Bacillus subtilis Bacteriophage SPP1 DNA Packaging Motor Requires Terminase and Portal Proteins*

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Bacillus subtilis phage SPP1 replication results in the formation of large head-to-tail concatemeric dsDNA. To initiate DNA packaging, the terminase, composed of small GIP and large G2P subunits, recognizes and cleaves the concatemeric DNA within the pac site (see Refs. 5 and 6 and Fig. 1). The SPP1 packaging site is divided into three discrete subsites: pacL, defining the non-encapsidated or left DNA end, pacC, the cleavage site, and pacR, the encapsidated or right DNA end (Refs. 7 and 8 and Fig. 1A). The terminase, bound to the encapsidated DNA end, interacts with the portal protein G6P. Then, the DNA is translocated into an empty procapsid until the head is full (5, 7, 9). Viral DNA translocation is a poorly understood mechanism. The SPP1 packaging motor lies at a unique vertex of the procapsid and contains the hetero-oligomeric SPP1 terminase enzyme, composed of two decameric ring-shaped GIP and two monomeric G2P, which assemble onto the pac site of SPP1 concatemeric linear dsDNA molecule, and the oligomeric G6P composed of 12 identical subunits (6, 8, 10). However, purified G6P in solution has 13 subunits (11).

Initiation of headful packaging of SPP1 DNA concatemers involves the interaction of the terminase, GIP and G2P, and the portal protein, G6P. GIP, which specifically recognizes the non-adjacent pacL and pacR subsites and directs loading of G2P to pacC, interacts with G6P. G2P, which has endonuclease, DNA binding, and ATPase activities, interacts with GIP and does it transiently with G6P. The stoichiometry of GIP on the GIP-G2P complex promotes the transition from a G2P endonuclease to an ATPase. G6P does not alter the endonuclease activity of G2P. Both GIP and G6P, which do not have endogenous ATPase activity, synergistically enhance and modulate the ATPase activity of G2P. Based on these results, we propose a model in which the modulation of the ATPase and endonuclease activities of G2P accounts for the role of the terminase in headful packaging.

Many cellular processes require the action of a biological motor protein that converts chemical energy into mechanical force or directional movement. Packaging of viral head-to-tail concatemeric dsDNA into viruses involves the specific interaction of virus DNA with the pre-assembled procapsid and subsequent translocation of the former into the latter, by the action of a DNA translocase, to render a highly condensed structure (1–4). DNA translocases are molecular motor proteins that use the energy of nucleoside triphosphate hydrolysis to package concatemeric dsDNA onto an empty procapsid. This is a common feature shared by the DNA packaging machinery of many bacterial, pox, and herpes viruses. Two general modes for packaging of concatemeric dsDNA into the capsid of a bacterial virus (also termed bacteriophage or phage) have been proposed. The first implies a site-specific packaging in which the recognition sequence (termed cos in phage λ) plays an important role in initiation and termination of DNA encapsidation. This packaging process, which generates unit-length encapsidated molecules, is well characterized in phages λ, T3, and T7 (1–4). The second mode implies headful packaging, in which the encapsidation initiates at a specific site in the genome (termed pac in phage SPP1), but with the capacity of the procapsid playing a predominant role in the termination step. The sequential headful packaging mechanism generates a heterogeneous population of terminally redundant and partially circularly permuted DNA molecules as in the cases of SPP1, P1, P22, or in the case of T4 whose DNA is totally permuted and terminally redundant (Refs. 1 and 3 and Fig. 1).

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† The abbreviations used are: dsDNA, double-stranded DNA; Myr, myristicin; SSR, square sumatory residual.

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Upon cleavage at pacC, the DNA end proximal to pacC is degraded by the G2P endonuclease (5, 6). We have proposed that the GIP molecule bound to pacC and interacting with the other GIP molecule bound to pacR is now free to interact with G2P that has cleaved the b-box proximal to pacR (Refs. 5 and 6 and Fig. 1A). The terminase with a GIP \( P \) molecule bound to \( P \) and an ATP concentration range of \( 10^{-5} \, \text{M} \) (with \( \alpha \) = 0.5 M) and GIP 60 nM G6P and G1P, was carried out with 5 M [\( \text{GTP} \) and G2P (3 μM) were preincubated in buffer B (50 mM Tris-HCl, pH 7.5 containing 10 mM MgCl2, 300 mM or 50 mM NaCl, over 15 min at 30 °C, loaded onto the gradient (10–30%), and centrifuged (SW50 Ti, 45,000 rpm, 6 h at 4 °C). The aliquots taken from the bottom of the tubes were separated by 15% (w/v) SDS-PAGE, electrophoresed onto polyvinylidene difluoride Immobilon-P transfer membrane (Millipore) according to standard procedures, and the proteins detected by a mixture of polyclonal antibodies raised against both proteins. The signals were quantified by laser densitometric scanning.

**ATPase Assay**—Standard reactions were incubated in buffer B (50 mM Tris-HCl, pH 7.5 containing 10 mM MgCl2, 50 mM NaCl, and 1 mM dithiothreitol) in a final volume of 20 μl. The reactions were initiated by the addition of the substrate after a preincubation of the proteins for 5 min at 37 °C and were allowed to proceed for 15 min more at 37 °C. ATPase activity was determined by measuring the amount of phosphate set free upon hydrolysis as previously described (21). Initial velocity studies were performed using 20 nM of G2P and an ATP concentration range of 0.0005–10 mM (10–0.05 μCl/μmol). The initial velocity of ATP hydrolysis was determined within the linear range of each reaction using the following protein concentrations: 20 nM G2P, 95 nM GIP, and 80 nM G6P.

ATP hydrolysis in the presence of different concentrations of G6P or/and GIP proteins and Myr, was carried out with 5 mM [\( \gamma\text{-32P} \)ATP, 0.05 μCi/μmol. \( K_a \) and \( V_{\text{max}} \) values were obtained by non-linear least-squares fit of the experimental data to the Michaelis-Menten equation with the Kaleidagraph® version 6.2.0 Abbebeck Software program. The mathematics software, MATLAB® version 5.1, has made possible an iterative analysis to fit the experimental data with several theoretical models.

For the binary interaction of G2P-GIP and G2P-G6P (see Fig. 3), considering E, the enzyme (G2P); M, the modulator (GIP or G6P); \( \alpha \), the ratio between \([EM]\) and \([E_M]\); \( \alpha \), the modulator stoichiometry coefficient; and \( x_0 \), the ratio between \([MI]\) and \([E_M]\). Equations 1 and 2 were applied.

\[
K_{E[M]} x_0 = \frac{\alpha}{(1 - \alpha)x_0 - \alpha} \quad \text{(Eq. 1)}
\]

\[
r_0 = V_{\text{max}} (1 - \alpha) + V_{\text{max}} \alpha \quad \text{(Eq. 2)}
\]

For the ternary interactions (Fig. 4): G2P-GIP-G6P, \( x_0 \) corresponds to the ratio between \([GIP]_{\text{free}}\) and \([G2P]_{\text{free}}\) and \( y_0 \) corresponds to the ratio between \([G6P]_{\text{free}}\) and \([G2P]_{\text{free}}\). The applied Equations 3–6 were as follows.

\[
x_0 = x + \frac{K_{E[M]}[C1_1]x_0}{(1 + K_{E[M]}[C1_1])^3} + \frac{2K_{E[M]}[C1_2]^3x^2 + 4K_{E[M]}[C1_2]^4x^4}{(1 + K_{E[M]}[C1_2])^3} \quad \text{(Eq. 3)}
\]
Endonuclease Activity Assay—The concentration of DNA was determined by using molar extinction coefficients of 6,500 \text{ M}^{-1} \text{ cm}^{-1} at 260 nm. Form I pBT363 (20 nM) was incubated with G6P (80 nM) and different concentrations of GIP and G6P over 60 min at 37 °C in buffer C (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM MgCl2). G2P exhibited endonuclease activity that converted Form I or supercoiled into Form II reaction Myr at 160 nM was used. Reactions were stopped by addition of 30 mM MgCl2, 50 mM NaCl, and 1.5 mM dithiothreitol with and without 1 mM (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM MgCl2). The pellets were dissolved in cracking buffer and loaded on a 15% PAGE, electroblotted, and revealed by Western blot analysis.

RESULTS

GIP Physically Interacts with G6P—Previously, it has been shown that G2P interacts with GIP (19, 6), but fails to form a stable complex with G6P (23, 19, 6). To learn whether GIP interacts with G6P a glycerol gradient was used. GIP (predicted molecular mass of 20.7 kDa), and the portal protein, G6P (predicted molecular mass of 57.3 kDa), have native molecular masses of 210 kDa (10 mer) and 745 kDa (13 mer), respectively (see Refs. 5, 8, 11, and 22). GIP (4.5 mM), G6P (2.9 mM), or both proteins in buffer A containing 50 or 300 mM NaCl were loaded onto a linear glycerol gradient and centrifuged. The aliquots, collected from the bottom of the tube, were separated by SDS-PAGE, electrophorellated, and revealed by Western blot analysis. When the collected fractions were analyzed, a G6P broad peak, with a maximal protein concentration between fractions 4 and 5, was observed (Fig. 2A), whereas a sharper peak, with maximal protein concentration between fractions 7 and 9, was detected for GIP (Fig. 2B).

The sedimentation coefficients corresponding to a molecular mass of 600–800 kDa for G6P (Fig. 2A) and of ~200 kDa for GIP were calculated (Fig. 2B). In the presence of 300 mM NaCl, the G6P and GIP peaks are similar to the ones corresponding to the individual proteins (Fig. 2C). In the presence of 50 mM NaCl, however, GIP shifted from fractions 7–9 to fractions 2–4, and G6P was observed between fractions 2 and 5. The estimated native mass of protein complex present in fraction 3 should be ~1,000 kDa (Fig. 2D). Furthermore, protein-protein interaction followed by immunoprecipitation allowed us to pull-down both GIP and G6P proteins (data not shown). These results are consistent with electron microscopy studies. Preliminary results, albeit with a limited resolution of the electron micrographs, suggest that GIP binds to the stem domain of G6P in several visualized structures. Since the G6P stem domain faces the capsid exterior (see Refs. 10 and 24) it is likely that the detected interaction, both in the absence of G2P and/or DNA, is relevant.

G6P and G1P Enhance Synergistically the G2P ATPase Activity—G2P hydrolyzed ATP and dATP to the corresponding diphosphate and inorganic phosphate, with a low affinity (Km ~950 μM) and activity (kcat 26 ± 1 min−1), but fails to hydrolyze other NTPs and dNTPs (6). To address whether G6P is able to interact and modify any of the G2P activities, we incubated both proteins and performed nuclease and ATPase assays. G6P does not have a nuclease or NTase activity of its own (data not shown). As revealed in Table 1, G6P is able to enhance ~2-fold the G2P ATPase activity but does not seem to modify the G2P nuclease activity (see below). The effect of G6P in lowering the Km and increasing kcat of the G2P ATPase is specific because both bovine serum albumin nor a heat-inactivated G6P is able to stimulate the ATPase activity of G2P (data not shown).

Previously, it has been shown that GIP lowers the Km and increases the kcat of the G2P ATPase (Ref. 6 and Table 1). The presence of both G6P and G1P increases the G2P ATPase or dATPase activity, regarding both the Km of the substrate and the reaction velocity of the enzyme. None of the other purified components of the SPP1 procapsid (major capsid protein, G1IP, scaffolding protein, G1P, and accessory protein, G7P) affect the ATPase activity of G2P (23).

In the G2P ATPase activity, ATP behaved as a Michaelis-Menten type substrate (see Table 1). The Km and Vmax values were obtained by non-linear least-squares fit of the experimental data to the Michaelis-Menten equation. Since we have failed to detect a stable G2P-G6P complex by affinity chromatography, we assumed that G6P might form a transient complex with G2P. It is likely, therefore, that G6P forms a complex with the terminase (G6P-G1P-G2P), and that G6P couples the stimulated ATPase to the functional SPP1 DNA packaging machinery.

G2P Interacts with G6P with a G6P1-G2P1 Stoichiometry—The ATPase activity of the G2P enzyme (E) in the presence of

\[ r_0 = \frac{V_{\text{max,1}} + V_{\text{max,2}}K_{\text{a1}}([C1]_0)^2x^2}{1 + K_{\text{a1}}([C1]_0)^2x^2 + K_{\text{a2}}([C2]_0)^2y^2} \]  

(Eq. 4)

\[ y_0 = \frac{K_{\text{a1}}([C1]_0)y + K_{\text{a2}}([C2]_0)y + 2K_{\text{a3}}([C2]_0)^2y^2}{1 + K_{\text{a1}}([C1]_0)y + K_{\text{a2}}([C2]_0)y + 2K_{\text{a3}}([C2]_0)^2y^2} \]  

(Eq. 5)

\[ r_0 = \frac{V_{\text{max,1}} + V_{\text{max,2}}K_{\text{a1}}([C1]_0)^2y^2}{1 + K_{\text{a1}}([C1]_0)^2y^2 + K_{\text{a2}}([C2]_0)^2y^2} \]  

(Eq. 6)

\[ V_{\text{max,1}} + V_{\text{max,2}} \] 

\[ K_{\text{a1}}([C1]_0)^2x^2 + K_{\text{a2}}([C2]_0)^2y^2 \] 

\[ 1 + K_{\text{a1}}([C1]_0)x^2 + K_{\text{a2}}([C2]_0)y^2 \] 

\[ 1 + K_{\text{a1}}([C1]_0)x^2 + K_{\text{a2}}([C2]_0)y^2 + 2K_{\text{a3}}([C2]_0)^2y^2 \] 

\[ 1 + K_{\text{a1}}([C1]_0)x^2 + K_{\text{a2}}([C2]_0)y^2 + 2K_{\text{a3}}([C2]_0)^2y^2 \] 

\[ V_{\text{max,1}} + V_{\text{max,2}} \] 

\[ K_{\text{a1}}([C1]_0)^2y^2 + K_{\text{a2}}([C2]_0)^2y^2 \] 

\[ 1 + K_{\text{a1}}([C1]_0)y^2 + K_{\text{a2}}([C2]_0)y^2 + 2K_{\text{a3}}([C2]_0)^2y^2 \]
increasing concentrations of G6P modulator (M) was analyzed using a mathematical program (see "Experimental Procedures"). This program made possible an iterative analysis to fit the experimental data with several theoretical models (one G2P with one, two, or three G6P molecules, E1M1, E1M2, E1M3, etc.).

The values of the catalytic constants were obtained by non-linear least-squares fit of the experimental data to the Michaelis-Menten equation in the presence of increasing ATP concentrations. ATPase assays were performed with a constant amount of G2P and increasing G6P concentrations. The experimental data suggest that the stoichiometry of the complex fits best with the theoretical E1M1 model, which corresponds to the lowest SSR value, with a very high constant of 1.58 nm⁻¹. It is noteworthy that this very high value for K, suggests a tendency for G2P to be in a complex with G6P with a 1:1 stoichiometry (Fig. 3A). When experiments containing a constant amount of G6P and increasing G2P concentrations were performed, a peak corresponding to a 1:1 stoichiometry was obtained, supporting the above data (data not shown). Since, there is only one G6P oligomer per empty procapsid it is likely that the stoichiometry of G2P and G6P in the packaging complex is G2P₁G6P₁.

G2P Interacts with G1P with a G1P₂₃G2P₁ Stoichiometry—G1P enhances the G2P ATPase activity (Ref. 6 and Table I). The ATPase assay was used to measure the stoichiometry of the G1P-G2P complex. As described in the previous section, the assay was carried out with a constant amount of G2P (E) and increasing G1P (M) concentrations. Initially, three possibilities have been considered (e.g., E1M1, E1M2, E1M3). The best fit (the lowest SSR value) corresponds to a stoichiometric coefficient of the modulator (a) = 2 (K = 6.12 × 10⁻⁴ nm⁻²) or (a) = 3 (K = 4.0 × 10⁻⁵ nm⁻²) and a stoichiometry of G1P₁G2P₁ or G1P₂G2P₁ (Fig. 3B). Since, in the packaging initiation complex there are two decameric G1P bound to the pacL and pacR DNA loop and one monomeric G2P molecule to the b-box proximal at pacR, it is likely that the stoichiometry of the active ATPase complex is G1P₁G2P₁ (8, 6). This is consistent with the observation that the second monomeric G2P protein, that degrades the non-encapsidated, leaves the packaging initiation complex (5, 6).

G1P and G6P Modulate G2P Activities—To study the G6P-G1P-G2P complex interaction, two sets of experiments were performed. First, constant G2P and G6P concentrations were incubated with increasing concentrations of G1P and, second, constant G2P and G1P were incubated with increasing concentrations of G6P, and ATPase and nuclease assays were performed. The G6P and G1P concentrations selected suggest that in the initial moment all the G2P molecules would be in the form of the corresponding adducts (G2P₁-G6P₆ or G2P₁-G1P₆). As revealed in Fig. 4A, a sharp peak corresponding to the first set of experiments, suggests that initially a very active species is formed when G1P is present in the reaction; however, when more G1P is added, the initial active adduct is transformed to a nearly inactive one. Such a negative effect on the ATPase activity is not observed when G6P is omitted from the reaction mixture (Ref. 6 and data not shown). Concerning the G2P endonuclease activity, there is a constant inhibition

| Protein added | $K_m$ (μM) | $V_{max}$ (μmol min⁻¹ mg⁻¹) | $h_{cat}$ (min⁻¹) | $k_{cat}/K_m$ (m⁻¹ s⁻¹) |
|---------------|------------|-----------------------------|-------------------|------------------------|
| G2P          | 913 ± 52   | 0.5 ± 0.01                  | 25                | 4.5 × 10²             |
| G2P + G1P    | 324 ± 81   | 1.7 ± 0.1                   | 83                | 4.3 × 10²             |
| G2P + G6P    | 605 ± 80   | 1.0 ± 0.2                   | 47                | 1.3 × 10³             |
| G2P + G1P + G6P | 219 ± 25 | 6.4 ± 1.2                  | 305               | 2.3 × 10⁴             |

Table I

**Catalytic parameters of G2P, G1P-G2P, G2P-G6P and G1P-G2P-G6P**

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Fig. 3. **Stoichiometry of ATPase complexes.** A comparison of experimental data and theoretical models, revealed different possible stoichiometries. The SSR value shows the deviation of the experimental data regarding each theoretical model. The experimental (filled circles) and theoretical (solid line) $A$, the ATPase activity of G2P (20 nm) in the presence of increasing concentrations of G6P (2.5, 5, 7, 9.4, 11.7, 14, 16.4, 80.5, and 161 nm). SSR values are as follows: $a = 1$, SSR = 0.0823; $a = 2$, SSR = 0.206; $a = 3$, SSR = 0.354. B, the ATPase activity of G2P (20 nm) in the presence of increasing concentrations of G1P (5, 9, 24, 42, 71, 95, 120, 190, and 275 nm). SSR values are as follows: $a = 1$, SSR = 0.077; $a = 2$, SSR = 0.019; $a = 3$, SSR = 0.023.
Myricetin Alters G2P Activities—The phosphoinositide 3-kinase protein has been co-crystallized with its inhibitor Myr. Myr in the Myr-phosphoinositide 3-kinase co-crystal fits and fills the ATP binding pocket (25). Furthermore, Myr blocks ATP hydrolysis and DNA translocation in certain DNA helicases (26). To determine whether Myr has any effect on the G2P activities, these assays were performed in the presence of increasing concentrations of Myr. The same Myr (~15 μM) concentration reduces ~50% of the ATPase activity of the G2P-G1P, G2P-G6P, or the G2P-G1P-G6P complexes (Fig. 5A).

To address whether any modification on the ATP binding pocket of G2P might also affect its endonuclease activity, ATPase and nuclease assays were performed. As revealed in Fig. 5B, 20 μM Myr reduces the G2P ATPase activity to nearly background levels and exerts a 50% inhibition on the nuclease activity of G2P.

The GIP modulator exerts a negative effect in the G2P endonuclease and enhances its ATPase activity (6). To learn if the GIP presence affects the action of Myr on G2P we have measured the activities associated with the GIP-G2P complex. GIP, at a concentration that stimulates the ATPase activity and reduces ~50% the G2P nuclease, was preincubated with G2P, then incubated with Myr and ATPase, and nuclease assays were performed. Myr inhibits the GIP stimulatory effect on the G2P ATPase activity, but is not able to inhibit the G2P nuclease activity (Fig. 5C). This is consistent with the observation that Myr (50 μM) is unable to disrupt the GIP-G2P complex, as addressed by protein-protein cross-linking in the presence of increasing concentrations of Myr (data not shown). Furthermore, in the presence of Ca2+, G2P binds to DNA, and such a binding is reduced by the addition of 20 μM Myr (data not shown). It is likely, therefore, that Myr introduces a conformational change in the G2P catalytic domain of the G2P ATPase and affects both the ATPase and nuclease activities in a manner that is different from the GIP-induced conformational change.
but the enzymatic data shown here demonstrate an interaction between both proteins. Using different approaches, it is suggested that G1P physically interacts with G6P. We showed that G1P-G6P synergistically increases the $v_{\text{max}}$ of the G2P ATPase. The ATPase activity associated with the terminase is likely to power DNA translocation in the SPP1 DNA packaging motor.

The major finding presented in this report is the regulation of the terminase ATPase activity by the portal protein G6P. G6P, which neither binds ATP nor hydrolyzes ATP on its own, stimulates by 2-fold the ATPase activity of G2P. This is consistent with the observation that no ATPase activity has been associated with any of the portal proteins described so far and with a 1.2-fold stimulation of the T4 gp17 ATPase by the addition of the gp20 portal protein (17).

G1P, which binds but does not hydrolyze ATP, might induce a conformational change on G2P. This postulated allosteric change in G2P leads to a stimulated ATPase and markedly reduced ATP-independent endonuclease, but does not affect sequence-independent DNA binding activity of G2P in the G1P-G2P complex, at any region other than at the pac site (6). This is consistent with the observation that both G1P and Myr modified the activities associated with G2P in a different manner.

G6P stimulates the G2P ATPase activity, but neither alters the endonuclease nor the DNA binding activity of G2P. In the presence of a large excess of G1P (G2P$_{\text{p}}$G1P$_{\text{2}}$G6P$_{\text{1}}$), both ATPase and endonuclease activities associated with G2P are drastically reduced, and a large excess of G6P reduced the G2P ATPase activity. Since the presence of an excess of G6P cannot reverse the negative effect of G1P on the endonuclease activity of terminase by titrating out G1P, but reduces the ATPase activity (Fig. 4B), we have to assume that both G1P and G6P are bound to G2P and act as modulators of the G2P activities. The global analysis of the ATP hydrolysis data showed that the most active ATPase species seems to have a G2P$_{\text{p}}$G1P$_{\text{1}}$G6P$_{\text{1}}$, or G2P$_{\text{1}}$G1P$_{\text{2}}$G6P$_{\text{1}}$ stoichiometry. The $k_{\text{cat}}$ is significantly reduced at higher G6P or G1P concentrations. On the basis of these data, we suggest that G1P and G6P are involved in promoting a conformational switching in G2P, thus performing a reorganization of the terminase subunits assembled at the procapsid to yield a catalytically competent DNA packaging motor complex with reduced endonuclease activity.

The experimental data regarding ATPase activity demonstrated that the $K_m$ and $k_{\text{cat}}$ had changed significantly in the presence of G1P and G6P. The affinity toward ATP has increased in the presence of both proteins, as observed by a decrease of 4-fold in the $K_m$ of the G2P$_{\text{1}}$G1P$_{\text{2}}$G6P$_{\text{1}}$ complex. At the same time, there is an increase of 13-fold in the turnover rate ($k_{\text{cat}}$) of the G2P ATPase.

The mechanism by which viruses translocate their genome into empty procapsids is still not understood. The translocation of SPP1 concatemeric dsDNA into a preformed viral capsid is a complex process that requires the concerted action of the terminase-DNA complex with the portal protein located at a unique vertex of the procapsid. This is consistent with the observation that: (i) in vivo, cleavage at pac is independent of G6P, but it is stimulated by its presence (5) and (ii) the purified major capsid, G13P, scaffolding, G11P, and accessory, G7P, procapsid protein are unable to interact with the terminase (19, 23, 27).

In many phage systems, the terminase large subunit physically interacts with the portal protein. Previously, we have failed to detect a G2P-G6P stable complex in solution (19, 6)
Previously, we have proposed that G1P induces a conformational change in G2P, with “modified G2P” having a stimulated ATPase and a shut off of endonuclease activity (6). The experiments reported here show that Myr inhibits the endonuclease and ATPase activities of G2P, whereas Myr exerts a negative effect on the ATPase but does not seem to affect the nuclease activity of the G2P/H18528G1P complex. Similar results are observed if G6P is added to the reaction G1P/H18528G2P complex. It is likely, therefore, that the nuclease and ATPase of G2P are present in discrete and related modules, but the presence of G1P uncoupled such domain interaction. This is consistent with T4 terminase mutants that showed a defect as a translocase but not in terminase cutting activity (16).

A Model for SPP1 DNA Packaging—The SPP1 packaging motor is a highly specific and processive enzyme starting within a concatemeric DNA substrate, which is able to translocate as many as 183.6-kb (4 processive headful packaging events) for an initial binding to the pac site (5, 18). The overall process begins with replication of the viral genome to generate head-to-tail concatemeric dsDNA. It is generally accepted that DNA translocation into the procapsid is powered by ATP hydrolysis; however there is much less agreement what general mechanism of ATP hydrolysis drives DNA into the procapsid (reviewed in Refs. 1–4). Unlike the Φ29 packaging motor, that is composed of twelve subunits of the portal, five or six pRNA and ATPase molecules (32–34), in SPP1 the packaging motor is composed of twelve subunits of G6P, more than one decameric G1P and a monomeric G2P (Refs. 8, 6, 10, and this work). At least in the SPP1 case the presence of the pRNA in the packaging motors has been ruled out (see Ref. 9).

After Hendrix (35), Dube et al. (11), Simpson et al. (33), and Guasch et al. (34), we propose a model for SPP1 DNA packaging that might apply to other bacteriophages and to herpesviridae. This is schematically depicted in Fig. 6.

I) Interaction of the Terminase with Concatemeric DNA—Specific recognition and endonucleolytic cleavage of the SPP1 pac sequence is the first step in DNA packaging. It requires exclusively two terminase subunits, although the terminase activity is enhanced by the presence of the portal protein (5, 6). Biochemical evidence shows that two molecules of G1P bind specifically and cooperatively to pacL and pacR subsites and interact and hold the two subsites together in a DNA loop containing the pacC subsite on a concatemeric substrate (Ref. 8, Fig. 6, step I). G1P loads G2P at each of the two b-boxes. G2P introduces an ATP-independent cut at each b-box, forming the nicking complex (6). The close interaction between the G1P oligomer bound at pacR and G2P positioned at its proximal b-box (see Fig. 1A) might favor the formation of the “modified” G1P2G2P1 at pacR. The modified G1P2G2P1 terminase has a shut off endonuclease activity and an activated ATPase activity (6). The G2P molecule, which had cleaved the b-box distal from the pacR subsite, now freed from G1P, initially bound to pacL and will degradate the non-encapsidated DNA end in a pac-independent manner (Refs. 5, 6, and 13 and Fig. 6, step II). This
observation imparts the unique directionality of the SPP1 packaging process. The GIP₂-GIP₁ complex bound to the DNA end carrying pacR is proposed to bind G6P localized at the vertex of the empty procapsid to initiate processive translocation of SPP1 DNA (Fig. 6, step II).

II) Interaction of the Terminase-DNA Complex with the Procapsid and Subsequent Transition to a Further Activated ATPase Complex—The interaction of G2P-GIP₁-GIP₂-pacR DNA (postcleavage complex) with G6P, at a unique vertex of the procapsid, should activate the ATPase activity that powers DNA packaging even more. Indeed, the G2P-GIP₁-GIP₂-G6P₁ complex increased the ATP affinity (Kₘ of 219 μM) and turnover (kₐₜ of 366 min⁻¹) of G2P (Tables I and II). There are no direct data on the location of G2P, although in Fig. 6, step II, we assumed that one G2P monomer is interacting stably with two G1P decamers and transiently with one G6P dodecamer.

III) Transition to an Active DNA Packaging Machine That Translocates DNA into the Procapsid Interior—The catalytically competent G2P₁-GIP₁-GIP₂-G6P₁-DNA packaging motor is believed to provide the energy for the linear translocation of DNA into the procapsid initiated by entry of the pacR DNA end. The DNA is pumped through the G6P pore until a threshold amount of DNA (headful) is reached inside the procapsid (5, 8, 13, 18, 22). Models for this DNA translocation mechanism normally take into consideration the symmetry mismatches between components of the DNA translocation machinery between the procapsid and portal protein, or between procapsid, portal protein, and DNA, or between the portal protein, the terminase, and DNA (that would permit rotations between the components of the DNA translocation machinery (32–35)). This rotation would be associated to sequential firing of the oligomeric terminase ATPase activity providing energy for a conformational change in one or in a few portal protein subunits that generates the power stroke for mechanical translocation of DNA to the capsid interior (33, 34). Although these models provide interesting test cases, direct evidence of rotation between components of the packaging machinery or the possibility that the portal protein is the mechanical device of the packaging machinery still lacks experimental proof.

It is difficult to envision how a portal protein rotary motion as described in the above packaging models could accommodate the observation that the SPP1 packaging ATPase is composed of one monomer of G2P, two or more decamers of G1P, and one dodecamer G6P (G2P₁-GIP₁-GIP₂-G6P₂) (Ref. 6 and this work). We propose here a model in which the G2P₁-GIP₁-GIP₂-DNA complex binds to G6P, embedded in the procapsid. The docking of the terminase-DNA complex at G6P might place the DNA end at the portal protein central channel. The ATP hydrolysis leads to a conformational change in G2P driving net translocation of DNA to the procapsid interior and “loose” grip of G2P on the DNA followed by a new translocation step (Fig. 6, stage IIIa and IIIb). This mechanism resembles the inchworm model initially described for DNA helicases (reviewed in Ref. 36). The G2P conformational change would provide energy for a conformational change in one or in a few portal protein subunits that might induce rotation of the portal and help in the mechanical translocation of the DNA. The structural organization of the G2P₁-GIP₁-GIP₂-G6P₁ complex at the procapsid portal vertex would ensure processivity of the reaction preventing G2P to fall off from the nucleoprotein complex during successive translocation steps.

DNA packaging leads to a drastic conformational change of the SPP1 major capsid protein, from a roundish procapsid structure to a capsid lattice, with sharp angles that highlight its icosahedral organization (Fig. 6, steps II and III).

IV) Transition to an Activated Cleavage Complex—When a threshold amount of DNA, representing about 103% of the SPP1 genome, has been packaged (headful), G2P shifts from translocase to a less specific endonuclease with a subsequent halt in DNA packaging. We could envisage that once the procapsid is full, G6P acting as a gauge (24) might lose affinity for G1P. GIP of the G2P₁-GIP₁-G6P₁ interacts with G6P of another spherical procapsid and promotes a change in the stoichiometry of the G2P₁-GIP₁-G6P₁ packaged complex toward the G2P₁-GIP₁-G6P₁ complex. The latter complex has low ATPase and a pac-independent cleavage activity with a subsequent release of DNA from the mature capsid. Alternatively, upon procapsid expansion G6P is unable to interact with the terminase; hence indirectly reducing the velocity of packaging or increasing the energy required to continue DNA packaging, which might attenuate translocation and by an unknown mechanism activate the G2P endonuclease activity. This is consistent with the observation that the efficiency of DNA packaging in SPP1szs mutant, which leads to undersizing of the DNA packaged, is reduced. It was shown that the SPP1szs mutations map in gene 6 coding for G6P and suggested that a trigger for headful cleavage could be the incapacity of the packaging machinery to encapsidate further DNA into the procapsid (22).

V) Termination of Packaging—DNA packaging terminates when the DNA inside the capsid is separated from the concatemer by a cutting process. The headful cleavage generates a new end, to which the packaging motor remains bound, serving as the starting point for the second round of DNA packaging (13, 15, 18, 22).

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