 1.0−2.5 kbar decrease the freezing point of water to negative values (Bridgman, 1964). For example, at 2.0 kbar, the freezing point of water decreases to −20 °C, providing the advantage that one can study cold denaturation without the addition of cryosolvents. This tool was used for the first time to study the dissociation and denaturation of phycobilisomes from cyanobacteria (Foguel *et al.*, 1992). The same approach has recently been used to study the cold dissociation and denaturation of allophycocyanin (Foguel & Weber, 1994) and to promote the denaturation of a protein−DNA complex (Foguel & Silva, 1994).

In this paper, we utilize this technique to examine the effects of single-site amino acid substitutions in a viral coat protein on the stability of the capsid of bacteriophage P22. Bacteriophage P22 is a double-stranded DNA containing phage whose morphogenetic pathway requires the formation of a precursor procapsid that subsequently matures to the capsid. The procapsid is composed of 420 molecules of coat protein arranged around an inner core composed of approximately 300 molecules of scaffolding protein (King & Casjens, 1974). The phage DNA is packaged into this preformed procapsid through a portal protein complex located at a single vertex (Bazinet *et al.*, 1988). As the DNA is packaged, the scaffolding protein exits and recycles to participate in further rounds of assembly. Empty shells of coat protein can be prepared from procapsids by extraction of the scaffolding protein with 0.5 M guanidine hydrochloride (GuHCl) (Fuller & King, 1981). These shells are topologically closed and are composed solely of coat protein. The bacteriophage P22 system has the advantages that biologically active monomeric coat protein can also be prepared (Prevelige *et al.*, 1988, 1993) and that the monomeric subunit is less stable compared to the polymerized form.

In a previous study, we measured the pressure stability of bacteriophage P22 coat protein in both monomeric and polymeric forms (Prevelige *et al.*, 1994). The monomeric protein is very unstable toward pressure and undergoes denaturation at relatively low pressures (below 1.5 kbar). In contrast to the monomeric protein subunit, the protein when assembled into procapsid shells is very stable to applied pressure and does not dissociate with pressure up to 2.5 kbar. However, under applied pressure the procapsid shells dissociate, and the subunits denature at low temperatures, suggesting they are entropically stabilized. In this paper we describe the effects of single amino acid substitutions on these two reactions. We find that the replacement of a nonpolar by a polar amino acid results in a direct destabilization of the particle that ultimately is related to loss of its entropic stabilization.

MATERIALS AND METHODS

Preparation of Protein Samples. The preparation of procapsids, empty shells, and coat protein monomers was as previously described (Prevelige *et al.*, 1988). The procapsids employed were purified from *Salmonella typhimurium* strain DB7136 which had been infected with P22 phage carrying 2^-am/13^-am mutations. The 2^- mutation blocks DNA packaging resulting in the accumulation of procapsids, while the 13^- mutation delays lysis, thereby increasing the yield. The preparation and properties of the coat protein mutants W48Q and G232D have been described by Gordon and King (1993).

The coat protein monomers were prepared by diluting 8 mg/mL capsid solution with 9 M urea in order to have in the final volume 2 mg/mL protein and 6.75 M urea. This solution was kept at room temperature for 2 h in a siliconized tube. The refolding was initiated by rapid dilution of the urea (final concentration was 0.34 M) with buffer containing 50 mM Tris, 25 mM NaCl, and 2 mM EDTA, pH 7.6. The refolding was performed on ice to avoid aggregation. This buffer was employed in all experiments reported.

Optical Methods. The high-pressure bomb, purchased from SLM-Aminco (Urbana, IL) has been described previously (Silva *et al.*, 1992). Fluorescence spectra were recorded on an ISS K2 computer-controlled spectrofluorometer (Champaign, IL).

The coat protein of bacteriophage P22 contains six tryptophan residues distributed more or less uniformly throughout the primary sequence (Eppler *et al.*, 1991). Fluorescence spectra at pressure p were quantified by specifying the center of spectral mass, $\langle \upsilon_p \rangle$.

$$\langle \upsilon_p \rangle = \sum \upsilon_i F_i / \sum F_i \qquad (1)$$

where F_i stands for the fluorescence emitted at wavenumber υ_i and the summation is carried out over the range of appreciable values of F. The degree of dissociation (α_p) is related to $\langle \upsilon_p \rangle$ by the expression

$$\alpha_p = (1 + Q(\langle \upsilon_p \rangle - \langle \upsilon_f \rangle)/(\langle \upsilon_i \rangle - \langle \upsilon_p \rangle))^{-1} \qquad (2)$$

where Q is the ratio of the quantum yields of dissociated and associated forms, $\langle \upsilon_p \rangle$ is the center of spectral mass at pressure p, and $\langle \upsilon_f \rangle$ and $\langle \upsilon_i \rangle$ are the corresponding quantities for dissociated and associated forms (Silva *et al.*, 1986; Royer *et al.*, 1986; Silva & Weber, 1988).

Light-scattering measurements were made in an ISS K2 spectrofluorometer (Silva *et al.*, 1989). Scattered light (320 nm) was collected at an angle of 90° to the incident light by integrating the intensity in the 315−325-nm window.

Urea Dissociation and Unfolding of Empty Shells. WT, W48Q, or G232D shells were incubated in increasing concentrations of buffered urea for 17−20 h at 20 °C in siliconized microfuge tubes. The final concentration of protein was 24 μg/mL. The fluorescence and light scattering of each sample were determined using a Hitachi F4500 spectrofluorometer. The settings for fluorescence were as follows: excitation wavelength, 280 nm; emission wavelength, 340 nm. The excitation and emission band-passes were 2.5 and 5 nm, respectively. Light scattering was monitored with both the excitation and the emission wavelength set at 340 nm with the band-passes as above. The values were corrected for baseline fluorescence or scattering. The fraction unfolded (F_u) was determined by the formula $F_u = (X_n - X_{obs})/(X_n - X_u)$ where X_n is the fluorescence or light scattering of the native shells, X_{obs} is the observed value,

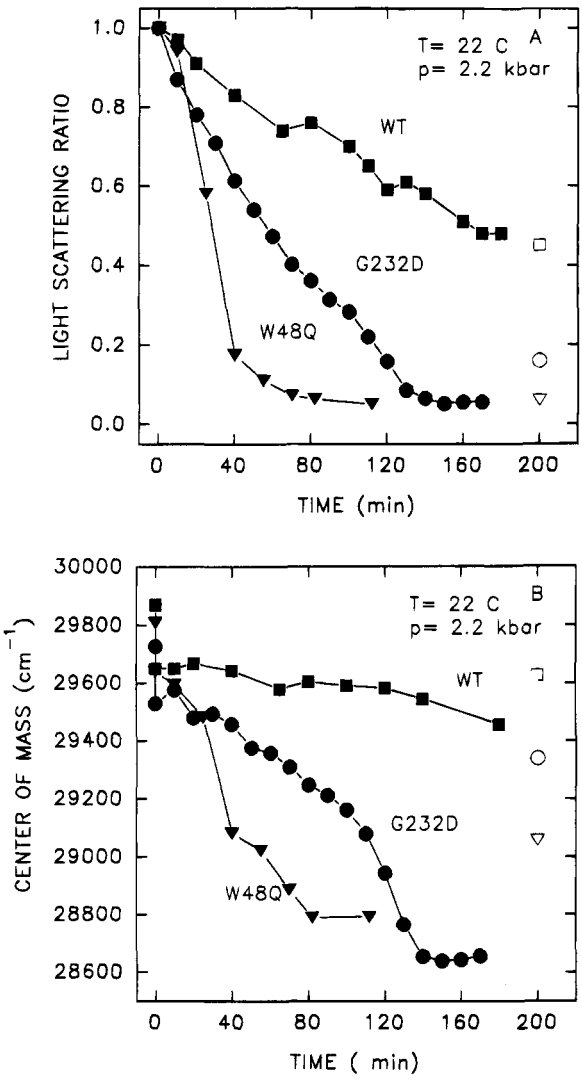

FIGURE 1: Kinetics of dissociation of wild-type and mutant shells at 22 °C. Panel A: Light scattering vs time at 2.2 kbar hydrostatic pressure. Panel B: Center of spectral mass vs time at 2.2 kbar hydrostatic pressure. Data is plotted as fraction of the value at zero time: wild-type (■), G232D (●), and W48Q (▼) shells. The open symbols near the right edge represent the values achieved after returning to atmospheric pressure. Protein concentration was 0.2 mg/mL.

and X_u is the fully denatured value. X_n and X_u were determined by linear extrapolation of the folding and unfolded plateaus into the region of the transition at each urea concentration (Creighton, 1987).

RESULTS AND DISCUSSION

The procapsid of bacteriophage P22 is composed of an outer shell of coat protein and an inner core of scaffolding protein. Empty coat protein shells can be prepared by extraction of the scaffolding protein with 0.5 M GuHCl. At 28-Å resolution, these shells are structurally identical to those containing the scaffolding protein (Prasad *et al.*, 1993). Empty coat protein shells (wild type and mutants G232D and W48Q) at a concentration of 0.2 mg/mL were subjected to pressure treatment, and their dissociation was monitored by fluorescence and light-scattering changes. Previous studies have shown that the fluorescence spectrum of the coat protein subunit is slightly red shifted relative to that of the polymerized subunit within the coat protein shell,

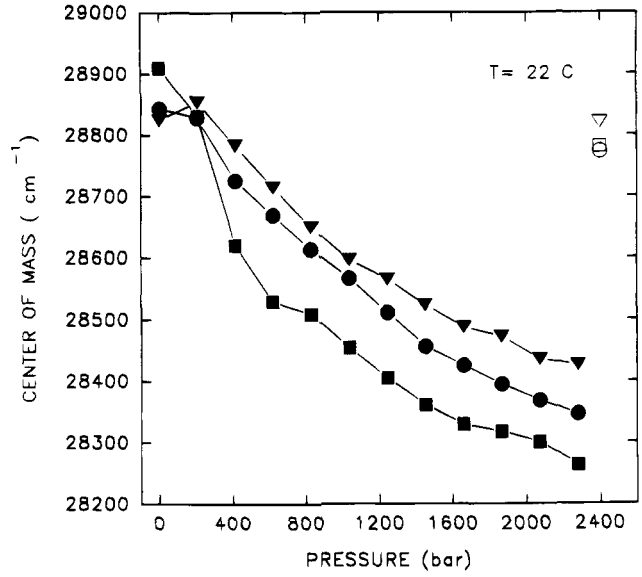

FIGURE 2: Pressure denaturation of the coat protein subunits for wild-type and mutant shells at 22 °C. The center of spectral mass versus pressure is plotted: wild-type (■), G232D (●), and W48Q (▼) shells. The open symbols near the upper right corner represent the center of mass after returning to atmospheric pressure. Protein concentration was 100 μg/mL.

indicating that the tryptophans are less solvent exposed in the polymerized form (Teschke & King, 1993; Prevelige *et al.*, 1994). Pressure-induced denaturation of wild-type coat protein subunits results in a further red shift (Prevelige *et al.*, 1994). The center of spectral mass in wavenumbers is a sensitive measure of the spectral envelope, with larger numbers indicating a blue shift. The following centers of spectral mass in wavenumbers were determined from the recorded spectra: wild-type shell, 29 850 cm^{-1}; W48Q mutant type shell, 29 800 cm^{-1}; G232D mutant type shell, 29 750 cm^{-1}. These values are considered to be within experimental error of each other.

The pressure-induced dissociation of shells was therefore followed by a decrease in the center of spectral mass, along with the decrease in light scattering. Figure 1 shows the kinetics of shell dissociation induced by pressure at room temperature (22 °C) for the wild type and the G232D and W48Q mutants as measured by light scattering (Figure 1A) and center of spectral mass (Figure 1B). Whereas the wild-type virus is stable against pressure at room temperature, the W48Q mutant is extremely unstable, and the G232D mutant displays intermediate stability. Once the shells are dissociated under pressure, they do not reassemble upon decompression as monitored by light scattering (isolated symbols near the right edge of Figure 1A). However, the center of mass values return to those corresponding to the folded dissociated coat protein subunit (isolated symbols in Figure 1B), indicating that the subunits have refolded. High-performance gel filtration chromatography of the pressure-treated mutants confirmed that they were dissociated (data not shown). The inability of the refolded subunits to reassemble in the absence of scaffolding protein is consistent with their biological activity *in vivo* (Prevelige *et al.*, 1988).

In order to evaluate whether the mutations affect the stability of the unpolymerized coat protein monomers, pressure denaturation curves were obtained for the wild-type and mutant forms of the coat protein subunit. As shown in Figure 2, very small differences were detected between the

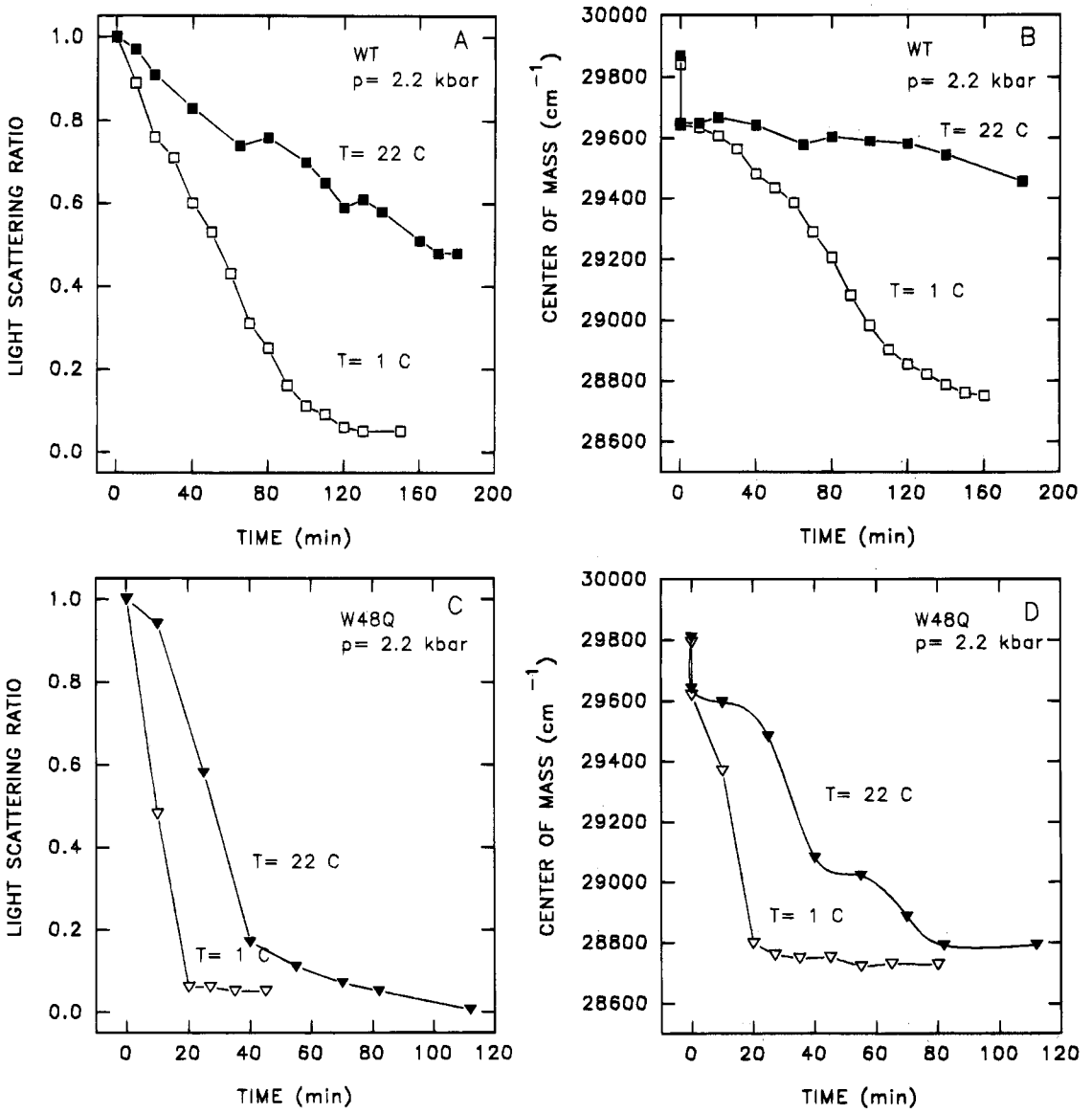

FIGURE 3: Effect of temperature on the dissociation of wild-type and W48Q mutant shells. Panels A and B: Dissociation of wild-type shells as followed by (A) light scattering and (B) center of spectral mass at a hydrostatic pressure of 2.2 kbar at 22 and 1 °C. Panels C and D: Dissociation of W48Q mutant shells as followed by (C) light scattering and (D) center of spectral mass at a hydrostatic pressure of 2.2 kbar at 22 and 1 °C. Protein concentration was 0.2 mg/mL.

three forms. We have reported previously that the wild-type coat protein monomer is very pressure labile (Prevelige *et al.*, 1993). The mutant subunits display a similar pressure sensitivity, although they may be slightly more stable than the wild type. These data indicate that the single-site substitutions markedly affect the stability of the assembled shell and yet have little effect on the stability of the coat protein subunit itself. This suggests that the substitutions are marking residues involved in inter-subunit interactions, either directly or through local conformational changes.

We have demonstrated previously that shells composed of wild-type protein subunits are stabilized by hydrophobic interactions (Prevelige *et al.*, 1993) and hence become labile with decreasing temperature. In order to evaluate the effect of the substitutions on the forces stabilizing the shells, we examined the effect of temperature on shell stability. Figure 3 shows the kinetics of dissociation of the wild-type shells (Figure 3A,B) and of the W48Q mutant (Figure 3C,D) at both 1 and 22 °C and 2.2 kbar as followed by light scattering (Figure 3A,C) and center of spectral mass (Figure 3B,D). As previously reported (Prevelige *et al.*, 1993), dissociation of the wild-type shells displays a large temperature dependence. However, the stability of the W48Q mutant is much less temperature dependent. We can conclude that the replacement of a single nonpolar amino acid (Trp48) by a polar residue (Gln) results in a significant loss of the entropic stabilization.

Shells composed of the G232D mutant subunits are more stable against pressure than the W48Q mutant but less stable than the wild type. In contrast to W48Q, shells composed of the G232D subunits maintain an appreciable dependence on temperature (Figure 4). In this mutant, a small uncharged side chain is replaced by a negatively charged side chain, suggesting that a packing defect in which an inter-subunit cavity is created may be responsible for the decrease in pressure stability.

The dissociation of shells by urea was also compared for the three forms (Figure 5). In these curves, the slope of the transition indicates the cooperativity of the denaturation, and a shift in the curve to lower denaturant concentration

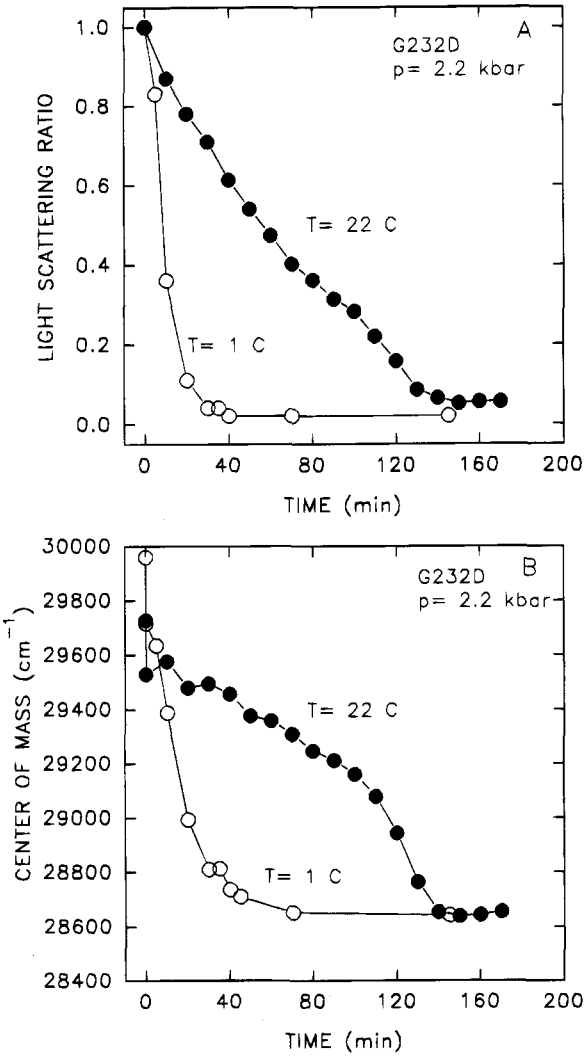

FIGURE 4: Effect of temperature on the kinetics of dissociation of G232D mutant shells. Panel A: Light scatttering vs time at a pressure of 2.2 kbar at both 22 and 1 °C. Panel B: Center of spectral mass vs time under identical conditions. Protein concentration was 0.2 mg/mL.

indicates a decrease in the stability of the capsid. A similar pattern was obtained wherein the wild-type shells were the most stable, followed by the G232D mutant and then the W48Q mutant shells.

The wild-type transition by both fluorescence and light scattering was quite cooperative, with the denaturation occurring from 4.8 to 6.0 M urea and the midpoint occurring at 5.2 M urea. The transitions by both light scattering and fluorescence were coincident, indicating that dissociation and denaturation are coupled. W48Q showed a decrease both in the cooperativity of denaturation, which occurred from 2.2 to 4.8 M urea, and in the midpont of the denaturation, which occurred at 3.2 M urea, indicating that shells made of the mutant W48Q protein are less stable than wild-type shells. As the W48Q mutant has lost a significant portion of its entropic stabilization, it would be expected to be more susceptible to urea dissociation than the wild type.

The behavior of G232D proved more complex. The dissociation/denaturation curve showed a distinct plateau by both light scattering and fluorescence. The plateau occurred in the range from 2.0 to 4.0 M urea, indicating the presence of a stable intermediate during dissociation. The plateau had

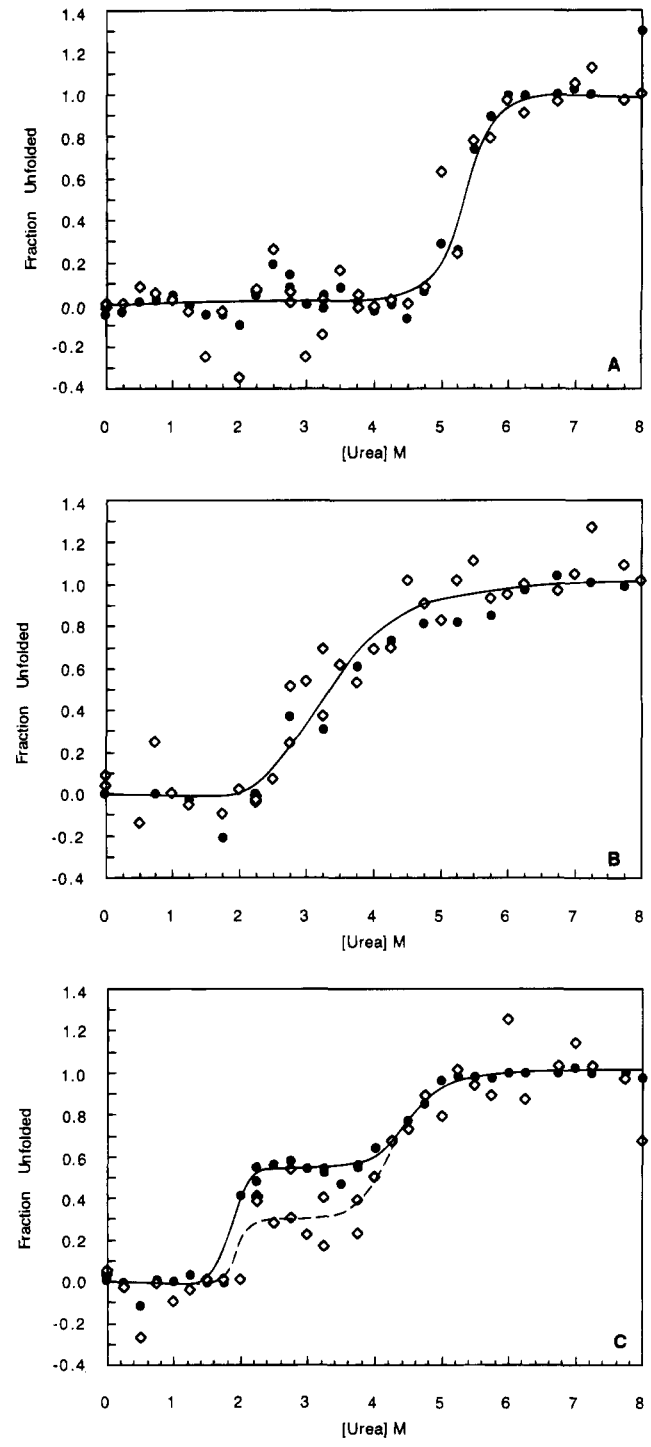

FIGURE 5: Urea denaturation of wild-type and mutant shells. Panel A: Dissoociation of wild-type shells with urea as followed by fluorescence (◇) and light scattering (●). Panel B: Same as panel A except shells are W48Q. Panel C: Same as panel A except shells are G232D.

73% of the fluorescence of the native G232D shells but only 46% of the light scattering, suggesting that the capsids were dissociating in a way that allowed the fluorescence to remain high, for instance, by removal of specific capsomeres. The transition from the native G232D shells to the plateau was extremely cooperative, occurring between 2.0 and 2.2 M urea with the midpoints determined by fluorescence and light scattering to be at 2.1 and 2.15 M urea, respectively. The second transition from the intermediate to the unfolded protein was broader, occurring from 4.0 to 5.2 M urea, and

it had midpoints of 4.3 M by fluorescence and 4.5 M urea by light scattering.

The capsids were inspected by negative stain electron microscopy after incubation in 3 M urea for 20 h (not shown). The micrographs revealed that wild-type shells mostly remained intact as expected. Shells of W48Q were primarily partial shells, but some intact shells were seen. Overall, there were fewer particles observed than for the wild type. In the case of G232D, we observed mostly intact shells whose edges were slightly fuzzy compared to those of the wild type. There were about the same number of particles in the G232D and wild-type samples. The appearance of a fuzzy edge in the micrographs is consistent with the loss of capsomeres.

The substitution of an aspartic acid residue in place of a proton may put the capsid under strain due to local packing deformities. The addition of urea could then weaken some interactions in the capsid, releasing a capsomere and thereby relieving the strain. Strain has been implicated in the maturation of nodaviruses where a specific proteolytic event causes release of strain in the capsid (Zlotnick *et al.*, 1994). Given that the coat protein subunits occupy different symmetry-related environments in the lattice, it is reasonable that the amount of strain induced by the substitution depends on the particular symmetry-related position being considered. This would explain the partial removal of capsomeres. The G232D substitution destabilized the capsid in general, as the second dissociation/denaturation transition occurred at lower urea concentrations than did the wild-type transition. In the case of the W48Q mutation, the substitution of a tryptophan residue for a glutamine appeared to have a general destabilizing effect on the capsid, consistent with the results seen by pressure denaturation.

In order to quantify the amount of entropic stabilization, we assumed the following model:

(1) First, dissociation is considered to be an irreversible reaction: $A_n \rightarrow nA$ with an apparent rate constant k.

(2) A value of $\Delta G^{\ddagger}$ (a standard free energy of activation) can be calculated according to the literature (Denbigh, 1981; Glasstone *et al.*, 1941; Guggenheim, 1937) from the equation

$$k = \frac{\kappa T}{h} \exp(-\Delta G^{\ddagger}/RT) \qquad (3)$$

where h is the Planck constant, κ is the Boltzmann constant, R is the gas constant, and T is the temperature.

(3) Equation 3 can be expressed in terms of entropy and enthalpy factors (Denbigh, 1981; Guggenheim, 1937):

$$k = \frac{\kappa T}{h} \exp(\Delta S^{\ddagger}/R - \Delta H^{\ddagger}/RT) \qquad (4)$$

The values of $\Delta S^{\ddagger}$ (entropy change of activation) and $\Delta H^{\ddagger}$ (enthalpy change of activation) were calculated from the data at the two temperatures and are given in Table 1. The table shows that the entropy term decreases drastically for W48Q.

These results demonstrate the importance of entropic contributions to stabilization of the procapsid lattice. The large unfavorable change in entropy of activation (-134.3 cal mol^{-1} K^{-1}) when wild-type shells dissociate to denatured coat protein reflects the exposure of nonpolar side chains to the solvent. Indeed, it has been shown that the hydrophobic dye bis-ANS binds well to coat protein monomers, but not to coat protein in shells (Teschke *et al.*, 1993). The W48Q mutant has much less unfavorable entropy of activation (-82.6 cal mol^{-1} K^{-1}), which is clearly reflected by the much smaller dependence on temperature (see the rate constant ratio column in Table 1). More recently, cold denaturation of cowpea mosaic virus was also observed under pressure and at subzero temperatures, and those dissociated coat subunits also bind bis-ANS (Da Poian *et al.*, 1995).

Table 1: Thermodynamic Activation Parameters of Dissociation of Wild-Type and Mutant P22 Shells

	rate constant ratio (k_1/k_{22})[a]	$\Delta S^{\ddagger}$ (cal mol^{-1} K^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$T\Delta S^{\ddagger}$ (22 °C) (kcal mol^{-1})	$\Delta G^{\ddagger}$ (22 °C) (kcal mol^{-1})
wild type	8.86	−134.3	−17.19	−39.62	22.43
W48Q	1.43	−82.6	−3.27	−24.37	21.1
G232D	6.38	−122.9	−14.73	−36.25	21.52

[a] Rate constants of dissociation were determined at 1 °C (k_1) and 22 °C (k_{22}).

Shells composed of either of the mutant forms of coat protein are less stable to pressure than the wild type. We have shown that for wild-type shell, there is a substantial entropic contribution to the stability. In the case of the W48Q mutant, the instability appears to arise from a decrease in the amount of entropic stabilization of the shell, while in the case of the G232D mutant, the effect appears to be volume related, with the shells maintaining entropic stabilization (Table 1). The interactions that lead to virus assembly are entropically driven, which means that they are dominated by weak interactions (nonpolar), and seem to have relatively less contribution from polar or H-bonding interactions. These results may have direct implications for the understanding of the switches that control assembly and disassembly of viruses *in vivo*. Any cellular process that ultimately leads to disassembly of the virus, as during uncoating, should perturb the weak van der Waals interactions ("hydrophobic bonds") that we show to be responsible for the stability of the virus. We show that a single alteration (Trp48 → Gln) can result in significant loss of the entropic stabilization of the capsid. The presence of a stabilizing "pocket factor" has been noted in the crystal structure of rhinovirus (Oliveira *et al.*, 1993). These factors may provide a means for modulating the strength of the hydrophobic interactions, and thereby virus stability.

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REFERENCES

Bazinet, C., Benbaset, J., King, J., Carazo, J., & Carrascosa, J. (1988) *Biochemistry 27*, 1849−1856.

Brandts, J. F. (1964) *J. Am. Chem. Soc. 86*, 4291−4301.

Bridgman, P. W. (1964) in *Collected Experimental Papers*, Vol. 1, pp 441−558, Harvard University Press, Cambridge, MA.

Chen, B., & Schellman, J. A. (1989) *Biochemistry 28*, 685−691.

Creighton, T. E. (1987) in *Protein Engineering* (Oxender, D. L., & Fox, C. F., Eds.) pp 83−90, Allen R. Liss Inc., New York.

Da Poian, A. T., Johnson, J. E., & Silva, J. L. (1994a) *Biochemistry 33*, 8339−8346.

Da Poian, A. T., Oliveira, A. C., & Silva, J, L. (1995) *Biochemistry* (in press).

Denbigh, K. G. (1981) in *The Principles of Chemical Equilibrium*, Chapter 15, pp 429−459, Cambridge University Press, Cambridge.

Eppler, K., Wyckoff, E., Goates, J., Parr, R., & Casjens, S. (1991) *Virology 183*, 519−538.

Foguel, D., & Weber, G. (1994) (submitted for publication).

Foguel, D., & Silva, J. L. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 8244−8247.

Foguel, D., Chaloub, R. M., Silva, J. L., Crofts, A. R., & Weber, G. (1992) *Biophys. J. 63*, 1613−1622.

Fuller, M. T., & King, J. (l98l) *Virology 112*, 529−547.

Glasstone, Laidler, K. J., & Eyring, H. (1941) in *The Theory of Rate Processes*, McGraw-Hill, New York.

Gordon, C. L., & King, J. (1993) *J. Biol. Chem. 268*, 9358−9368.

Griko, Y. V., Privalov, P. L, Sturtevant, J. M., & Venyaminov, S. Y. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3343−3347.

Guggenheim, E. A. (1937) *Trans. Faraday Soc. 33*, 607.

King, J., & Casjens, S. (l974) *Nature 251*, 112−119.

Lauffer, M. A., Ansevin, A. T., Cartwright, T. E., & Brinton, C. C.(1958) *Nature 181*, 1338−1339.

Oliveira, M. A., Zhao, R., Lee, W.-M., Kremer, M. J., Minor, I., Rueckert, R. R., Diana, G. D., Pevear, D. C., Dutko, F. J., McKinlay, M. A., & Rossmann, M. G. (1993) *Structure 1*, 51−68.

Prasad, B. V. V., Prevelige, P. E., Marietta, E., Chen, R. O., Thomas, D., King, J., & Chiu, W. (1993) *J. Mol. Biol. 231*, 65−74.

Prevelige, P. E., Thomas, D., & King, J. (1988) *J. Mol. Biol. 202*, 743−757.

Prevelige, P. E., Thomas, D., & King, J. (1993) *Biophys. J. 64*, 824−835.

Prevelige, P. E., King, J., & Silva, J. L. (1994) *Biophys. J. 66*, 1631−1641.

Privalov, P. L. (1990) *Crit. Rev. Biochem. Mol. Biol. 25* (4), 281−305.

Privalov, P. L., Griko, Y. V., Venyaminov, S. Y., & Kutyshenko, V. P. (1986) *J. Mol. Biol. 190*, 487−498.

Rossman, M. G., & Johnson, J. E. (1989) *Annu. Rev. Biochem. 58*, 533−573.

Royer, C. A., Weber, G., Daly, T. J., & Matthews, K. S. (1986) *Biochemistry 25*, 8308−8315.

Silva, J. L. (1993) in *High Pressure Chemistry, Biochemistry and Material Sciences* (Winter, R., & Jonas, J., Eds.) NATO ASI Series C401, pp 561−578, Kluwer Academic Publishers, Dordrecht.

Silva, J. L., & Weber, G. (1988) *J. Mol. Biol. 199*, 149−161.

Silva, J. L., & Weber, G. (1993) *Annu. Rev. Phys. Chem. 44*, 89−113.

Silva, J. L., Miles, E. W., & Weber, G. (1986) *Biochemistry 25*, 5780−5786.

Silva, J. L., Silveira, C. F., Correia, A., & Pontes, L. (1992) *J. Mol. Biol. 223*, 545−555.

Silva, J. L., Villas-Boas, M., Bonafe, C. F. S., & Meirelles, N. C. (1989) *J. Biol. Chem. 264*, 15863−15868.

Stutervant, J. M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2236−2241.

Teschke, C. M., & King, J. (1993) *Biochemistry 32*, 10839−10847.

Teschke, C. M., King, J., & Prevelige, P. (1993) *Biochemistry 32*, 10658−10665.

Weber, G. (1992) *Protein Interactions*, Chapman & Hall, New York and London.

Weber, G. (1993) *J. Phys. Chem. 97*, 7108−7115.

Zlotnick, A., Reddy, V. S., Dasgupta, R., Schneenann, A., Ray, W. J., Rueckert, R. R., & Johnson, J. E. (1994) *J. Biol. Chem. 266*, 13680−13684.

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