Effects of Surface Active Compounds on Thermal Denaturation of DNA

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ABSTRACT AND SUMMARY

The interaction of surface active compounds (including N-lauroyl amino acids) with *calf thymus* DNA in aqueous solutions has been studied by measurements of the melting temperature of the DNA. The results suggest that the surface active nature of these additives is not the primary factor governing the interaction with DNA, but their molecular structure is very important in the interaction.

INTRODUCTION

A number of studies have reported on the interactions of surfactants with proteins or synthetic polymers in aqueous solutions. However, few studies have been made on the interaction between surfactants and DNA in spite of the biological importance of DNA. The present communication deals with the effects of surface active compounds on the thermal denaturation of *calf thymus* DNA to study the interaction of these additives with the DNA. The surfactants examined here include those derived from amino acids that are expected to interact with the DNA since protein-DNA interactions have already been reported (1).

EXPERIMENTAL PROCEDURES

The melting curves of *calf thymus* DNA were obtained by optical density measurements at 260 nm, utilizing the hyperchromicity of DNA (2). Absorbance was measured with a JASCO UVIDEC-2 spectrophotometer with Teflon-stoppered quartz absorption cells thermostated by circulating water. The temperature of sample solutions was measured directly with a copper-constantan thermocouple. The concentration of the DNA was kept constant at 30 μg/ml; the absorbance at this DNA concentration was about 0.4 at room temperature. The buffer solution used was 0.01 x SSC (=1.5 m mol/l sodium chloride + 0.15 m mol/l trisodium citrate, pH 7.0).

The sample of *calf thymus* DNA was obtained from Miles Laboratories (Elkhart, IN), and deoxycholic acid from Difco Laboratories (Detroit, MI). Surfactant samples including N-lauroyl amino acids were synthesized and purified by recrystallization except polyoxyethylene dodecyl ether.

RESULTS AND DISCUSSION

Figure 1 shows typical examples of the melting curves of the DNA from which the melting temperature, T_m, in the presence of each additive, was determined. The T_m is defined as a temperature at the midpoint of thermal transition. The T_m of the DNA is significantly affected by the addition of benzopyrenes and p-dimethylaminoazobenzene which are known to interact strongly with DNA (Fig. 1 and Table I). The result shows that the T_m measurement is a useful means for investigation of the interaction between DNA and additives.

It is well known that the T_m of DNA highly depends upon the ionic strength, I, of the solution (3). The relation of T_m vs. I, which was obtained by measuring T_m values at different concentrations of the buffer, is expressed by the following equation.

$$T_m = 16.5 \log I + 98$$

Schildkraut and Lifson (3) reported similar relations for the T_m of *E. coli* DNA in solutions of various salts. Their data were also given by an expression similar to the above with an accuracy of ± 1 C, independent of the kind of ions. Therefore, the T_m change due to the interaction of the DNA with an additive, when the additive is electrolytic, may be evaluated by subtracting the change due to the increase of ionic strength from the total T_m change.

The effects of additives on the T_m of the DNA are summarized in Table I where ΔT_m expresses the extent of T_m change due to the interaction. All anionic surfactants have slight or no appreciable interaction with the DNA except N-lauroylprolylprolylglycine and deoxycholic acid which will be discussed later. This may be interpreted as showing that the surfactant molecules cannot approach the DNA molecules to a distance enough to interact with each other because of their ionic repulsion. On the other hand, the nonionic surfactant which has no charge in the molecule and the zwitterionic surfactant could approach the DNA molecule more closely than anionic surfactants. With myristyltrimethylammonium chloride, precipitation at 1.0 m mol/l can be explained as a result of the ionic bond formation with the phosphate

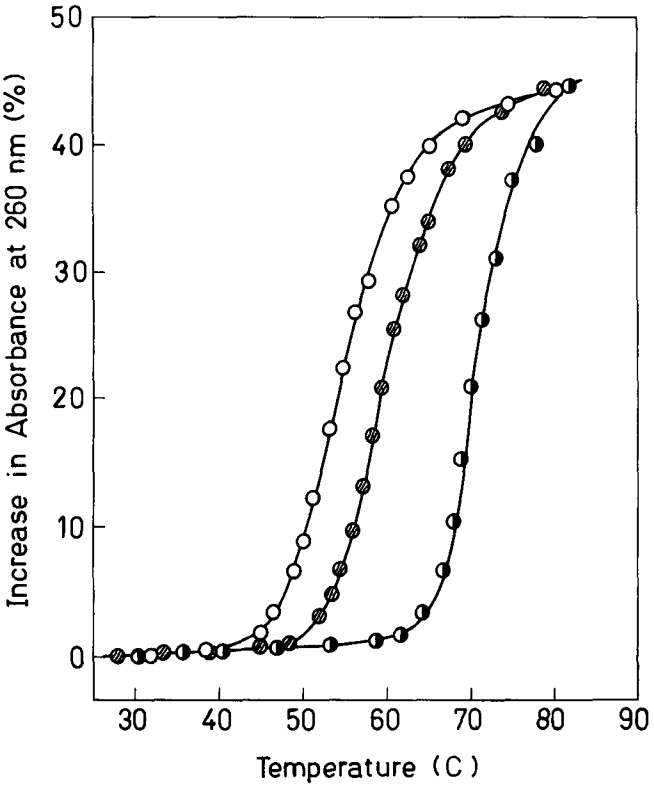

FIG. 1. The melting curves of *calf thymus* DNA in 0.01 x SSC buffer in the absence and presence of additives. Additives are none (○), 1.1 m mol/l sodium dodecyl sulfate (⊜), and benzo (α) pyrene (◑).

TABLE I

Effects of Additives on Melting Temperature of DNA

Type of additive	Additive	Concentration of additive[a] (m mol/l)	Ionic strength ($I \times 10^3$)	Melting temperature, T_m (C)	ΔT_m (C)
Control	None	—	2.4	55.0	—
Anionics	Sodium dodecyl sulfate	1.10	3.5	60.4	2.8
	N-lauroylglycine	1.00	3.4	60.0	2.7
	N-lauroylproline	1.00	3.4	59.0	1.6
	N-lauroylprolylproline	1.00	3.4	58.7	1.3
	N-lauroylprolylprolylproline	1.00	3.4	58.6	1.2
	N-lauroylglutamic acid	1.00	5.4	59.6	-1.1
	N-lauroylprolylprolylglycine	0.01	2.4	60.0	5.0
		0.05	2.5	60.9	5.9
		0.50	2.9	64.5	8.3
		1.01	3.4	67.3	10.0
	Deoxycholic acid	saturated	2.4[b]	65.0	10.0
Nonionics	Polyoxyethylene dodecyl ether	0.10	2.4	59.0	4.0
		1.11[b]	2.4	59.5	4.5
		10.0[b]	2.4	60.5	5.5
Zwitterionics	3-(Dimethylmyristylammonio)-propane-1-sulfonate	1.00	2.4	58.0	3.0
Cationics	Myristyltrimethylammonium chloride	1.00	3.4	precipitated	—
		99.7*	—	74.5	—
Reference	Benzo(α) pyrene	saturated	2.4	70.4	15.4
	Benzo (e) pyrene	saturated	2.4	71.6	16.6
	p-Dimethylaminoazobenzene	saturated	2.4	72.0	17.0

[a]Concentrations of surfactants were below CMC except *.
[b]Slight increase of ionic strength by addition of deoxycholic acid was neglected.

groups of the DNA. Further addition of the cationic surfactant, however, dissolves the precipitate. The ΔT_m value at 99.7 m mol/l cannot be evaluated because of much higher concentration than the Critical Micelle Concentration (CMC), but it can be roughly estimated to be -1 C assuming the degree of ionization of micelles to be 0.4 (4). As mentioned above, it is thus unlikely that the typical surface active compounds significantly affect the DNA structure, contrary to the fact that they strongly interact with proteins to denature them (5).

Deoxycholic acid is a surface active substance existing in nature with a steroid skeleton. The interaction of steroidal amines with DNA was studied by Gabbay and Glaser (6) who presumed that the steroid skeleton interacts with the hydrophobic groove of the DNA surface. The high value of ΔT_m for deoxycholic acid, ca. 10 C, may be explained by this hydrophobic interaction. The interaction of N-laurolyprolylprolylglycine with the DNA is worth noting, since the similar types of surfactants derived from amino acids exhibit slight or no interaction, as seen in Table I. This suggests that the amino acid sequence of prolyl-prolyl-glycine in the molecule would play an important role in the interaction with the DNA, although the mechanism is not clear. Two examples shown by deoxycholic acid and N-lauroylprolylprolylglycine suggest that the primary factor governing the interaction of additives with DNA is the molecular structure of additives rather than their surface activity and ionic nature.

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