

Influence of formamide on the thermal stability of DNA

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MS received 5 May 1984; revised 20 August 1984

Abstract. The utility of formamide in the denaturation and renaturation of DNA has been examined. The melting temperature of duplex DNA is lowered by 0.6°C per per cent formamide. The depression of melting temperature is independent of the GC content. Formamide also increases the width of the thermal transition. Upto 30%, it does not affect the rate of DNA reassociation.

Keywords. DNA melting; formamide; T_m ; DNA base composition; DNA reassociation.

Introduction

The optimum rate for DNA-DNA reassociation occurs at about 25°C below the melting temperature (T_m), (Wetmur and Davidson, 1968; Britten *et al.*, 1974). The incubation temperature (T_i) for reassociation, on account of its dependence on T_m varies with the GC content of the DNA. For DNA samples of GC content of 50% or less. T_i is within the normally accessible range for optical and hydroxyapatite chromatographic studies. For instance, the optimum temperature for monitoring reassociation of *Escherichia coli* DNA (GC content, 50%) in the presence of 1M NaCl, is around 75°C. For DNA having 70% GC, in the presence of 1 M NaCl, T_i will be around 90°C. Incubation at such a high temperature often causes strand breakage and depurination of DNA. Hence the use of chemical agents whose presence lowers T_m without any specificity for base composition and does not hinder reassociation rates is advocated.

Formamide seems to be the choicest of the agents and has been in use more than any other compound both in solution and in filter hybridization studies. The effect of formamide on the destabilization of DNA duplex was first studied systematically by McConaughy *et al.* (1969) using *Bacillus subtilis* DNA (40% GC). According to them the rate of reduction of T_m was 0.72°C per per cent formamide. Later, Casey and Davidson (1977) reported a value of 0.63°C for *E. coli* DNA. In this communication we report studies on the effect of formamide on denaturation of DNAs with a wider variation in base composition.

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Abbreviations used: EDTA, Ethylenediamine-tetraacetic acid; RNA, ribonucleic acid; SDS, sodium dodecyl sulphate; T_i , temperature of incubation; T_m , temperature midpoint of melting.

Materials and methods

Bacterial strains

Mycobacterium smegmatis SN2, *E. coli* B and *Bacillus cereus* were obtained from our laboratory culture collection.

DNA isolation

The cells were lysed by lysozyme-EDTA-sodium dodecyl sulphate (SDS), and the DNA was isolated by the phenol extraction procedure (Kirby, 1959). To achieve lysis of *M. smegmatis* cells sensitization of cells by pretreatment with glycine was necessary. Glycine (0.15 M) was added to the growing culture 2 h before harvesting. RNA was removed from the nucleic acid preparation by treatment with RNase *A*. In the case of *B. cereus*, a combination of RNase *A* and RNase *T1* was used. After RNase digestion, sodium acetate was added (final concentration, 0.3 M) and the samples were extracted with (24:1) chloroform-isoamylalcohol (Marmur, 1961). DNA was precipitated with 2.5 volumes of ethanol, pooled and dissolved in 10mM Tris pH 7.0, containing 1 mM EDTA and 10mM NaCl.

Reagents

Lysozyme, RNase *A*, RNase *T1* formamide, Tris (hydroxymethyl) aminomethane and SDS were from Sigma Chemical Company, St. Louis, Missouri, USA. All other reagents were of analytical grade. Purification of formamide has been described elsewhere (Sadhu *et al.*, 1984).

Melting and reassociation of DNA

Thermal denaturation was done according to the procedure described by Mandel and Marmur (1968) using a Pye-Unicam SP700A spectrophotometer fitted with an electrical heating system. Absorbance was monitored at 270 nm because of the inherent absorbance of formamide at 260 nm. For reassociation studies, stoppered cuvettes containing DNA was heated by circulating water. Reassociation was initiated by the addition of prewarmed sodium-phosphate buffer and the decrease in absorbance at 270 nm was recorded for 1 h using Shimadzu ultra-violet recording spectrophotometer. In all the reassociation experiments T_i was maintained at 25°C below T_m at that condition. Results were plotted as suggested by Wetmur (1976).

Results and discussion

Melting of DNA in the presence of formamide

The T_m values for *M. smegmatis*, *E. coli* and *B. cereus* DNA in the absence of formamide were 90°C, 83.5°C and 78.5°C respectively (data not shown). Base compositions of these DNA species were determined from the equation (Mandel and Marmur, 1968): $\Delta GC = \Delta T_m \times 2.44$ using *E. coli* DNA as the standard. Assuming the GC content of *E. coli* DNA as 50% (Marmur, 1961) the GC contents of *M. smegmatis* and *B. cereus* DNA were found to be 66% and 38% respectively.

T_m values of each of these DNA samples in the presence of varying concentrations of formamide were determined. With increasing concentration of formamide T_m values of all the DNA samples were lowered. For each DNA sample, the T_m values were plotted against formamide concentration (figure 1). The slopes of all the lines were same, indicating that DNAs with varying GC content behave in an identical fashion in the presence of formamide. The drop in T_m with increasing concentration of formamide is linear and corresponds to 0.6°C per per cent formamide. As mentioned above, the DNAs employed in these studies varied in GC content from 38–66%. It is, therefore, possible that formamide does not interact with the bases in a differential manner.

For each melting profile, the standard deviation, σ , which is a relative measure of cooperativity, was calculated by the equation (Wada *et al.*, 1980): $\sigma = (\Delta T_{14.1}^{85.9} - 0.6) \times 1.25$. The results are presented in table 1. The value of σ increased with an increase in

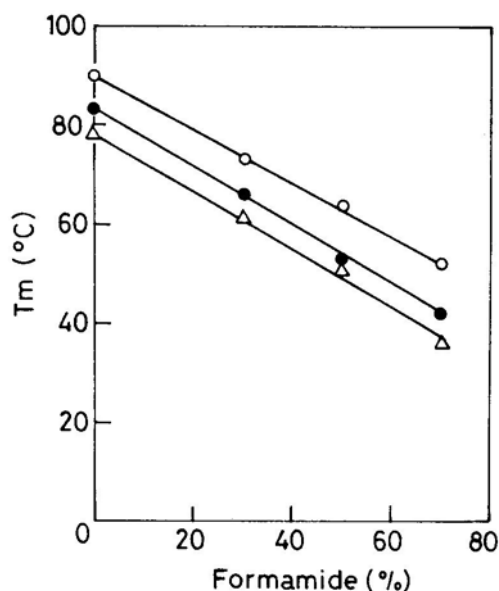


Figure 1. Effect of formamide on T_m of different DNA samples. (○), *M. smegmatis*; (●), *E. coli*; (Δ), *B. cereus*.

Table 1. Effect of formamide on σ value.

Source of DNA	σ value			
	a	b	c	d
<i>B. cereus</i>	4.75	5.75	6.50	8.50
<i>E. coli</i>	4.50	4.80	5.50	8.62
<i>M. smegmatis</i>	4.00	5.25	5.50	10.25

(a), 0% formamide; (b), 30% formamide;
(c), 50% formamide; (d), 70% formamide.

the formamide concentration. However, a quantitative correlation between GC content, σ values and formamide concentration could not be obtained. Cooperativity of the helix to coil transition in DNA is intensified by weakening the shielding effect of the ionic cloud (Wada *et al.*, 1980). The shielding effect can be altered by changes in ionic strength, dielectric constant etc. At a fixed ionic strength, dielectric constant of the medium is increased from 81 of the aqueous phase to 96 by the addition of formamide (1:1, v/v) (Record, 1967). This may be the cause of the observed increase of σ in the presence of formamide.

Effect of formamide on the rate of reassociation

We have examined the effect of formamide on the rate of DNA reassociation using *E. coli* DNA. With increasing concentrations of formamide, reassociation was carried out at lower temperatures to maintain a difference of 25°C between T_m and T_i . The results are shown in figure 2. Upto 30%, formamide showed no appreciable effect on the rate of reassociation. At higher percentages of formamide, the reassociation rate was reduced.

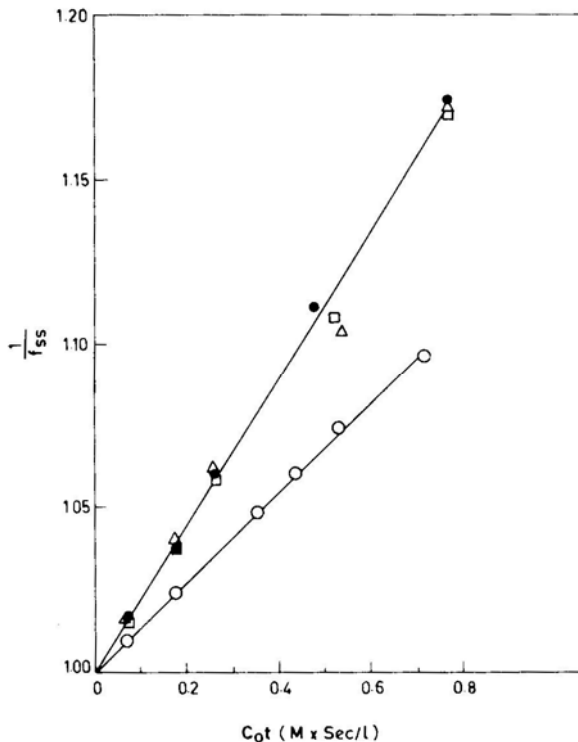


Figure 2. Effect of formamide on the rate of reassociation. Reassociation was carried out in the presence of 0.8 M phosphate buffer (pH 6.9). The T_i in the presence of 0, 15, 30 and 45% of formamide were 78°C, 69°C, 60°C and 51°C respectively. f_{ss} , the fraction of single stranded DNA was calculated from the relation, $1/f_{ss} = (A_0 - A_\infty)/(A_t - A_\infty)$ where A_0 is absorbance of entirely denatured molecules, A_∞ is absorbance at infinite time, and A_t is absorbance at time, t . (Δ), 0% formamide; (\square), 15% formamide; (\bullet), 30% formamide; (\circ), 45% formamide.

The rate of reassociation in the presence of formamide can be influenced by the following factors:

(i) Increased accessibility of the complementary strands to each other due to an increase in the dielectric constant of the medium causing a decrease in the interstrand electrostatic repulsion.

(ii) Decreased bimolecular collisions due to the reduction of T_i .

A balance between these two opposing factors probably kept the rate more or less unaltered upto 30% formamide. Further increase in formamide concentration may give rise to the condition where the temperature factor overtakes the former causing the observed decreased in the rate of reassociation.

Acknowledgements

We thank Dr. Hirak Basu for his keen interest in the work. Financial support from Department of Atomic Energy, Bombay and University Grants Commission, New Delhi, is gratefully acknowledged.

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