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KINETICS OF THE T4 GENE 32 PROTEIN-SINGLE-STRANDED NUCLEIC ACID INTERACTION

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The kinetics of the interaction of the T4 bacteriophage coded gene 32 protein (GP32) with single-stranded nucleic acids have been investigated. The GP32 is a helix destabilizing protein necessary for DNA replication, recombination, and repair. GP32 functions through its interaction with single-stranded DNA and RNA to which it binds cooperatively (1). Its main function during replication is thought to be the stabilization of transient single-stranded regions in the replication fork. Kinetic studies are necessary to understand how GP32 interacts with a moving replication fork.

Fluorescence and stopped-flow methods have been used to investigate the association and dissociation kinetics of GP32 with a series of single-stranded homopolynucleotides and intact single-stranded DNA from bacteriophage M13. The time-course was monitored by observing the quenching of the intrinsic tryptophan fluorescence of the GP32 upon its binding to the single-stranded nucleic acids.

ASSOCIATION KINETICS

Polynucleotides and GP32 were mixed (0.1 M NaCl, 10 mM Tris, pH 8.3, 25°C) in a Gibson-Durrum stopped-flow spectrophotometer and the resulting fluorescence decrease was monitored. In all association experiments, the nucleic acid (N.A.) was in great excess over the [GP32] so that isolated binding of the GP32 was the major mode of binding. For poly (rA) and poly(dA) two relaxations are observed (τ_f and τ_s).

The slow relaxation (τ_s) is essentially independent of [N.A.] [for poly(rA) τ_s = 3s], whereas τ_f is a function of [N.A.]. The [N.A.] dependence of τ_f is consistent with the following mechanism for GP32 ($\equiv P$) binding in the isolated mode to single-stranded N.A. ($\equiv D$)

$$P + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PD^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PD. \qquad (1)$$

The observed fluorescence change occurs in the second step presumably involving a conformational change in the GP32 N.A. complex. Evidence of a conformational change has also been obtained from equilibrium studies.[1] The best fit parameters for the scheme in Eq. 1 are

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[1]Kowalczykowski, S., J. Newport, N. Lonberg, and P. von Hippel. Submitted for publication; see also these discussions.

[poly(rA), 0.1 M NaCl, 25.0°C, pH 8.3] $k_1/k_{-1} = 2.0 \pm 0.5 \times 10^4$ M^{-1} (nucleotides), $k_2 = 7.5 \pm 0.5$ s^{-1}, $k_{-2} = 0.8 \pm 0.2$ s^{-1}. The presence of this intermediate in the association to single-stranded N.A. may explain the inability of GP32 to denature native DNA even though it is a helix destabilizing protein (1).

DISSOCIATION KINETICS

The dissociation experiments consisted of mixing GP32-N.A. complexes [preformed at low salt (0.1 M NaCl) where binding is stoichiometric] with buffer containing [NaCl] great enough to insure complete dissociation of the complex. Depending on ionic conditions, temperature, pH, polynucleotide, and initial binding density, the relaxation times, τ, are in the range 10 ms $< \tau <$ 10 s.

TIME-COURSE

Both the dissociation rate and the qualitative behavior of the time-course are functions of initial fractional saturation of the nucleic acid, f_{sat}. When $f_{sat} < \sim 0.50$, a single relaxation is observed for dissociation from homopolynucleotides, whereas two relaxation processes are observed for dissociation from single-stranded DNA of heterogeneous base composition (2) (Lohman, unpublished findings). When $f_{sat} > \sim 0.5$, deviations from a single exponential decay are observed in the final 20–40% of the process such that the apparent dissociation rate constant increases with time.

The dissociation rate depends on the following variables (in addition to temperature and pH): (*a*) Polynucleotide lattice. There is a strong dependence of the dissociation rate on both sugar type and base composition. In order of increasing half life: poly(rA) < poly(rU) < poly(dA) < poly(dC) << S.S.M13DNA < poly(dT). In general, GP32 has a shorter half life on RNA than DNA. (*b*) [NaCl]. The dissociation rate increases with increasing [NaCl]. $\partial \log \tau^{-1}/\partial \log$ NaCl = 4.0 ± 0.3 for all polynucleotides investigated. This is in contrast to the [NaCl] dependence of the equilibrium constant ($\partial \log K_{obs}/\partial \log$ [NaCl]) = -6 ± 1 (see footnote 1). The dissociation rate is sensitive to both cation and anion effects. (*c*) Initial fractional saturation (f_{sat}) of the nucleic acid. The apparent dissociation rate constant increases with decreasing initial f_{sat} (below 0.30). This is in qualitative agreement with the conclusion that dissociation occurs from the ends of protein clusters and the number of cluster ends increases as the initial f_{sat} decreases (2). However, the dependence of τ at very low f_{sat} indicates that either the isolated and singly contiguous GP32 molecules do not dissociate independently, or that the rate limiting steps for the two modes are quite similar in magnitude.

These results seem to indicate that GP32 does not slide along the lattice very quickly. For example, the data are not consistent with Epstein's (3) limiting case in which there is instantaneous redistribution of GP32 on the lattice throughout the course of dissociation. However, limited redistribution by sliding can not be eliminated as a possibility. It is difficult to imagine, therefore, that GP32 can slide along with the replication fork. However, since the T4 replication system consists of several proteins, movement of GP32 may be facilitated by some or all of these proteins.

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THE SELF-ASSEMBLY OF TOBACCO MOSAIC VIRUS
Influence of the Viral RNA and Protein Components upon the Assembly Process

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The initial stage in the self-assembly of tobacco mosaic virus (TMV) RNA and coat protein into virions involves insertion of a loop of TMV RNA between layers of a double-layered disk of TMV protein (containing 17 coat protein subunits per layer) via the central channel of the disk, followed by conversion of the disk-RNA complex into a two-turn protohelix and addition of more protein (elongation). This mechanism leads to assembling particles in which one of the uncoated RNA tails, that containing the 5′-terminus, runs back along the central channel of the growing rod (1, 2).

Bidirectional Elongation

The nucleotide sequence recognized during initiation is located 950 residues (~15% total chain length) from the 3′-end of the RNA (3). Thus, the looped-back 5′-tail of short incompletely assembled rods is much longer than the 5′-tail. It has been suggested that the proximity of the looped-back 5′-tail to the growing point of the particle in the 3′-direction may hinder encapsidation of the 3′-tail (1, 4). Study of the reconstitution of nearly complete particles shows that, for such particles at least, encapsidation can proceed simultaneously on both tails although growth is on the average much faster in the 5′ than in the 3′-sense if we consider the whole of the assembly process. Surprisingly, the ultimate step in assembly appears to be encapsidation of the last several hundred nucleotides of the 5′-tail, as incomplete particles having about nine-tenths the full length (but with no visible RNA tail) may persist for several hours after the beginning of reconstitution.

Heterologous Reconstitution and the Specificity of Initiation

The mechanism for assuring the specificity of self-assembly is thought to reside in the initiation stage of the reaction. The portion of the RNA chain which first binds to coat protein during initiation has been characterized and shown to possess a hairpin loop structure