CORRELATION BETWEEN POLAROGRAPHIC REDUCIBILITY AND CIRCULAR DICHROISM OF DNA AT SUBMELTING TEMPERATURES

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SUMMARY

The properties of calf-thymus DNA were studied in dependence on temperature with the aid of circular dichroism, pulse polarography, and spectrophotometry. In the region of submelting temperatures the positive CD band amplitude of DNA increased linearly with rising temperature. The polarographic reducibility of DNA grew parallel to the increase of the positive CD band. It has been concluded that the temperature rise in the submelting zone results in changes of DNA conformation. The regions of the DNA molecule with changed conformation are, in comparison with native DNA, more opened, while stacking of bases is not substantially influenced.

The original conception of thermal denaturation of DNA (1) has somewhat changed in the past 10 years. In has been found that already at temperatures far below denaturation temperature the DNA double-helical structure undergoes certain changes (e.g. 2-4). The first information indicating changes in DNA properties at submelting temperatures has been obtained with the aid of polarography (5,6). By this method it has been found that the potentially reducible bases (adenine and cytosine) are, at room temperature, polarographically non-reducible if they form part of native double-helical DNA (7,8). If the

temperature is increased (without the denaturation temperature being reached) a certain portion of these bases becomes available for the electroreduction. An important evidence of the conformational changes in DNA at submelting temperatures was the measurement of circular dichroism (CD) performed by Brahms and Mommaerts (9), who observed an increase of the positive CD band as a result of temperature elevation in the region of submelting temperatures. These authors, however, presented only one CD spectrum measured at submelting temperature (at 45°).

In the present paper, the temperature dependence of CD, the optical density, and the polarographic reducibility of DNA have been followed with the view of obtaining further data on CD of DNA at submelting temperatures and of comparing the results of the measurements obtained by means of the above mentioned methods.

MATERIALS AND METHODS

The method of isolation of calf-thymus DNA and its characterization were the same as previously published (2). The measurements were performed in 0.0075 M sodium phosphate with 0.001 M EDTA, pH 6.9 and in 0.5 M and 4 M $NaClO_4$ with 0.05 M sodium phosphate, pH 6.5. In 4 M $NaClO_4$, DNA melts at relatively low temperatures ($T_m \sim 65°$ C); this medium is also suited for polarographic measurements (at ionic strengths of about 0.01 polarographic measurements are difficult). The measurements were carried out on a Roussel-Jouan Dichrograph model CD 185 and on an A 3100 Southern-Harwell pulse polarograph (Southern Analytical Ltd.) with a mercury dropping electrode; details concerning the polarographic measurements have been

published earlier (10). For spectrophotometric measurements a Unicam SP 700 was employed.

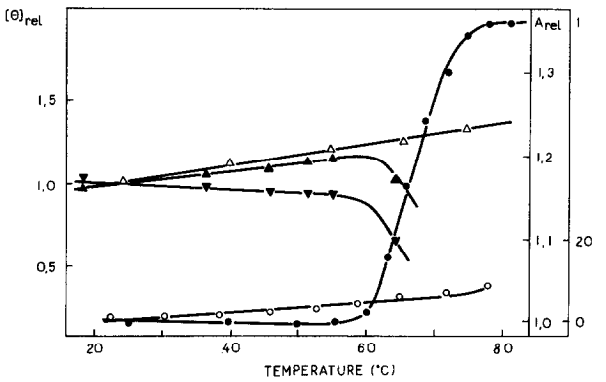

Fig. 1 Spectrophotometry, circular dichroism and polarography of DNA: temperature dependence. Calf-thymus DNA in 0.0075 M sodium phosphate with 0.001 M EDTA, pH 7; ●——● , optical density at 260 nm; ▲——▲ , positive CD band (275 nm); ▼——▼ , negative CD band (245 nm); 0.5 M $NaClO_4$ with 0.05 M sodium phosphate pH 6.5; △——△ , positive CD band (275 nm); o——o , pulse polarography.

Θ_{rel}, the ratio between the ellipticity at the given temperature and at 25° C.

A_{rel}, the ratio between the absorbancy (260 nm) at the given temperature and the absorbancy at 25° C.

I, the height of the pulse-polarographic wave in divisions. The pulse-polarographic measurements were carried out at an amplifier sensitivity of 1/8, the number of divisions being calculated for a sensitivity of 1/32. All data were corrected for thermal expansion.

RESULTS

In 0.075 M sodium phosphate (Fig. 1) the amplitude of the positive CD band increased linearly with elevating temperature until the beginning of denaturation indicated by the rise of the optical density at 260 nm. The amplitude of the negative band changed only slightly with temperature in the submelting

region. In the region of denaturation temperatures a marked decrease of the amplitude of the positive and negative bands occurred.

Simultaneous pulse-polarographic and CD measurements were carried out in 0.5 M and 4 M $NaClO_4$ (Figs. 1 and 3). In 0.5 M $NaClO_4$ the slope of the dependence of the positive CD band amplitude on temperature (Figs. 1,2) differed only slightly from that of the same dependence in 0.075 M sodium phosphate.

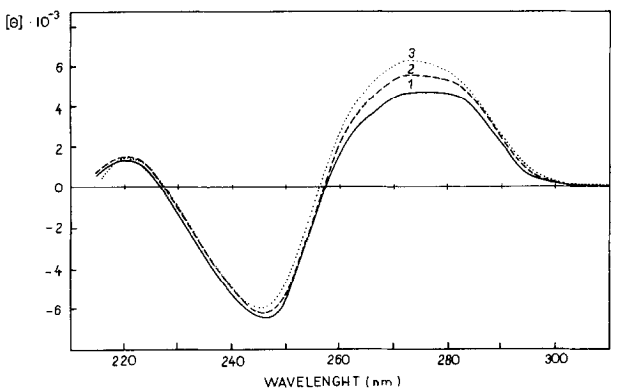

Fig. 2 Temperature dependence of circular dichroism of DNA at submelting temperatures. Calf-thymus DNA in 0.5 M $NaClO_4$ with 0.05 M sodium phosphate pH 6.5.
1 - 25°; 2 - 55°; 3 - 80°. Mean residue ellipticity is reported in degrees centimeter^2 per decimole of nucleotide residues.

In 4 M $NaClO_4$ the increase of this band in dependence on temperature in the submelting region, was substantially steeper (Fig. 3). The pulse-polarographic wave increased in this region almost parallel to the growth of the positive CD band amplitude both in 0.5 M $NaClO_4$ (Fig. 1) and in 4 M $NaClO_4$ (Fig. 3). In 4 M $NaClO_4$ the negative CD band somewhat decreased with temperature, while in 0.5 M $NaClO_4$ it remained practically uninfluenced (Fig. 2). As it follows from the spectrophotometric

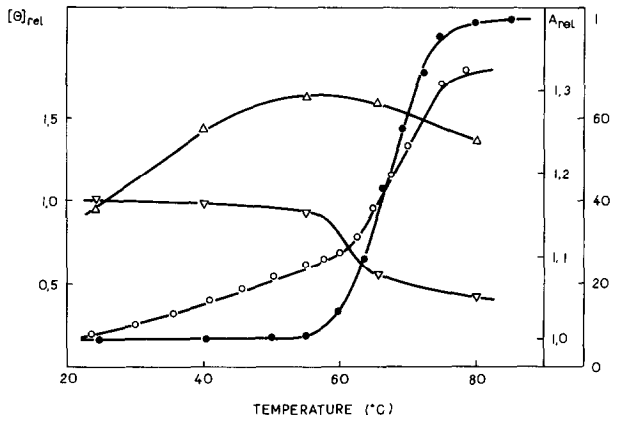

Fig. 3 Thermal transition of calf-thymus DNA followed by circular dichroism, polarography and spectrophotometry. DNA in 4 M $NaClO_4$ with 0.05 M sodium phosphate pH 6.5. Circular dichroism: △——△ , positive band (275 nm); ▽——▽ , negative band (245 nm); o——o , pulse polarography; ●——● , absorbancy at 260 nm.

Θ_{rel}, the ratio between the ellipticity at the given temperature and at 25° C.

A_{rel}, the ratio between the absorbancy (260 nm) at the given temperature and the absorbancy at 25° C.

I, the height of the pulse-polarographic wave in divisions.

Pulse-polarographic measurements were carried out at an amplifier sensitivity of 1/8 or lower, the number of divisions being calculated for a sensitivity of 1/32. All data were corrected for thermal expansion.

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measurements (not shown), no DNA denaturation occurred in the latter medium within the temperature range used in the experiment (Fig. 1). In 4 M $NaClO_4$, DNA denaturation resulted in a sharp decrease of the negative and in a moderate decrease of the positive CD band as well as in a sharp increase of the pulse-polarographic wave, similar to the increase of the optical density in the melting region (Fig. 3). The pulse-polarographic behaviour of DNA in 0.5 M $NaClO_4$ in dependence on tem-

perature corresponded, in principal, to the measurements performed earlier in other media (7, 8, 11).

DISCUSSION

The good correlations between CD and polarography suggest that both methods reflect different aspects of the same changes taking place in the DNA molecule due to the temperature increase in the premelting temperature zone. Polarographic measurements show that at elevated (submelting) temperatures DNA is present in solution in a structural form, which, in comparison with its form prevailing at room temperature, is more opened, i.e. the bases in this form may interact more easily with their environment than the bases contained in the native form. On the other hand, the difference in the potentials at which the polarographic currents of denatured and native DNAs at submelting temperatures appear (11) suggests that the changes occurring in DNA at these temperatures differ from those taking place in DNA during denaturation. Brahms and Mommaerts (9) explained the changes in CD at submelting temperatures by formation of an intermediary conformation of DNA. For the time being, the limited experimental material does not permit to draw detailed conclusions on the character of the conformational changes occurring in the premelting temperature zone. As fairly important we do consider the fact that the changes in the CD spectra in dependence on temperature (Fig. 1, 2, 3) are rather marked. The relative increase of the ellipticity of the positive CD band is, in the given temperature range, over 30 per cent in 0.5 M $NaClO_4$ and 60 per cent in 4 M $NaClO_4$. (Note: the given percentage values are not simply comparable,

for the ellipticity value at 25° C, to which they are related, depends on the concentration of $NaClO_4$.) On the other hand, the optical density of the DNA solutions practically does not change under the given conditions (Figs. 1 and 3). The difference in the results of spectrophotometric and CD measurements reflects the different sensitivity of these two methods to minor conformational changes (12). The results obtained by both methods can be explained by the presumption that at premelting temperatures relatively minor conformation changes occur which do not substantially influence the stacking of the bases, but which affect extensive regions of the DNA molecule.

It follows from our polarographic (2,13) and preliminary CD measurements (14) that the above mentioned conformational changes take place preferably in the adenine-thymine rich regions. A more thorough study of the dependence of CD of DNA on temperature as well as a more detailed analysis of DNA conformation in solution will be published elsewhere.

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