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On the Alkaline Denaturation of DNA: the Rise-Time of the Hyperchromic Effect in the Transition Region

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Biofisica. — *On the Alkaline Denaturation of* DNA: *the Rise-Time of the Hyperchromic Effect in the Transition Region* (*). Nota di MARIO AGENO (**), ELISABETTA DORE (**), CLARA FRONTALI (**) e FRANCA PODO (**), presentata (***) dal Corrisp. M. AGENO.

RIASSUNTO. — (Sulla denaturazione alcalina del DNA: il tempo di salita dell'effetto ipercromico nella regione di transizione) – Vengono riportati nel presente lavoro i dati relativi all'andamento del tempo di salita dell'effetto ipercromico dovuto a denaturazione per alcali. Questi risultati, uniti a quelli ottenuti in precedenti lavori, permettono una più completa descrizione del processo di denaturazione alcalina. Nella transizione del DNA da nativo a denaturato si possono distinguere due zone: una in cui l'effetto ipercromico è totalmente reversibile, e il suo tempo di salita è regolato dalla formazione dell'equilibrio tra molecole di DNA e agenti deprotonanti presenti in soluzione (ioni OH^-); una seconda in cui le molecole, in presenza di una maggiore concentrazione di ossidrili, vengono deprotonate assai rapidamente e il tempo di salita totale è regolato dal tempo di salita dell'effetto ipercromico irreversibile.

Previous work on phage DNA solutions in saline media [1–4] has shown that when the pH values are such as to cause complete denaturation of the DNA molecules (pH 12.4 — 12.6), the following three characteristic intervals of time have to be considered:

1. The rise-time t_1 of the hyperchromic effect which, for the pH values considered, is totally reversible for some tenths of a second after denaturation conditions have been established. It gives a measure of the intramolecular melting rate under those conditions; e.g. at pH 12.5 its value is less than 0.1 s.

2. The interval of time t_2 required for strand separation in a single DNA molecule. Its order of magnitude was evaluated as one second, using the CsCl density gradient technique in the analytical ultracentrifuge.

3. The rise-time t_3 of the residual hyperchromic effect (i.e. the fractional increase in absorption at 260 mμ measured between the initial state of the native DNA solution and the final state of the solution when, after denaturation for an interval of time τ, the pH is rapidly changed again to 7.0). It depends on the length of the DNA molecules, the viscosity of the medium, and the nature of the small positive ions in the solution. Its order of magnitude is of several seconds (about 20 seconds for T_2 DNA and about 5 for α DNA [2]).

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The experimental conditions in which most of this work was done were particularly simple and were appropriate for separately studying the two processes of the melting of the hydrogen–bonds and of strand separation. When the pH was suddenly raised to 12.5 melting was already complete, while the two strands of the molecule still remained almost in the relative positions they occupied in the native DNA. In fact t_1 is much shorter than t_3.

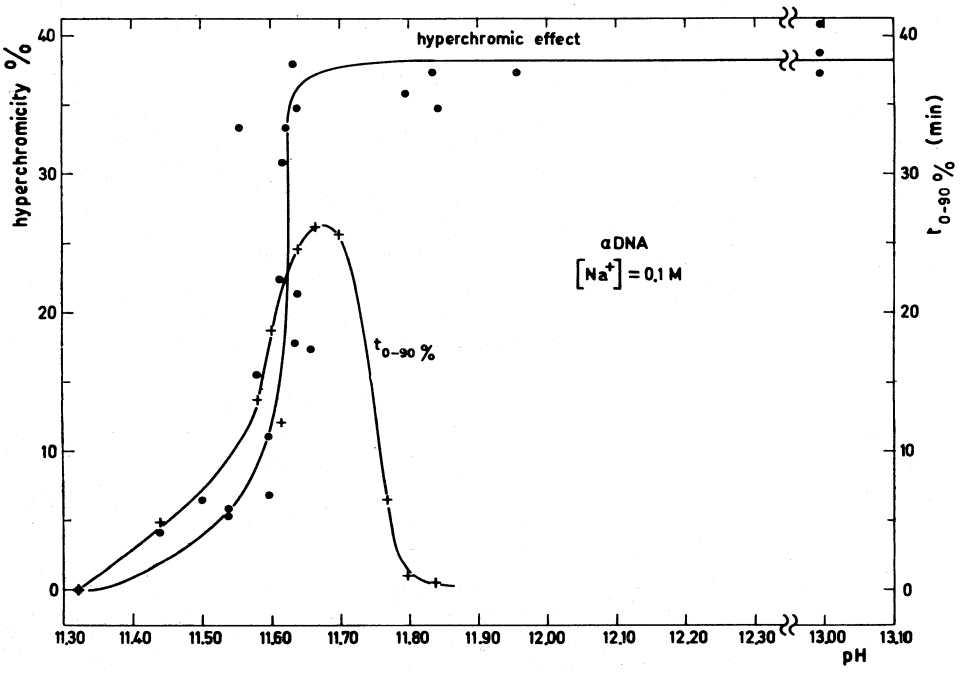

Fig. 1.

The experimental results, in this range of the pH, showed that *a*) the actual value of the residual hyperchromic effect (below its maximum value). is a measure of the fraction of the DNA molecules which have undergone total strand separation. It was also shown that *b*) for a particular molecule, strand separation is a quasi–catastrophic event, which takes place randomly in time. When the denaturation conditions are suddenly established, some molecules almost immediately denature, but some others remain in the double stranded state for a time comparable to t_3 and then they denature quite suddenly in a time t_2 much shorter than t_3. These processes raise two questions. The first refers to the source of energy required to overcome the viscous drag in a time so short as t_2, and this was found in the " deprotonation " of the H–bridges, between complementary bases, by the hydroxil–ions in the solution. The second question refers to the nature of the chance event necessary to start the unwinding process in a particular molecule, and this was considered to be due to a fluctuation in the number of positive ions which must surround the " deprotonated " sites.

However, denaturation also takes place in very different conditions when the pH is not so high as to make melting a very rapid event in comparison with strand separation. In this case the two processes cannot be considered separately and it is conceivable that the rate limiting factor for the denaturation process in this case is set by the detachment of the protons by the hydroxil-ions.

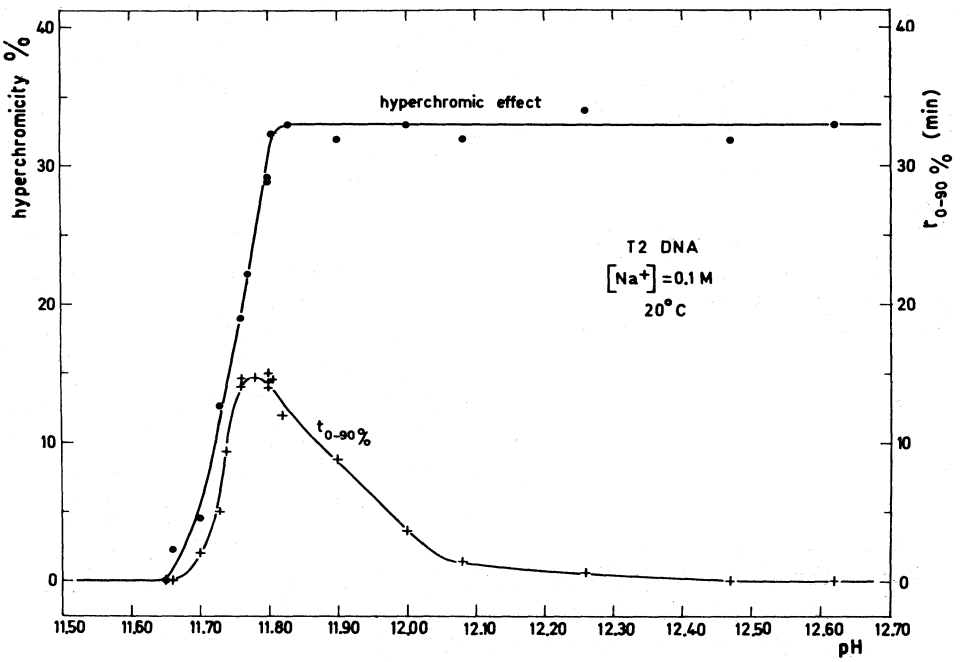

Fig. 2.

In this paper the denaturation process is studied in the pH range in which the DNA solution undergoes the hyperchromic transition. In particular, the rise-time t_1 of the hyperchromic effect was measured, as a function of pH, for two DNAs, that of phage T_2 and that of phage α. The following procedure was adopted: the pH of the DNA sample was suddenly raised to φ by mixing with alkali and the actual absorption of the sample at 260 mμ was measured as a function of time with a spectrophotometer. In the case of the α DNA a Beckmann mod. D.U. Spectrophotometer at room temperature, and in the case of T_2 DNA a Cary mod. 15 Recording Spectrophotometer thermally regulated at 20° C, were used. For each φ value, the final hyperchromic effect and the corresponding rise-time (defined as the interval of time in which the hyperchromic effect passes from zero to 90 % of its final value) were read from the graph.

The results are plotted in fig. 1 for α and in fig. 2 for T_2 DNA. The most striking feature of the plot is the rise-time peak corresponding to the 80 ÷ 90 % of the maximum hyperchromic effect. The difference between

this plot and the curve published in a previous paper [5] is essentially due to the fact that in that paper the rise-time was arbitrarily taken as infinitely long for the pH values at which no denaturation occurs.

It must be noted that the upper parts of the curves of the hyperchromic effect in the transition region go up very steeply and it is difficult to see if they have a sharp upper knee, or if there is a region of instability. It must be remembered that above pH 11.8 the hyperchromic effect of the T_2 DNA becomes increasingly irreversible [5]. Only in this region will a fraction of the molecules of the sample be completely denatured.

It is now possible to give a fairly complete description of the denaturation process. Two regions must be separately considered.

1) In the first region, on the left side of the peak of the rise-time, the hyperchromic effect is totally reversible and no denatured molecules are found in the sample. An equilibrium is established between the solution and the DNA molecules in which a number of H–bridges between pairs of complementary bases are deprotonated, but this number is not sufficient to cause strand separation. Careful examination of the spectrophotometric graphs showed that, when the pH was suddenly raised to a given value, a very rapid initial increase of the absorbing power of the sample (lasting not more than a few seconds) was followed by a much slower subsequent variation. It may be that the initial increase was due to random deprotonation of each molecule and that the following slower increase was due to an internal rearrangement of each molecule. In fact, deprotonated sites are likely to form clusters, owing to the free energy of stacking stored between neighbouring bases of the same strand [6–7].

2) In the second region, on the right side of the peak of the rise-time, an increasing irreversible hyperchromic effect takes place. However, it is not clear if there is a transition region in which, at constant pH and room temperature, denatured molecules and double-stranded ones can coexist in equilibrium. As the pH increases, the deprotonation and unwinding processes are more and more separated in time, and in the end the conditions of the system become the same as those considered in previous papers.

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