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# CALORIMETRIC STUDIES OF THERMAL DENATURATION OF DNA AND tRNAs*

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## Abstract

The thermal denaturation of DNA and tRNAs was investigated by differential scanning calorimetry (DSC). Endothermic peaks were observed in the DSC curves when the samples were heated. The denaturation temperatures ($T_d$) and denaturation enthalpies ($\Delta H_d$) of samples of lyophilized powder and of samples in 4 mol/dm$^3$ urea solution were obtained. The experimental results showed that the thermal stabilities of DNA and tRNAs were obviously different under these conditions.

**Keywords:** DNA and tRNA calorimetry, thermal denaturation

## Introduction

The thermal stability of the spatial structure of biopolymers can be estimated by means of calorimetry [1–6]. Investigations [7, 8] indicate that the denaturation of biopolymers depends on the solvent system. The present report compares the differences in the thermodynamic properties of DNA and tRNAs for lyophilized powder and for samples in 4 mol/dm$^3$ urea solution. The effects of the urea solvent on the thermal denaturation of two biopolymers are discussed.

## Experimental

### *Materials*

The calf thymus DNA was obtained from Department of Biochemistry of the Dongfang Instrument Co., with a content >95%. The tRNA (*E.coli*) and tRNA (wheat germ) were purchased from Sigma Chemical Corporation. Their activities were 21.0 and 18.1 $A_{260}$ units/mg, respectively. Urea (A. R. reagent) was obtained from the Beijing Chemical Plant.

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## Instruments and methods

A Perkin-Elmer Model DSC-2C differential scanning calorimeter with a cryostat was used for the thermal measurements. This instrument was fitted with a Model 3500 data station and a Model 7225A plotter. The balance was a Model AD-2Z electromagnetic ultramicrobalance from the P-E Corporation. The samples were placed in stainless steel sample pans and sealed. Preparation of sample solution: a sample of known mass and 20 μl of 4 mol/dm$^3$ urea solution were sealed in a stainless steel sample pan, with 20 μl of redistilled water sealed in a reference pan. The solutions of samples were equilibrated for 5 h at room temperature before calorimetric measurements. The mass of sample for each experiment was about 3.0 mg. The experimental temperature range was from 290 to 390 K. The heating rate was 10 deg·min$^{-1}$. The temperature and energy calibrations were performed with indium (99.999%) and zinc (99.999%).

## Results and discussion

The DSC curves of DNA and tRNAs are shown, respectively, in Figs 1 and 2. Endothermic transition peaks were observed. As can be seen in Fig. 1, the shapes of the denaturation peaks of the DNA for lyophilized powder and that in 4 mol/dm$^3$ urea solution are obviously different. The endothermic transition shows that the structure of the DNA double helix was destroyed. Two peaks are seen in curve *a*, but only one is observed in curve *b*. The smaller peak at lower temperature (see curve *a*) was due to a change in the order part, with lower stability in the DNA structure. It is seen from Fig. 2 that the denaturation peaks of tRNA lyophilized powder appeared in the higher temperature region. The

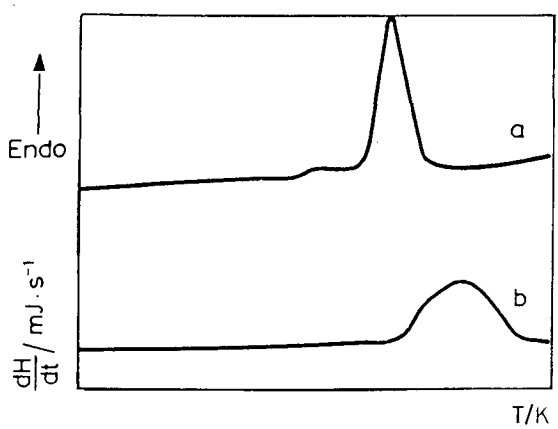


**Fig. 1** DSC curves of calf thymus DNA. a: solid-state DNA; b: DNA in 4 mol/dm$^3$ urea solution

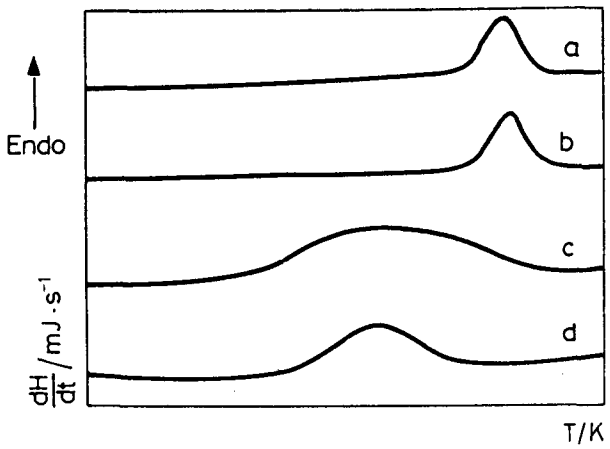


**Fig. 2** DSC curves of two tRNAs. a: tRNA (*E.coli*) lyophilized powder; b: tRNA (wheat germ) lyophilized powder; c: tRNA (*E.coli*) in 4 mol/dm$^3$ urea solution; d: tRNA (wheat germ) in 4 mol/dm$^3$ urea solution

shapes of the denaturation peaks of the two tRNAs are similar. After the addition of 4 mol/dm$^3$ urea solution, the shapes of the denaturation peaks of the two tRNAs were quite changed.

Experimental thermodynamic data on the thermal denaturation of the DNA are summarized in Table 1. The denaturation temperatures and enthalpies of denaturation of the two tRNAs are listed in Table 2.

**Table 1** $T_d$ and $\Delta H_d$ of DNA

| Sample | $T_d$ /K | $\Delta H_d$/J·g$^{-1}$ |
|---|---|---|
| Solid-state DNA | 358.6 | 26.42 |
| DNA in 4 mol /dm$^3$ urea solution | 368.7 | 33.06 |

**Table 2** $T_d$ and $\Delta H_d$ of tRNAs

| Sample | $T_d$ /K | $\Delta H_d$/J·g$^{-1}$ |
|---|---|---|
| tRNA (*E.coli*) lyophilized powder | 369.5 | 12.04 |
| tRNA (wheat germ) lyophilized powder | 368.2 | 12.33 |
| tRNA (*E.coli*) in 4 mol /dm$^3$ urea solution | 349.3 | 29.68 |
| tRNA (wheat germ) in 4 mol /dm$^3$ urea solution | 350.2 | 32.98 |

As shown in Table 1, the $T_d$ of solid-state DNA was 358.6 K and that of DNA in 4 mol/dm$^3$ urea solution was 368.7 K. The $\Delta H_d$ of the DNA in 4 mol/dm$^3$ urea solution was also higher. This shows that the thermal stability

of DNA in 4 mol/dm$^3$ urea solution is increased. It may be caused by the direct action of urea on the double helix or via the water molecule, i.e. hydration of the DNA molecule [9, 10]. The $T_d$ values of the two tRNAs in 4 mol/dm$^3$ urea solution were decreased, but their denaturation enthalpies were increased. Investigations [11, 12] indicate that the thermodynamic properties of thermal unfolding of tRNA are different, probably because of the differences in their ionic conditions and the source of tRNA used in the studies. The effects of 4 mol/dm$^3$ urea solution on the DNA and tRNA differ, because their structures are not the same.

## Conclusions

1. The shapes of the denaturation peaks of the lyophilized powder and samples in 4 mol/dm$^3$ urea solution for DNA and tRNAs are different.

2. The effects of 4 mol/dm$^3$ urea solution on the thermal denaturation differ for DNA and tRNAs, i.e. they have different thermal stabilities.

3. The thermodynamic parameters for tRNA (*E.coli*) and tRNA (wheat germ) are not obviously different.

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**Zusammenfassung** — Mittels DSC wurde die thermische Denaturierung von DNA und tRNA untersucht. Beim Erhitzen der Proben werden an den DSC-Kurven endotherme Peaks festgestellt. Temperatur ($T_d$) und Enthalpie ($\Delta H_d$) der Denaturierung der Proben von liofilisiertem Pulver und von Proben in einer 4 mol/dm$^3$ Harnstofflösung wurden ermittelt. Die experimentellen Ergebnisse zeigen, daß sich die thermische Stabilität von DNA und tRNA unter diesen Umständen eindeutig unterscheidet.