New and Notable

CONFORMATIONAL CHANGE—AN ALTERNATIVE ENERGY SOURCE?: Exothermic Phase Transition in Phage Capsid Maturation

Alasdair C. Steven
Laboratory of Structural Biology, National Institute of Arthritis, Musculoskeletal, and Skin Disease, National Institutes of Health, Rockville, Maryland 20892 USA

CAPSID EXPANSION AND CONFORMATIONAL CHANGE

Although the capsids of the large double-stranded (ds) DNA bacteriophages vary substantially in such basic aspects as the molecular weights, amino acid sequences, and packing geometries of their protein subunits, their assembly pathways are organized along remarkably uniform lines (1). Their shared properties include a striking example of a large-scale, concerted, irreversible, conformational change that is undergone by the capsid subunits. In this transition, the round, thick-walled, precursor capsid (procapsid) converts to the polyhedral, thinner-walled, mature capsid (Fig. 1): moreover, it expands by about 15% in linear dimensions, corresponding to an approximately 50% increase in its capacity for DNA. New work by Galisteo and King (2), based on differential scanning calorimetry (DSC) of *Salmonella* phage P22 procapsids, brings fresh insight into the energetic transactions that accompany capsid expansion.

CALORIMETRIC SIGNATURE OF PROTEIN CONFORMATION

DSC measures directly the exchanges in energy that accompany phase transitions in macromolecules, as induced by thermal scanning. With proteins, these transitions are generally endothermic, and correspond to thermal denaturation of entire molecules or individual domains (3). The temperature (T_m), denaturational enthalpy, and change of specific heat across the transition are characteristic of a given protein under specified buffer conditions and reflect the combination of forces that stabilize its conformation. The sharpness of an endotherm relates to its cooperativity. Endotherms of this kind are interpreted in terms of the thermodynamics of a reversible partitioning between two states (folded/unfolded), and much experimental work with globular, monodomainal, proteins supports this scenario. However, many other proteins, whose folding and assembly are presumably more elaborate, do not spontaneously renature after thermal denaturation. Nevertheless, their thermograms qualitatively resemble those of "well-behaved" model proteins, and are interpreted in terms of kinetically limited rather than thermodynamically reversible processes.

THERMAL BEHAVIOR OF P22 PROCAPSIDS

The experiments of Galisteo and King show that P22 procapsids exhibit four well-resolved thermal events—endotherms at 49, 71, and 87°C, and an exotherm at 61°C (2). The molecular consequences of each event were worked out by using other techniques to compare procapsid samples after incubation at strategically chosen temperatures. The methods employed were negative staining electron microscopy, gel filtration chromatography, agarose gel electrophoresis, and density gradient centrifugation. Thus, the 49°C endotherm was found to correspond to denaturation of the (internal) scaffolding protein; the 71°C endotherm, to a localized denaturation event in the shell; and the 87°C endotherm (energetically, by far the major event recorded), to denaturation of the capsid shell. The sharpness and even the T_m of the latter event closely match those previously recorded for the T4 capsid (4, 5). Importantly, after incubation of procapsids at 65°C, only capsids of the mature (expanded) type were present, implying that the 61°C exotherm correlates with expansion!

LOCAL AND GLOBAL FREE ENERGY MINIMA: PROTEIN CONFORMATION AS AN ENERGY SOURCE?

Assuming that the efflux of energy in the 61°C exotherm does indeed derive from capsid expansion (and not from some other phenomenon, such as capsid aggregation, with expansion being an energetically unobtrusive event

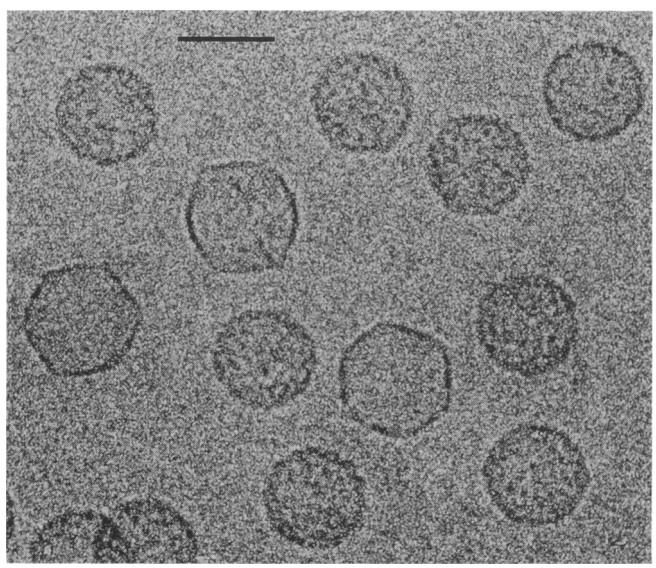

FIGURE 1 Cryoelectron micrograph of precursor and mature (expanded) capsids of bacteriophage T7 (M. Cerritelli, N. Cheng, J. Conway, and A. Steven, unpublished results). The P22 capsid undergoes a similar transition (7). Bar = 50 nm.

that happens to occur around the same temperature), its implications are considerable. That expansion should, in fact, release energy is implied by earlier DSC experiments in the T4 system which detected a difference in denaturational enthalpy between the precursor and the expanded states of the capsid surface lattice (6). Unlike the T4 precursor lattice which denatures upon heating, the P22 procapsid first expands, making possible a direct monitoring of this reaction by DSC.

The amount of energy released in the 61°C exotherm (90 kJ/mol of capsid protein) is about 5% of the energy required to denature the capsid at 87°C, and approximately equivalent to the hydrolysis of three ATP molecules per monomer or about 1200 ATP hydrolyses per procapsid.

The observation of an efflux of energy upon switching of a protein between two physiologically defined states is novel and suggests a mechanism in which protein folding and a requirement for biomechanical work at the molecular level may be coupled (6). The protein initially assumes a conformation that corresponds to a local minimum of free energy. Subsequently, in response to some regulatory trigger, it switches into a different state that represents another, lower, local or global minimum-energy state, with a concomitant release of energy (Fig. 2). The released energy is channeled into some otherwise forbidden process.

RAMIFICATIONS

In phage capsid assembly, the most obvious candidate for coupling to an energy-requiring process would be packaging of DNA (8). However, the requirement for ATP of in vitro packaging, and the observed packaging of DNA into pre-expanded T4 capsids appear to rule this out (9). Conversely, the

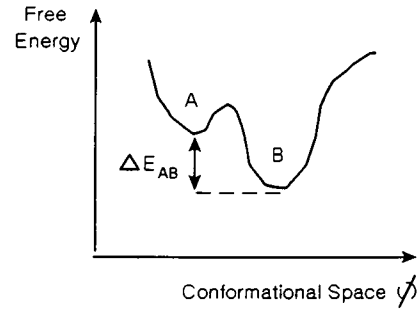

FIGURE 2 Schematic diagram indicating local, and global minima of free energy in conformational space. In principle, transition between states A and B corresponds to an energy release of ΔE_{AB}.

onset of DNA packaging may well trigger capsid expansion in vivo (1, 9). The main gain from procapsid expansion transformation is probably a stabilization of this relatively labile particle.

Another example of such a reaction is represented by the contraction of the tail sheath of phages that use this mechanism to penetrate host cell membranes (10). Contraction of the T4 tail sheath has been correlated with an exothermic transition at 72°C with an emission of 180 kJ/mol of sheath protein (11), approximately twice as much as in P22 capsid expansion. This observation prompts the speculation that exothermic conformational changes may be more widespread in other processes in which membrane penetration is again the energetic barrier: e.g., the translocation of secretory proteins across membranes, or the insertion of integral membrane proteins.

Are exothermic conformational changes of proteins real? Do phage hold the monopoly? Do dsDNA phage capsid proteins fold into novel variations of the jelly-roll β-barrel motif that is common to so many other, generally smaller, viruses (12)? Or into some entirely different conformation(s)? And how are they reorganized upon expansion? These and other questions, underscored by the recent developments in the P22 system (2), provide a strong incentive for enhanced activity in this picturesque arena where bioenergetics, protein folding, and viral multiplication converge.

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