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A DSC study of the liquid crystalline phases of salmon sperm DNA

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Thermodynamic stabilities of the liquid crystalline (LC) and gel (G) phases of salmon sperm DNA were investigated by differential scanning calorimetry (DSC). The enthalpic and the entropic components of the thermal denaturation free energy are briefly discussed. In order to evaluate the thermodynamic data for the denaturation of the gel (G) phase of DNA and compare it with those for the LC DNA phase, a hypothesis for the thermal denaturation mechanism of G DNA is proposed. A comparison between these two sets of data has shown that DNA in the LC phase is thermally and thermodynamically more stable than in the G phase over the temperature range which was investigated.

1. Introduction

The DNA molecule in living systems is highly packed inside the cell; a tremendous packing ratio must be reached to allow a biopolymer molecule one metre long to be confined within a single nucleus. This topological problem is not limited only to the achievement of the packing aspect: the DNA replication *processes* in nature demand a particular spatial *precision* unattainable in the absence of a more widespread spatial *order*. A partial answer to the above problem can be found in the particular structures in which the DNAs 'live' in cell nuclei, sperm heads and virus capsids: these structures show the distinctive characteristics of liquid crystalline (LC) phases [1–3].

DNA has been shown to form LC phases in concentrated aqueous solution [4–11] and different phases have been described depending on the DNA concentration [12–14]. The condensation of DNA can also be induced by adding 'condensing' agents like polymers and salts [15–17], polylysine [18], polyhistidine [19], divalent metallic ions [20, 21], alcohols [22, 23] or neutral polymers such as polyethylene glycol (PEG) [24–26] to a dilute buffered solution of DNA to form a suspension of LC microphase. The LC phase of DNA has been chiefly studied by polarized light microscopy [26, 27] and chiro-optical techniques, such as circular dicroism (CD) and linear dicroism (LD) [28–31], as well as by X-ray diffractometry [32, 33].

Recently, using differential scanning calorimetry (DSC), we have shown that the thermal behaviour of LC DNA is different in the isotropic gel (G) and LC phases [34, 35]. In particular renaturation is more extensive for DNA denaturated in its LC state, rather than in its G state. Starting from previous results, by means of the same microcalorimetric approach, the present paper reports a study of the thermodynamic stability of salmon sperm DNA in the LC phase, and compares the results with those obtained from thermal denaturation of DNA in the G phase.

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2. Experimental

Salmon sperm DNA samples in the G and LC phases were prepared as described in [34, 35]. A PEG water–salt solution (170 mg ml^{-1}) was added to the G phase to give a LC microphase (LC droplets suspended in the isotropic medium). Because of its helical structure, the formation of this phase was monitored by CD. The LC DNA phase used in our microcalorimetric measurements was obtained from this microphase by centrifugation. The calorimetric measurements were carried out with a Micro-DSC differential scanning calorimeter (Setaram, France). About 0·7 ml of water–salt solution containing the appropriate PEG concentration and 1–2 mg of DNA phase was used in the sample vessel (with the same quantity of solution in the reference cell).

Both sample and reference cells were scanned from 30 to 100°C with a precision of ±0·08°C at a rate of 0·5°C min^{-1}. The average noise was about 0·5 μW and the reproducibility on refilling was about 0·1 mJ K^{-1} ml^{-1}. Calibration of the energy was obtained by supplying a finite power, generated from the Joule effect, (EJ2 Joule calibrator by Setaram) into one of the cells.

In order to obtain quantitative data, a 'zero' line was recorded before each measurement: reference and sample cells filled with the same volume of buffer solution used in the denaturation experiment were scanned under the same experimental conditions. This 'zero' line was then subtracted from the experimental denaturation curve to obtain the $C_{p_{tot}}(T)$. In order to obtain the $C_{p_{exc}}(T)$ for the denaturation process, the method of Privalov [36] was used: a straight line was drawn from the start to the end of the peak of the $C_{p_{tot}}(T)$.

3. Results and discussion

It is commonly supposed that when DNA molecules are exposed to thermal denaturation processes, a 'melting' phenomenon occurs, with partial or complete separation of the two strands, depending on the temperature and base sequence [37–42]. On cooling, the original double stranded DNA tends to reform, depending on experimental conditions such as temperature, annealing time, ionic strength, pH, etc. [43]. Both of these phenomena (denaturation and renaturation) were experimentally followed by microcalorimetric DSC measurements.

The output of the apparatus, suitably calibrated in energy, gives the C_p of the sample as a function of the temperature over the scanned range. From these results, values for $C_{p_{exc}}$ and ΔC_p for the thermal denaturation process can be obtained. Thermodynamic parameters $\Delta H(T)$, $\Delta S(T)$ and $\Delta G(T)$ are then obtained according to the thermodynamic equations given below:

$$\Delta H(T) = \Delta H(T_m) + \int_{T_m}^{T} \Delta C_p \, dT, \tag{1}$$

$$\Delta S(T) = \Delta H(T_m)/T_m + \int_{T_m}^{T} (\Delta C_p/T) dT, \tag{2}$$

$$\Delta G(T) = \Delta H(T_m)(T_m - T)/T_m + \int_{T_m}^{T} \Delta C_p \, dT + T\int_{T}^{T_m} (\Delta C_p/T) \, dT, \tag{3}$$

where T_m is the thermal denaturation temperature, corresponding to the maximum of the C_p curve, ΔG, ΔH and ΔS represent respectively the free energy, enthalpy and entropy changes for the denaturation process; ΔC_p corresponds to the difference between $C_{p_{excess\,(denatured)}}$ and $C_{p_{excess\,(non\text{-}denatured)}}$.

The enthalpy change associated with the denaturation, $\Delta H(T_m)$ is in turn obtained directly by integrating the area enclosed between the transition peak and baseline by using equation (4):

$$\Delta H(T) = \int_{T_i}^{T_f} \Delta C_{p_{excess}} dT, \tag{4}$$

where T_i and T_f represent, respectively, the initial and final temperatures of the transition.

Thus, the determination of the differences in enthalpy, entropy and free energy between the native and denaturated states is practically reduced to determining the denaturation heat effects and the difference in their heat capacities ΔC_p. Before dealing with the calorimetric data in terms of thermodynamic relationships, it is necessary to verify the thermodynamic reversibility of the denaturation process.

As reported in our previous paper [36], denatured LC DNA renatures almost entirely (90 per cent); so we regard this denaturation process as 'reversible'. This high 'reversibility' was also confirmed by CD [36] and suggests the following hypothesis: native double stranded DNA is 'forced' into a LC arrangement because of the decreasing dielectric constant of the PEG solution. Single strands obtained by thermal denaturation of native double stranded DNA maintain a residual order which, on cooling, induces the reformation of hydrogen bonds and the stacking interactions, renaturing the DNA. The energy involved in the breaking and reconstruction of hydrogen bonds and the stacking interactions is remarkable.

The whole process can be presented schematically as:

$$\text{LC DNA}_{\text{double stranded}} \rightleftharpoons \text{DNA}_{\text{ordered single stranded}}$$

On the contrary, only about 20 per cent of denatured G DNA renatures and this denaturation process cannot be regarded as reversible. This behaviour can be explained by adding to the previous scheme a further 'irreversible' step leading to the randomization of the single strands as follows:

$$\text{DNA}_{\text{double stranded}} \rightleftharpoons \text{DNA}_{\text{ordered single stranded}} \rightarrow \text{DNA}_{\text{random coil}}$$

Unlike the LC phase, in this case, the intermediate state of 'ordered single strands' is not stabilized by PEG and evolves to a 'random coil' state in an irreversible way. The energy involved in the second step of the denaturation process, which would correspond to the randomization of separated strands, should be small compared with the energy involved in the first step, corresponding to the breaking of hydrogen bonds and stacking interactions. The hypothesis given above is quite similar to that proposed by Privalov [43] to explain the thermally irreversible denaturation of proteins. If this hypothesis is correct, all data referring to the irreversible thermal denaturation phenomena can be treated using thermodynamic equations, in order to obtain the corresponding thermodynamic parameters.

From the apparent specific heat (C_p) values, the C_p excess curves for the LC state (curve (*a*)) and the G state (curve (*b*)) versus temperature are obtained for salmon sperm DNA (see Experimental § as shown in figure 1.

Referring to figure 1, it should be noted that the right end of LC curve extends beyond the experimental temperature range. This phenomenon, due to the greater thermal stability of LC phase compared to the G phase, keeps us from measuring

directly the ΔC_p value necessary to calculate the values of the thermodynamic parameters. In order to overcome this problem, the experimental curve was fitted to a gaussian curve (shown in figure 1 as a dotted line). This approach allowed us to calculate a ΔC_p value of about 0·05 kJ K^{-1} mbp^{-1}. ΔC_p values between 0 and 0·17 kJ K^{-1} mbp^{-1} have been reported [44]. In order to evaluate the effect of varying ΔC_p values on ΔG, ΔH and ΔS, we have calculated thermodynamic parameters assuming ΔC_p values of 0 and also 0·1 kJ K^{-1} mbp^{-1}: the results obtained do not modify the conclusions reported below. Figure 2 shows the ΔG, ΔH and ΔS trends with temperature (top, middle and bottom, respectively) for LC DNA (1) and G DNA (2). In the table, melting temperatures and thermodynamic parameters for both LC and G phases at temperatures of 37, 50, 70 and 90°C are reported.

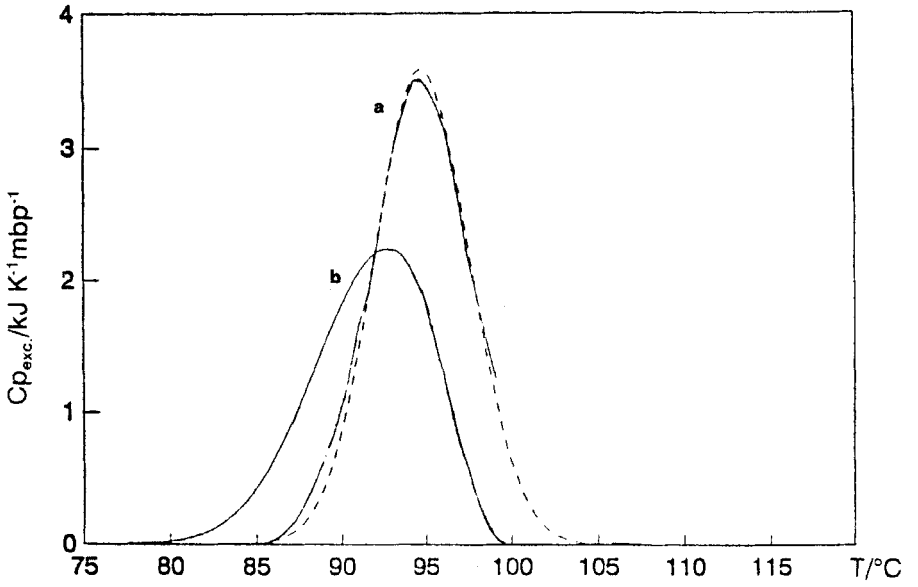

Figure 1. $C_{p_{excess}}$ (kJ K^{-1} mbp^{-1}) thermal profiles for LC DNA (curve (*a*)) and G DNA (curve (*b*)) versus temperature (°C). The gaussian curve fitted to the curve for the LC phase is reported as a dotted line.

Thermal denaturation and thermodynamic parameters referring to LC ($\Delta C_{p_{excess}}$ = 0·05 kJ K^{-1} mbp^{-1}) and G ($\Delta C_{p_{excess}}$ = 0·00 kJ K^{-1} mbp^{-1}) DNA phase.

Phase	T/°C	ΔH/kJ mbp^{-1}	ΔS/J K^{-1} mbp^{-1}	ΔG/kJ mbp^{-1}
G DNA	37	32·8	0·091	4·634
(T_m = 87·9°C)	50	32·8	0·091	3·453
	70	32·8	0·091	1·635
	90	32·8	0·091	−0·09
LC DNA	37	39·4	0·106	6·434
(T_m = 95·0°C)	50	40·04	0·108	5·04
	70	41·04	0·111	2·84
	90	42·04	0·114	0·584

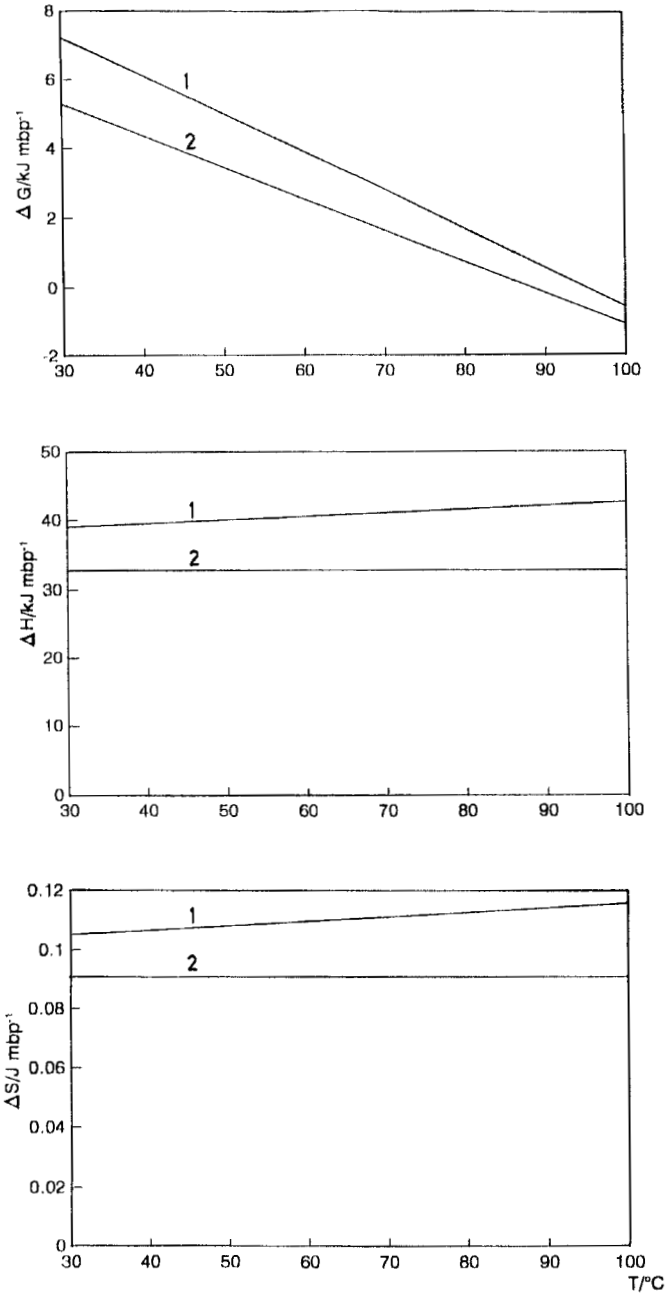

Figure 2. ΔG, ΔH and ΔS denaturation trends (top, middle and lower part, respectively) for LC (1) and G (2) phases, respectively) versus temperature (from 30 to 100°C).

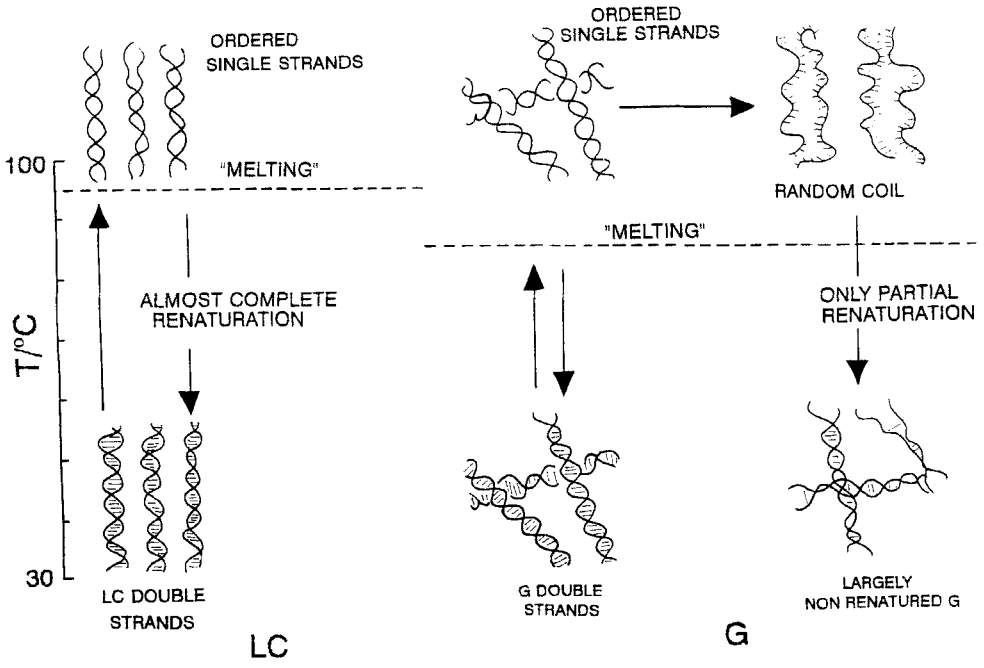

Figure 3. Diagrammatic representation of the denaturation and renaturation processes with reference to the LC and G DNA phases, respectively.

An analysis of figure 2 allows us to derive some interesting conclusions:

(*a*) The LC phase is *thermodynamically* more stable than the G phase in the overall temperature range considered. In particular this greater stability ($\Delta\Delta G$) decreases with increasing temperature and at 37°C its value is about $2\,\mathrm{kJ\,mbp^{-1}}$.

(*b*) The LC phase is thermally more stable than the G phase: the difference between T_m of the LC phase and T_m of the G phase is about 7°C.

(*c*) Denaturation is always an endothermic process: in particular the demolition of the LC phase requires more energy than for the G phase in the overall temperature range which was considered (see figure 2). This result can be explained by considering that, in the presence of PEG, the different double strands forming the LC phase are closer; therefore, their separation demands more energy.

(*d*) The 'demolition' process requires an increasing entropy that is greater for the LC phase than for the G phase and rises over the temperature range. This result can be qualitatively explained by considering that the LC DNA is in a more 'ordered' state then G DNA and therefore the denaturation process involves a higher degree of disordering.

(*e*) Finally the signs of ΔH and ΔS for the denaturation process show that the stability of native LC DNA is enthalpy driven.

A sketch designed to assist the reader in understanding the equilibria and the disordering process proposed in the Results and discussion for the denaturation and renaturation of LC and G DNA is given in figure 3. The whole thermodynamic data relating to the LC and G phases may explain why naturally occurring DNAs under particular conditions forms LC phases rather than G phases.

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References

[1] DU PRAW, E. J., 1970, *DNA and Chromosomes* (Holt, Rinehart & Winston).
[2] EARNSHAW, W. C., and CASJENS, S. R., 1980, *Cell*, **21**, 319.
[3] SIPSKI, W., and WAGNER, T. E., 1977, *Biopolymers*, **16**, 573.
[4] ROBINSON, C., 1961, *Tetrahedron*, **13**, 219.
[5] ROBINSON, C., 1966, *Molec. Crystals*, **1**, 467.
[6] LERMAN, L. S., 1973, *Cold Spring Harb. Symp. quant. Biol.*, **38**, 59.
[7] LIVOLANT, F., 1984, *Eur. J. Cell. Biol.*, **33**, 300.
[8] LIVOLANT, F., 1986, *J. Phys. Paris*, **47**, 1605.
[9] LIVOLANT, F., 1987, *J. Phys., Paris*, **48**, 1051.
[10] LIVOLANT, F., and BOULIGAND, Y., 1986, *J. Phys., Paris*, **47**, 1813.
[11] RILL, R. L., 1986, *Proc. natn. Acad. Sci. U.S.A.*, **83**, 342.
[12] LIVOLANT, F., and MAESTRE, M. F., 1988, *Biochemistry*, **27**, 3056.
[13] Strzelecka, T. E., DAVIDSON, M. W., and RILL, R. L., 1988, *Nature, Lond.*, **331**, 457.
[14] LIVOLANT, F., LEVELUT, A., DOUCET, J., and BENOIT, J., 1989, *Nature, Lond.*, **339**, 724.
[15] JORDAN, C. F., LERMAN, L. S., and VENABLE, J. H., 1972, *Nature, Lond., New Biol.*, **236**, 67.
[16] LAEMMLI, U. K., 1975, *Proc. natn., Acad. Sci. U.S.A.*, **72**, 4288.
[17] EVDOKIMOV, Y. M., PYATIGORSKAYA, T. L., POLYSTEV, D. F., AKIMENKO, N. M., KADYKOV, V. A., TSVANKIN, D. Y., and VARKAVSKY, Y. M., 1976, *Nucl. Acids Res.*, **3**, 285.
[18] HAYNES, M., GARRETT, R. A., and GRATZER, W. B., 1970, *Biochemistry*, **9**, 4410.
[19] BURCHARDT, G., ZIMMER, C., and LUCK, G., 1973, *FEBS Lett.*, **30**, 35.
[20] SIMPSON, R. T., and SOBER, H. A., 1970, *Biochemistry*, **9**, 3103.
[21] SHIN, Y. A., and EICHHORN, G. L., 1977, *Biopolymers*, **16**, 225.
[22] REICH, C., MAESTRE, M. F., EDMONSON, S., and GRAY, D. M., 1980, *Biochemistry*, **19**, 5208.
[23] HUEY, R., and MOHR, S. C., 1981, *Biopolymers*, **20**, 2533.
[24] LERMAN, L. S., 1971, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1886.
[25] EVDOKIMOV, YU. M., PLATONOV, A. L., TIKHONENKO, A. S., and VARSHAVSKY, YA. M., 1972, *FEBS Lett.*, **23**, 180.
[26] YEVDOKIMOV, YU. M., SKURIDIN, S. G., and SALYANOV, V. I., 1988, *Liq. Crystals*, **3**, 1443.
[27] SKURIDIN, S. G., BADAEV, N. S., LAVRENTOVICH, O. D., and YEVDOKIMOV, YU. M., 1987, *Dokl. Akad. Nauk SSSR*, **295**, 1240.
[28] BRUNNER, W. C., and MAESTRE, M. F., 1974, *Biopolymers*, **13**, 345.
[29] YEVDOKIMOV, YU. M., SALIANOV, V. I., and PALUMBO, M., 1985, *Molec. Crystals liq. Crystals*, **131**, 285.
[30] SAMORI', B., DOMINI PELLERANO, I., ROSSI, A., GRASSO, D., and ALBERTINI, G., 1990, *Nuovo Cim. D*, **12**, 1317.
[31] KELLER, D., and BUSTAMANTE, C., 1986, *J. chem. Phys.*, **84**, 2961.
[32] SKURIDIN, S. G., BADAEV, N. S., DEMBO, A. T., LORTKIPANIDZE, G. B., and YEVDOKIMOV, YU, M., 1988, *Liq. Crystals*, **3**, 51.
[33] SKURIDIN, S. G., DEMBO, A. T., OSIPOV, M. A., DAMASCHUN, H., DAMASCHUN, G., and YEVDOKIMOV, YU. M., 1985, *Dokl. Acad. Nauk SSSR*, **285**, 713.
[34] GRASSO, D., FASONE, S., LA ROSA, C., and SALYANOV, V. I., 1991, *Liq. Crystals*, **9**, 299.
[35] GRASSO, D., GABRIELE-CAMPISI, R., and LA ROSA, C., 1992, *Thermochim. Acta*, **199**, 239.
[36] PRIVALOV, P. L., and POTEKHIN, S. A., 1986, *Methods Enzymol.*, **131**, 4.
[37] HINZ, H.-J., FILIMONOV, V. V., and PRIVALOV, P. L., 1977, *Biochem. J.*, **72**, 79.

[38] FILIMONOV, V. V., PRIVALOV, P. L., HINZ, H.-J., VON DER HAAR F., and CRAMER, F., 1976, *Eur. J. Biochem.*, **70,** 25.
[39] MAEDA, Y., KAWAI, Y., FUJITA, T., and OHTSUBO, E., 1985, *Thermochim. Acta*, **88,** 235.
[40] MAEDA, Y., and OTHSUBO, E., 1987, *J. molec. Biol.*, **194,** 691.
[41] CANTOR, C. R., and SCHIMMEL, P. R., 1980, *Biophysical Chemistry, Part III: The Behaviour of Biological Macromolecules* (Freeman, W. H. and Co.).
[42] EDGE, V., ALLEWELL, N. M., and STURTERVANT, J. M., 1987, *Biochemistry*, **24,** 5899.
[43] PRIVALOV, P. L., and KHECHISNASHVILI, N. N., 1974, *J. molec. Biol.*, **86,** 665.
[44] KLUMP, H., and ACKERMANN, T., 1971, *Biopolymers*, **10,** 513.