INTERACTION BETWEEN COLICIN E2 AND DNA *IN VITRO*

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1. Introduction

Colicins are highly specific antibacterial proteins. It has been proposed that they initiate a lethal sequence of events in sensitive bacteria by adsorbing to specific cell-surface receptor-sites and disrupting membrane-mediated cellular control mechanisms [1]. Investigations into colicin binding [2] and trypsin-induced reversal of colicin action [1, 3] have indicated that the colicin molecule remains at the bacterial cell surface.

Recent work [4, 5] has shown that pure colicin E3 interacts *in vitro* with its intercellular target, the ribosome, and initiates cleavage of 16 S ribosomal RNA. RNA cleavage had previously been shown only to occur with colicin E3 *in vivo* [6, 7]. Colicin E2 resembles E3 since *in vivo* it acts by initiating a nucleolytic event i.e. single strand scission of the DNA duplex [3]. The possibility that colicin E2 also interacts directly with its target, the bacterial chromosome, has been investigated [8]. Colicin E2 is found to destabilise the DNA helix under certain conditions *in vitro* and this may be the necessary event required to activate latent cell nucleases.

2. Materials and methods

Collicin E2 and E3 were purified and characterised as previously described [3, 9]. DNA was extracted from *E. coli* strain CL 142 and purified by chloroform/octanol extraction, ethanol precipitation and RNase treatment followed by phenol extraction and a second ethanol precipitation [8, 10, 11]. The DNA had an A_{260}/A_{280} ratio of 1.96–2.01, a melting temperature (T_m) of 63–66° in SSC/10 (fig. 1) and hyperchromicity of 25–30%. Its molecular weight was 10^7 as determined by sedimentation.

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3. Results

DNA destabilisation and DNA-protein interactions can be detected by changes in T_m and slope of the melting profile ($\sigma^{2/3}$) [12]. Heating colicin E2 to approx. 70° in the absence of DNA produced turbidity due to protein aggregation. The effect of colicin E2 on the melting of *E. coli* DNA is shown in fig. 1a. At 30 and 40 μg colicin E2 per ml, there was a premature rise in the melting profile at 50° but no protein aggregation. On increasing the colicin concentration to 60 and 80 μg/ml, the profile became biphasic (fig. 1b) and the $\sigma^{2/3}$ value increased approx. 4 times (fig. 2). The two composite curves indicated the presence of DNA with a T_m of approx. 49°. Further increase in colicin E2 concentration resulted in a reduction of the $\sigma^{2/3}$ value and total conversion of DNA to a form having the lower T_m. The percentage of the total DNA destabilised was obtained from the hyperchromicities of profiles in fig. 1a and this value is plotted against colicin E2 concentration (fig. 3). The relationship is linear and between 100 and 400 colicin molecules were bound per DNA molecule.

Different colicin E2 preparations produced a 10% variability in the protein concentration required to destabilise 50% of the same DNA preparation. The decrease in T_m with colicin E2 varied between 5° and

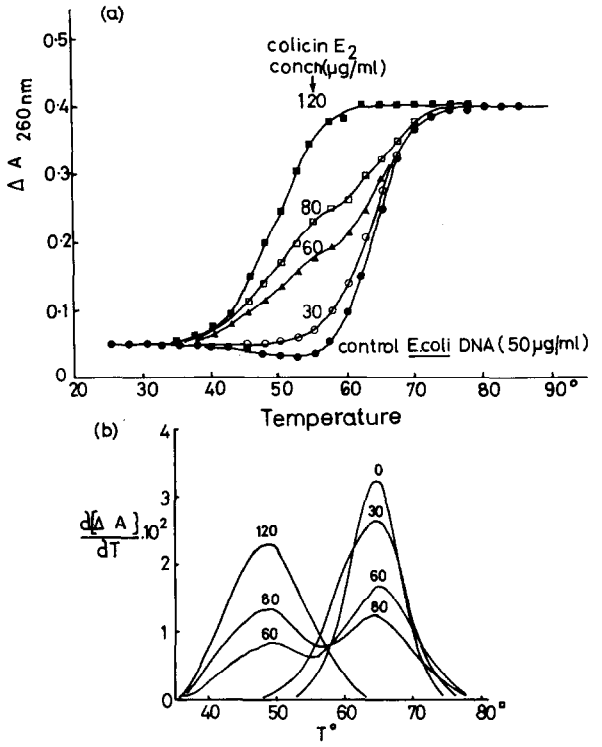

Fig. 1. Effect of colicin E2 on thermal denaturation of DNA. (a) *E. coli* DNA (50 μg/ml) was mixed with colicin E2 in 15 mM NaCl–1.5 mM trisodium citrate (SSC/10) at 25° as indicated. The temperature was increased at a rate of 0.5°/min and the absorbance recorded using a Cary 15 spectrophotometer. (b) Derivative curves of the melting profiles in fig. 1(a) were calculated. The numbers above the curves correspond to colicin concentration in μg protein per ml.

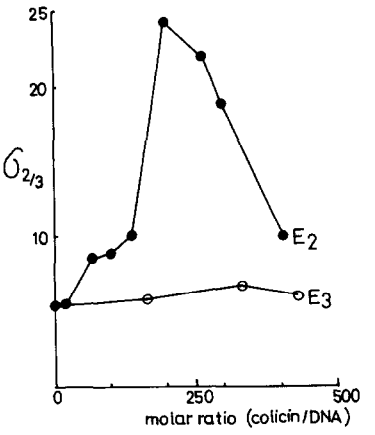

Fig. 2. Effect of colicins E2 and E3 on the $\sigma^{2/3}$ values of DNA melting profiles. The $\sigma^{2/3}$ value or dispersion of thermal transition corresponds to the temperature difference between 17% and 83% of the hyperchromic effect [12]. This is plotted against colicin:DNA molar ratio assuming molecular weights of 6×10^4 and 10^7, respectively.

15° with different DNA preparations from the same strain of *E. coli*, whereas the $\sigma^{2/3}$ value at 60 μg colicin E2 per ml only varied between 18.1 and 23.2. 0.1 mM phenol enhanced the colicin–DNA destabilisation effect although it did not affect the T_m and $\sigma^{2/3}$ values for DNA alone. This may explain the variability with different DNA preparations since traces of phenol may have been present.

Addition of colicin E3 to DNA resulted in a slight increase in T_m. Colicin E2 interacted with DNA's extracted from colicin resistant and immune strains of *E. coli* equally well and it is unlikely that the specificity of colicin E2 action results from specific binding sites on bacterial DNA [8]. Commercially prepared calf-thymus DNA interacted less effectively with colicin E2.

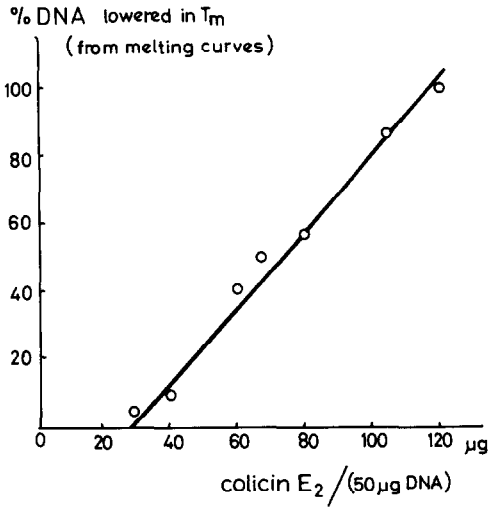

Fig. 3. Biphasic analysis of melting profiles. The percentage DNA lowered in melting temperature on mixing with colicin E2 was derived from the hyperchromic effects in fig. 1 and plotted against colicin E2 concentration.

4. Discussion

Colicin E2 interaction with DNA *in vitro* may be related to the initiation of nucleolytic destruction of the bacterial chromosome *in vivo*. The nuclease initially involved could be activated by regions of DNA destabilised by colicin E2 [3]. As yet no exo- or endonucleolytic activity has been detected in preparations of colicin E2, although colicin treated DNA was found to be more susceptible to DNase digestion [8].

Interaction of colicin E2 with DNA depends on the method of DNA purification and requires some degree of initial distortion of the helix. Similarly the peptide bleomycin only interacts with DNA *in vitro* causing a lowering of the T_m if DNA is pretreated with a thiol [13]. However thiols have little effect on colicin E2–DNA interaction [8].

Binding of colicin E2 to DNA has been confirmed by nitrocellulose-filter retention [14] of radioactively labelled DNA–colicin complex [8], but this only occurred at low ionic strengths. The molar ratio of colicin:DNA was again found to be between 100–400. This suggests that one colicin E2 molecule is bound every 200 nucleotide pairs assuming a DNA molecular weight of 10^7. Ribonuclease also lowers DNA melting temperature [15]. Sekine et al. [16] propose that ribonuclease A binds to local regions of distortion energy 5×10^6 daltons along DNA strands and this may be relevant to colicin E2 binding. Ribonuclease and bleomycin are the only two proteins so far demonstrated to destabilise DNA.

The similarity between colicins and diphtheria toxin is worthy of speculation. The toxin is a protein of similar molecular weight and is inactive on its target (transferase II) *in vitro*. When split into its A and B fragments, A is active *in vitro* but requires B for cell penetration *in vivo* [17]. Similarly colicin may consist of two moieties; one remaining at the cell surface, thus enabling the other to penetrate.

The detection of colicin activity *in vitro* fundamentally affects the dogma of an allosteric membrane-conformational change as the source of colicin action and may provide a simpler explanation as to how these intriguing protein molecules work.

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