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# THERMAL STABILITY OF HERRING DNA IN THE PRESENCE OF CLUPEINE FRACTIONS *

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## ABSTRACT

The denaturation temperatures and enthalpies of the complexes formed between herring DNA and each of the three clupeines (protamine fractions replacing histones in fish seminal fluid and vescicles) have been determined. An increase of about 20 K was found for the melting temperature and of about 100% for the denaturation enthalpy (compared with free DNA). The large increase in the stabilisation of DNA against denaturation after formation of its complexes with clupeines is expressed by the temperature dependence of the unfolding free energy.

## INTRODUCTION

In fish, during spermatogenesis histones are fully replaced by basic polypeptides of lower molecular weight known as protamines [1–3].

The DNA–protamine complexes are compact structures that completely inhibit transcription and protect the nucleic acid from enzymatic hydrolysis. Herring protamines (clupeines) have been resolved into three main fractions YI, YII and Z, formed of 31, 30 and 31 amino acid residues (mostly arginines) respectively [4–7]. Their primary sequences are:

YI
$Ala^{1}ArgArgArgArg^{5}SerSerSerArgPro^{10}IleArgArgArgArg^{15}Pro-ArgArgArgThr^{20}ThrArgArgArgArg^{25}AlaGlyArgArgArg^{30}Arg$

YII
$Pro^{1}ArgArgArgThr^{5}ArgArgAlaSerArg^{10}ProValArgArgArg^{15}ArgPro-ArgArgVal^{20}SerArgArgArgArg^{25}AlaArgArgArgArg^{30}$

Z
$Ala^{1}ArgArgArgArg^{5}SerArgArgAlaSer^{10}ArgProValArgArg^{15}ArgArg-ProArgArg^{20}ValSerArgArgArg^{25}ArgAlaArgArgArg^{30}Arg$

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Denaturation temperatures and enthalpies of the three herring DNA–clupeine complexes have been determined by means of differential scanning calorimetry and compared with those of pure DNA. To avoid fast precipitation of the complexes and to limit the turbidity of the solutions, sonicated DNA was incompletely complexed and the arginine/phosphate ratio was kept as low as 0.7.

## EXPERIMENTAL

### *Materials*

The herring DNA used was a commercial product (Sigma D-6898), dissolved without further purification in a 0.01 M phosphate buffer, containing 0.01 M NaCl at pH 7.0. Its concentration was detected spectrophotometrically using a $\epsilon_{260} = 6300\ cm^{-1}\ mol^{-1}\ l$ [7]. The solutions were prepared by dissolving DNA with gentle stirring, for 72 h at 4°C [8]. The clupeine fractions, YI, YII and Z, were obtained chromatographically from the commercial mixtures (SIGMA product), according to a described procedure [9].

Reproducible results were obtained only by using sonicated DNA to prepare the DNA–clupeine complexes and by slowly adding clupeine to the buffered DNA solution, with rapid stirring at 0°C.

### *Instrumentation*

A Branson J-17 Sonifier was used to sonicate the DNA. The solutions were treated for 2.5 min at 0°C and 60 W.

The microcalorimeter was a Setaram, second-generation, MICRO-DSC apparatus, explicitly designed for studies on dilute aqueous solutions of biological macromolecules. Its temperature programme covers the range 0–100°C and the scanning rate can be changed to suit widely differing conditions. For the present study, a scanning rate of $1°C\ min^{-1}$ was chosen after several trial experiments.

## RESULTS AND DISCUSSION

### *Characterisation of herring DNA*

A characteristic curve of the apparent excess heat capacity of DNA versus temperature is presented in Fig. 1. The curve, when normalised according to the electrical calibration of the instruments, gives important parameters, such as the initial ($T_i$) and final temperatures ($T_f$) of the denaturation

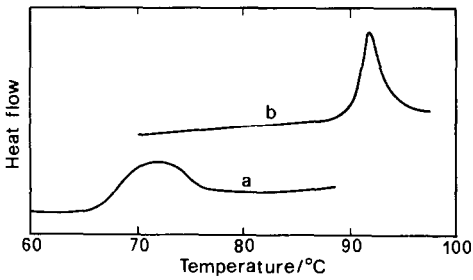


Fig. 1. a, Scanning calorimetry recording of the sonicated herring DNA solution. b, Scanning calorimetry recording of the sonicated DNA–YII-fraction complex: Arg/phosphate = 0.7. Phosphate buffer, pH 7.0; total ionic strength 0.01 M; scanning rate 1° C $min^{-1}$.

process and the temperature of the maximum ($T_d$), which can be assumed to correspond to the mid-point denaturation temperature. The denaturation enthalpy is directly obtained by integrating the area under the denaturation peak

$$\Delta_d H = \int_{T_i}^{T_f} C_p \, dT - \int_{T_i}^{T_d} C_p^N \, dT - \int_{T_d}^{T_f} C_p^D \, dT \quad (1)$$

where $C_p^N$ and $C_p^D$ are the apparent excess specific heats below and above the denaturation range and are extrapolated into this range up to $T_d$. In the case of DNA, denaturation can be interpreted in terms of successive steps, microscopically reversible, throughout most of the process.

In other words, owing to the size of the DNA molecule, there is a distribution of defects on each DNA double-chain and, hence, of differently affected helices during each denaturation step. This fact, as well as the cooperativity of the denaturation process, makes the changes of the physical parameters that monitor the transition nearly symmetrical with respect to the mid-point. The validity of eqn. (1) is based on the experimental data only and derivation of the other thermodynamic parameters does not need any model.

The denaturation temperatures and enthalpies at pH 7.0 for sonicated $0.7 \times 10^{-3}$ (nucleotide equivalent $l^{-1}$) DNA, at various NaCl concentrations are reported in Table 1. The increase in DNA stability with increasing activity of monovalent ions is well known [10–13]. The $T_d$ value, 72° C, (at 0.01 M NaCl) is in agreement with the expected value for 42.2% G–C bases [10,14,15]. However, the $\Delta_d H$ is about 20% higher than the average value expected for this base content [16]. It is very likely that residual contamination of the material is responsible for this discrepancy. It must be noted that unsonicated DNA displays the same $T_d$, but a higher $\Delta_d H$ value (that is therefore less reproducible).

Calorimetric measurements can also provide the analytical dependence of the degree of advancement of the denaturation on temperature. This is usually obtained from other non-thermodynamic parameters. From this

TABLE 1

Denaturation enthalpies and temperatures of sonicated herring DNA as a function of NaCl concentration: concentration $0.7 \times 10^{-3}$ nucleotide equivalent $l^{-1}$; pH 7.0

| NaCl concentration (M) | $\Delta_d H$ (kJ $mol^{-1}$) | $T_d$ (°C) |
|---|---|---|
| 0.01 | 19.5 | 72.0 |
| 0.02 | 31.5 | 76.0 |
| 0.10 | 36.8 | 87.0 |

analysis, a value of the denaturation enthalpy can be obtained assuming a temperature-shifted equilibrium model and applying the van't Hoff relationship. However, for DNA this procedure cannot be based on a two-state denaturation model. Rather, the analysis of the sigmoid curve representing the degree of advancement of the denaturation provides empirical relations accounting for the cooperativity of the process. These relations are usually expressed in terms of the so-called van't Hoff enthalpies and compared with the total denaturation enthalpy measured by calorimetry. A commonly used relation for internuclear complexes between two strands is [17,18]

$$\frac{6RT_d^2\ \Delta C_p^{\star}(T_d)}{\Delta_d H^2_{(\text{calorim.})}} = \frac{\Delta_d H_{(\text{v.H.})}}{\Delta_d H_{(\text{calorim.})}} = \langle m \rangle \tag{2}$$

where $\Delta C_p^{\star}(T)$ is defined as

$$\Delta C_p^{\star} = C_p - C_p^{\mathrm{N}} \tag{3}$$

and $\langle m \rangle$ is a measure of the average number of nucleotide pairs that must melt together in order to unfold a single nucleotide pair. It is, therefore, also a measure of the cooperativity of the transition.

*Characterisation of the DNA–clupeine complexes*

The calorimetric study was undertaken to obtain a quantitative evaluation of the DNA stabilisation induced upon complexation with clupeine. The experimental curve of the apparent excess heat capacity versus temperature for the DNA–Z-fraction complex at Arg/phosphate ratio 0.7 is shown in Fig. 1b.

The $\Delta_d H$, $T_d$ and $\langle m \rangle$ values for the three complexes at the same Arg/phosphate ratio are compared with similar values for sonicated DNA in Table 2. $T_d$ increases by 20 °C for all three clupeine fractions in agreement with the optical density measurements [7].

The denaturation enthalpies (at this molar ratio) are about twice those of free DNA. The DNA–Z-fraction complex underwent the lowest enthalpy change.

TABLE 2

Values of enthalpies, temperatures and cooperativity parameters for herring DNA and DNA–clupeine fraction complexes; conditions as in Table 1

| | $\Delta_d H$ (kJ mol$^{-1}$) | $T_d$ (°C) | $\langle m \rangle$ |
|---|---|---|---|
| DNA | 12.5 | 72.0 | 9.0 |
| DNA+YI | 24.3 | 92.0 | 9.5 |
| DNA+YII | 24.4 | 92.0 | 9.5 |
| DNA+Z | 19.6 | 92.0 | 11.0 |

It is not easy to explain why the DNA–Z-fraction complex (21 arginines) has an enthalpy of denaturation about 5 kJ (nucleotide equivalent)$^{-1}$ lower than the other two complexes. The only difference between the Z-fraction and clupeine YII is that it has 4 Arg instead of 3 Arg in the *N*-terminal part (where a Pro residue also replaces Ala).

In any case, an entropy–enthalpy compensation must take place, leading to the same denaturation temperature for all three complexes. Cooperativity, on the other hand, is only slightly increased with respect to DNA alone.

*Relative stability of DNA–clupeine complexes*

In addition to the denaturation enthalpy, the calorimetric data enable the $\Delta_d C_p$ values from the native to the denatured form of DNA and of complexes, to be estimated. From this parameter, $\Delta_d H$ can be evaluated as a function of temperature even beyond the denaturation range [19–21]

$$\Delta_d H(T) = \Delta_d H(T_d) - \int_T^{T_d} \Delta_d C_p \, dT \tag{4}$$

The enthalpy and free energy are also obtained in the same way

$$\Delta_d S(T) = \frac{\Delta_d H(T_d)}{T_d} - \int_T^{T_d} \frac{\Delta_d C_p}{T} dT \tag{5}$$

and

$$\Delta_d G(T) = \Delta_d H(T) - T\,\Delta_d S(T) \tag{6}$$

The $\Delta_d C_p$ values were about 200 J K$^{-1}$ (nucleotide equivalent)$^{-1}$ for both DNA and the three complexes. This is somewhat small if compared with the $\Delta_d H$ values. As a first approximation it was assumed to be constant with temperature in the absence of other experimental evidence. Consequently, Fig. 2 compares the trends of the denaturation free energy for the three complexes with that of DNA alone. The shift toward more positive values for the complexes extends the stability range.

It is estimated that the maximum stability of DNA is reached at 15°C, whereas the complexes reach maximum stability at lower temperatures.

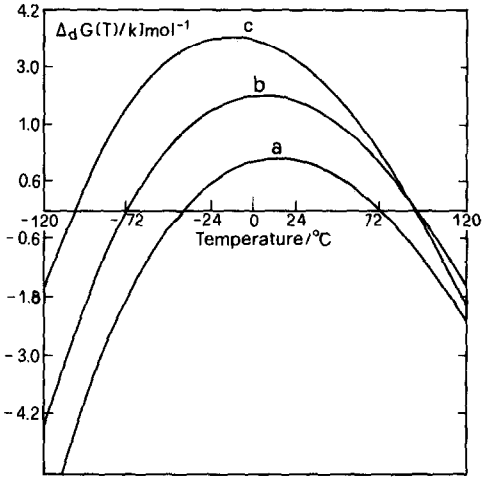


Fig. 2. Temperature dependence of the denaturation Gibbs energy differences for: a, sonicated herring DNA; b, DNA–Z-complex and c, DNA–YI and DNA–YII complexes. Phosphate buffer, pH 7.0; total ionic strength 0.01 M.

In view of the different dependence of the enthalpy and entropy functions on temperature, the stability of macromolecules and their complexes appears to reach a maximum with decreasing temperature, with a "cold denaturation" taking place at very low temperatures; this has been demonstrated for a few proteins [22,23].

In the present case, we report the possible trend of $\Delta_d G$ versus $T$ plots as the obvious consequence of the thermodynamic treatment (eqns. (4)–(6)). It must be noted that (even with the oversimplified and not strictly necessary assumption of constant $\Delta_d C_p$) the $\Delta_d G$ curve is not symmetrical, so that an inversion of the roles of enthalpy and entropy before "cold denaturation" should be attained.

Over most of the temperature range, where the native DNA is stable, the enthalpic contribution maintains the normal conformation with respect to the entropic driving forces. At temperatures below the maximum, the entropic contribution becomes negative, thus promoting the stability of the ordered structure beside the enthalpic contribution. Near the "cold denaturation" point, finally, the enthalpic contributions become positive, destabilising the native ordered structure. This undoubtedly fascinating paradox should stimulate more extensive theoretical speculations and experimentations.

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