CONFORMATIONAL CHANGES IN THE REGION OF THE ENDS OF THE DNA MOLECULE AT PREMELTING TEMPERATURES

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1. Introduction

Since 1962 there have been increasingly frequent reports of the changes in the properties of DNA as a function of temperature at temperatures below the melting zone [1–9]. The assumption has been made that these changes are conditioned by conformational changes in the DNA and that the nature of these changes (vertical base stacking is probably not substantially affected) excludes the possibility of their detection by spectrometry, which is usually used for studying helix-coil transition. One of the methods suitable for studying premelting changes is polarography which allows the release of reducible groups, which are unavailable to electrode processes at room temperature, to be followed [10]. Recently it was demonstrated that the introduction of single-strand breaks (s.s.b.) into the DNA molecule (by DNAase-I or by γ-radiation) leads to increased premelting changes [11]. At the same time it was found that the slight lowering of the molecular weight of DNA caused by small doses of ionising radiation, does not influence the oscillopolarographic behaviour of the DNA sample.

This work is concerned with the question as to whether a greater lowering of M.W. of DNA (roughly to one-tenth of the original value) will affect the extent of premelting changes and, therefore, if the ends of the DNA molecule represent one of the labile parts of the molecule, in which these changes take place preferentially.

2. Materials and methods

Calf-thymus DNA was isolated and characterized as before [12]. The DNA sample did not contain denatured DNA in an amount detectable by oscillopolarography [13]. The lowering of M.W. was achieved by degradation in a capillary. Molecular weights of DNA samples were estimated with the aid of a four-gradient Ubbelhode type viscometer [14]. The formation of s.s.b. during shear degradation was controlled viscometrically [15] on the basis of Zamenhof's finding [16] that viscometrical denaturation curves are influenced by the presence of s.s.b. in the DNA molecule. It was found by this method that as long as s.s.b. were formed during shear degradation, their number was so small that they could not influence the results of our measurements. Direct current polarographic measurements were made with a Radelkis polarograph type OH 102 (Metrimpex, Hungary). For the oscillopolarographic measurements the universal oscillopolarograph designed in our Institute [17] was used. The dropping mercury electrode was polarized with a square-wave current, frequency 50 Hz. In the "first curve technique" a single current cycle was applied at a definite time (9.0 sec) after the fall of the preceding drop; the drop time (10.0 sec) was controlled mechanically.

3. Results

The thermal denaturation curves of control (M.W. 9×10^6 daltons) and shear-degraded DNA (M.W. 9×10^5 daltons), followed by classical polarography, are shown in figure 1a. The figure shows that the degraded

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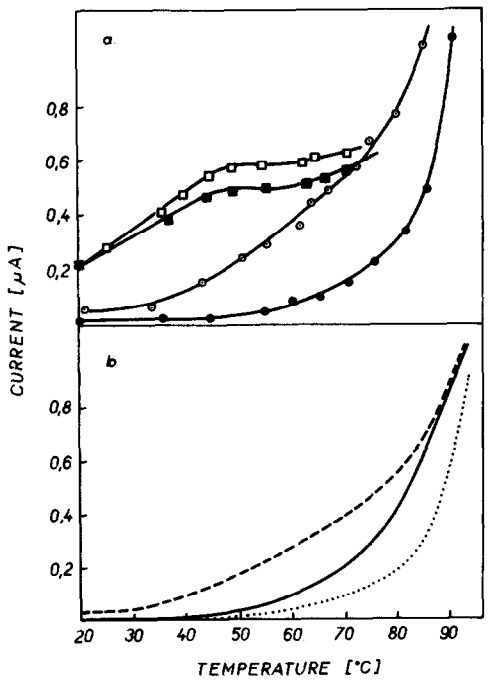

Fig. 1. Dependence of polarographic current on temperature
a) Native DNA: ○ degraded, ● undegraded. Denatured DNA: □ degraded, ■ undegraded. Polarographic measurements were carried out with native DNA at a concentration of 97.5 μg/ml and with denatured DNA at a concentration of 32 μg/ml in 0.6 M ammonium formate with 0.1 M sodium phosphate pH 6.8.
b) The correction of currents for temperature increase: - - - degraded sample, ... undegraded control, —— theoretical current-temperature curve of the degraded sample.

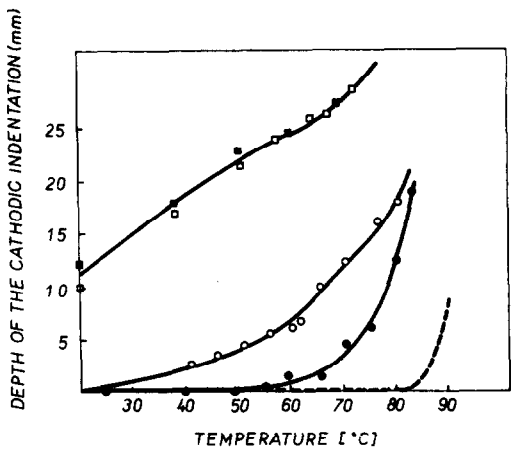

Fig. 2. Dependence of depth of oscillographic indentation on temperature. Native DNA: ○ degraded, ● undegraded. Denatured DNA: □ degraded, ■ undegraded. Measurements were made in 0.3 M ammonium formate with 0.1 M sodium phosphate pH 6.8 at the DNA concentratration of 97.5 μg/ml.

DNA sample provides substantially higher polarographic currents at premelting temperatures than does the undegraded control. The correction of measured values for increase of temperature does not substantially change these results (fig. 1b). It is, however, necessary to remember that the current increase produced by the degraded sample is, at least partly, caused by the change in the diffusion coefficient, due to the decreased M.W. of the DNA. The relation between the polarographic current and the diffusion coefficient of substances tested is, for the diffusion-controlled processes, contained in the Ilkovič equation [18]. Polarographic measurements were, therefore, made in conditions where the characteristics of the current produced by DNA agree reasonably with the characteristics of diffusion-controlled currents [15, 19]. The theoretical current-temperature curve of the degraded sample was then calculated on the basis of the experimental curve of the control sample and of the increased diffusion coefficient of the degraded DNA. The diffusion coefficient values were taken from the literature [20]. Even after these corrections the curve of the degraded DNA reached higher current values than that of undegraded DNA (fig. 1b).

In an attempt to obtain other experimental evidence, independent of diffusion coefficient values, the course of denaturation curves of DNA samples of different molecular weights were also compared by the oscillopolarographic "first curve technique" [5]. In this the dropping mercury electrode is immersed in the solution for a certain time, during which the depolarizer may adsorb on the surface of the electrode. Only then one cycle of alternating current is applied, which causes the reduction of almost all adsorbed molecules of the depolarizer. The depth of the cathodic indentation on the $dE/dt = f(E)$ curve is, in this case, not determined by the diffusion rate of the depolarizer but by its adsorbability [21]. As is seen in figure 2, even in this case the sample with lower M.W. shows a marked extension of the premelting changes. The DNA samples were denatured and the dependence of the depth of

their cathodic indentations on temperature were followed. Both samples yielded almost identical curves.

In contrast to the polarographic curves, the curves of the dependence of optical density at 260 nm on temperature, produced by degraded and undegraded DNA, were identical. Our prelimiary results [15] obtained by the non-electrochemical method of Glišin and Doty [8] were in agreement in principle with the results of this work.

4. Discussion

The conformational changes preceding thermal denaturation of DNA are mostly considered as events which affect any part of the DNA molecule [3, 4, 8]. Quite recently it has been shown that these changes occur preferentially in the (adenine + thymine) -rich regions [9] and in the vicinity of s.s.b. [11]. It follows from our results that other regions, relatively important in bringing about premelting conformational changes, are the ends of the DNA molecule. In contrast to this the length of the molecule has no influence on T_m, which characterizes the denaturation of DNA [22]; changes in T_m were observed only when the M.W. of DNA dropped to 2×10^5 daltons [23]. The preliminary results of the study of premelting changes by electrochemical and non-electrochemical methods suggest that the premelting changes in DNA conformation occur preferentially in a number of other regions of the DNA molecule, especially where the regularity of the double helix is disturbed by slight modifications caused by chemical and physical factors [24]. Up to now great attention has been given to the study of DNA denaturation [25]; the study of conformational changes preceding the thermal denaturation of DNA deserves at least the same attention. These changes which occur in conditions substantially closer to the physiological than those in which denaturation takes place, may play an important role in the realization of DNA function.

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