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RAPID COMMUNICATIONS

Interactions of the E. coli Single Strand Binding (SSB) Protein with ss Nucleic Acids. Binding Mode Transitions and Equilibrium Binding Studies.

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The E. coli Single Strand Binding (SSB) protein is a helix destabilizing protein that is required for replication, recombination and repair of the host genome. It binds selectively and cooperatively to single stranded (ss) nucleic acids; however, the interactions are quite complex, in vitro. We have recently identified four distinct modes for the interaction of the E. coli SSB protein with ss DNA and RNA, that differ by the number of nucleotides occluded per bound SSB tetramer (i.e. site size, n) and their fluorescence properties (Lohman, T. & Overman, L. (1985) J. Biol. Chem. 260, 3594; Bujalowski, W. & Lohman, T. (1986) Biochem. 25, 7799). Binding modes with n=35±2, 56±3 and 65±3 nucleotides per tetramer are observed at 25°C, whereas an additional binding mode with a site size of 40±2 nucleotides per tetramer is observed at 37°C. We find that the transitions among these binding modes depend on the cation and anion concentration and type and pH, as well as the temperature and protein binding density, as described below. Divalent cations facilitate the transitions to the higher site size binding modes at 50 to 100-fold lower concentrations that NaCl. The different site sizes seem to reflect different degrees of wrapping of the DNA around the SSB protein, and possibly different aggregation states of the bound SSB tetramer. Based on these data and the electron microscopy studies of Griffith and colleagues, we have suggested that the ss DNA interacts with only two subunits of the SSB tetramer, whereas the ss DNA binds to all four subunits in both the $(SSB)_{56}$ and $(SSB)_{65}$ binding modes. The $(SSB)_{65}$ binding mode seems to exist as an equilibrium mixture of DNA bound tetramers and octamers, due to slight positive cooperativity between ss DNA-bound tetramers; the $(SSB)_{56}$ complex may also form octamers, although this is not certain.

We have extended our investigations of the multiple binding modes that form between the E. coli SSB protein and single stranded (ss) DNA by examining the effects of anions, pH, $BaCl_2$ and protein binding density on the transitions among these binding modes. "Reverse" titrations that monitor the quenching of the intrinsic tryptophan fluorescence of the SSB protein upon addition of poly(dT) have been used to measure the apparent site size of the complex at 25°C in pH 8.1 and 6.9 as a function of NaF, NaCl, NaBr and $MgCl_2$ concentrations. Under all conditions in which "reverse" titrations were performed, we observe three distinct binding modes with site sizes of 35±2, 56±3 and 65±3 nucleotides per SSB tetramer; however, the transitions among the three binding modes are strongly dependent upon both the cation and anion valence, type and concentration as well as the pH. A net uptake of both cations and anions accompanies the transition from the $(SSB)_{35}$ to the $(SSB)_{56}$ binding mode at pH 6.9, whereas at pH 8.1, this transition is anion independent and only a net uptake of cations occurs. The transition from the $(SSB)_{56}$ to the $(SSB)_{65}$ binding mode is dependent upon both cations and anions at both pH 6.9 and 8.1 (25°C), and a net uptake of both cations and anions accompanies this transition. We have also examined the transitions by monitoring the change in the sedimentation coefficient of the SSB protein-poly(dT) complex as a function of $MgCl_2$ concentration (20°C, pH 8.1) and observe an increase in s_{20w}, which coincides with the increase in apparent site size of the complex, as measured by fluorescence titrations. The frictional coefficient of the complex decreases by a factor of two in progressing from the $(SSB)_{35}$ to the $(SSB)_{65}$ binding mode, indicating a progressive compaction of the complex throughout the transition, although the majority of the compaction occurs during the first transition. The transition between the $(SSB)_{35}$ and the $(SSB)_{56}$ complex is also dependent on the protein binding density, with the lower site size $(SSB)_{35}$ complex favored at higher binding density. These results indicate that the transitions among the various SSB protein-ss DNA binding modes are complex processes that depend on a number of solution variables that are thermodynamically linked.

We have also developed a statistical thermodynamic model ("tetramer/octamer" model) which describes the equilibrium binding of the E. coli SSB protein to ss nucleic acids in its "beaded" binding mode (Chrysogelos, S. & Griffith, J. (1982) Proc. Natl. Acad. Sci., U.S.A. 79, 5803), which seems to be equivalent to the high site size, $(SSB)_{65}$ binding mode. The method of sequence generating functions is used to derive the model, which accounts for the observation that clustering of bound SSB tetramers is limited to the formation of octamers, which have been observed as "beads" in the electron microscope. The model also accounts for the overlap of potential protein binding sites on the nucleic acid. The "tetramer/octamer"

model is fully described by only three parameters: the site size, n; the intrinsic equilibriuim constant, K; and the cooperativity parameter, $\omega_{T/O}$, and we obtain exact, closed form expressions for the binding isotherm as well as the distribution of DNA bound SSB tetramers and octamers. The closed form expressions allow one to easily calculate average binding properties and analyze experimental binding isotherms to obtain estimates of K and $\omega_{T/O}$. The "tetramer/octamer" model provides a much better description of the experimental isotherm over the entire binding density range than a model which assumes the formation of clusters of unlimited size. A cooperativity parameter of $\omega_{T/O}$=420±80 provides a good fit to data for SSB binding to poly(dA) and poly(U), corresponding to an interaction-free energy of -3.6 kcal per mole of SSB octamer formed. Based on this moderate value of $\omega_{T/O}$, the "tetramer/octamer" model predicts that at low to intermediate binding densities, a significant fraction of bound SSB exists in the form of tetramers coexisting with octamers.

Equilibrium binding constants for the interaction of the E. coli SSB protein with poly(A), poly(U), poly(dA) and poly(dT) have been measured over a range of monovalent salt concentrations and types under conditions which favor only the high site size, $(SSB)_{65}$ binding mode. The binding isotherms were determined using a general method of analysis of ligand-macromolecule binding (Bujalowski, W. & Lohman, T., (1987) Biochem. 26, 3099) for systems in which the observed signal originates from the ligand (SSB protein). The isotherms are analyzed using the statistical thermodynamic "tetramer/octamer" model that assumes cooperative binding of SSB is limited to the formation of octamers (Bujalowski, W. & Lohman, T., (1987) J. Mol. Biol. 195, 897), rather than the indefinite clustering of tetramers. The dependence of the intrinsic association equilibrium constant, K_{obs}, and cooperativity parameter, $\omega_{T/O}$, on salt concentration has been determined by titrations which monitor the fluorescence quenching of the SSB protein upon complex formation. In the $(SSB)_{65}$ binding mode, SSB binds with only moderate cooperativity to ss nucleic acids (Lohman, T., Overman, L. & Datta, S. (1986) J. Mol. Biol. 187, 603). The cooperativity parameter derived from the "tetramer/octamer" model, which represents the equilibrium constant for formation of a nucleic acid bound SSB octamer from two nucleic acid bound tetramers, has a value of $\omega_{T/O}$=410±120, independent of salt concentration and type for poly(dA), poly(U) and poly(A) (25°C, pH 8.1). However, K_{obs} decreases steeply with increasing salt concentration, such that $(\partial \log K_{obs}/\partial \log[NaCl]) = -7.4\pm0.5$ for poly(U), -6.1±0.6 for poly(dA), and -6.2±0.3 for poly(A) (25°C, pH 8.1). The SSB protein-poly(dT) affinity is too high to measure in buffers containing even 5 M NaCl; however, in 1.8 to 2.5 M NaBr we measure $(\partial \log K_{obs}/\partial \log[NaBr]) = -5.7\pm0.7$, with a slightly lower value of $\omega_{T/O}$=130±70. A dramatic effect of anion type on both the salt dependence and magnitude of K_{obs} is also observed such that for poly(U): $(\partial \log K_{obs}/\partial \log[Kglutamate]) = -5.7\pm0.4$, $(\partial \log K_{obs}/\partial \log[NaF]) = -4.3\pm0.4$, $(\partial \log K_{obs}/\partial \log[NaCH_3COO]) = -6.5\pm0.2$ and $(\partial \log K_{obs}/\partial \log[NaBr]) = -6.7\pm0.6$; in 0.35 M monovalent cation: $K_{obs}(glu) = 5.6\ K_{obs}(F) = 11\ K_{obs}(CH_3COO = 1.1x10^3\ K_{obs}(Cl) = 1.1 \times 10^4\ K_{obs}(Br)$. These data indicate that significant electrostatic interactions occur in the $(SSB)_{65}$ complexes formed with all polynucleotides, resulting in a net release of both cations and anions, although from the analysis of the site size transitions, it is clear that there are also contributions due to cation and anion uptake.

Our studies indicate that the E. coli SSB protein interacts with ss nucleic acids in a number of different binding modes and the transitions between these modes are a sensitive function of solution conditions. Furthermore, the positive cooperativity that can exist between DNA bound-tetramers also is a function of solution variables and may be different for the different binding modes. Since the E. coli SSB protein is involved in DNA replication, recombination and repair processes, it is possible that the different binding modes are used selectively for these different functions. In any event, the results of multiprotein reactions that involve the E. coli SSB protein, in vitro, such as replication and recombination studies, will most certainly depend on the particular binding mode that the SSB protein adopts; therefore, caution must be exercised when comparing data collected under different sets of solution conditions in any experiments involving the E. coli SSB protein, in vitro.