STABILITY AND DYNAMICS OF THE COMPLEXES OF THE SINGLE STRAND DNA BINDING PROTEIN (E.COLI) WITH SINGLE STRANDED DNA

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The single strand DNA binding protein (ssB protein) from E. coli is essential for replication. It binds cooperatively to single stranded DNA and does not bind to double stranded DNA. The protein consists of four identical subunits each having a molecular weight of 20,000 daltons. We have determined stability constants and rate constants for the complex formation of the ssB protein with oligonucleotides of defined composition and length: each of the four subunits of the ssB protein has a binding site for oligonucleotides of 8 - 9 residues length. The binding sites are nearly equivalent and independent. The intrinsic binding constant K for the complex formation between ssB protein and the various oligonucleotides and their ionic strength dependences are given below :

	KCL (mM)	K (M^{-1})
$d(pT)_{16}$	50	$1.4 \cdot 10^6$
	200	$6 \cdot 10^5$
	400	$6 \cdot 10^5$
$d(pT)_{30-40}$	200	$>3 \cdot 10^8$
$d(pA)_{16}$	200	$5 \cdot 10^3$
$d(pA)_{40-60}$	50	$1.5 \cdot 10^7$
	200	$3 \cdot 10^6$
	400	$1.5 \cdot 10^5$

buffer:
20.mM potassium phosphate,
pH : 7.4

T = 8°C

Oligoadenylates bind about two orders of magnitude weaker than the corresponding oligo(dT) species. This behaviour is ascribed to the stacking tendency of the adenylate oligomers. The binding of oligo(dT) is weakly dependent on ionic strength, in contrast to the oligo(dA)-ssB complex formation. Stopped flow and temperature jump experiments on the interaction between ssB protein and (dT)-oligonucleotides of various chainlength show that the complex formation can be described by a simple one step reaction. The rate constants of association for all systems studied are of the same order of magnitude ($\vec{k} \sim 10^8$ M^{-1} sec^{-1}), whereas the rate constants of dissociation differ strongly with chainlength in correspondence with the different degree of complex stability. Similar studies on the interaction of ssB protein with oligo(dA)-systems are in progress. The cooperative binding of ssB protein to polymeric single stranded DNA's was demonstrated in the complex formation between ssB protein and poly(dT), poly(dA), and single stranded circular fd-DNA using fluorescence titrations as well as sedimentation velocity runs in the analytical ultracentrifuge. All four binding sites on the ssB tetramer are occupied each covering 8 - 9 nucleotides. The complex stabilities are higher than 10^8 M^{-1}. It is assumed that the ssDNA is wound around the ssB tetramer in a manner similar as described for the binding of DNA to histones. Fluorescence melting experiments of the complexes show that the conformation of the single stranded DNA has a strong influence on the stability of the complexes.