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A Comparison between the Absorption Spectra of Heat and Alkali-Denatured DNA

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SEZIONE II

(Fisica, chimica, geologia, paleontologia e mineralogia)

Biofisica. — *A Comparison between the Absorption Spectra of Heat and Alkali-Denatured* DNA (*). Nota di MARIO AGENO (**) ELISABETTA DORE (**) e CLARA FRONTALI (**), presentata (***) dal Corrisp. M. AGENO.

RIASSUNTO. — (Confronto tra spettri di assorbimento del DNA denaturato al calore e per alcali) — Dagli spettri di assorbimento del DNA nativo e denaturato, al calore e per alcali, vengono calcolati in funzione della lunghezza d'onda i rispettivi effetti ipercromici. Si mette così in evidenza che, l'effetto ipercromico residuo è nei due casi quasi identico, mentre l'effetto ipercromico a 88° C differisce notevolmente da quello a pH 12,5, in accordo con quanto da noi precedentemente esposto sulla particolare natura della denaturazione alcalina.

Evidence that the two states in which the DNA molecule is left after thermal and alkaline denaturation are different was obtained from spectrophotometric absorption measurements. For this purpose a Cary mod. 15 Recording Spectrophotometer, flushed with dry nitrogen, and Quartz Suprasil cuvettes 1 cm thick were used.

The absorption spectrum of heat-denatured DNA of Phage T 2 in 0.1M NaCl at 88° C was compared with the absorption spectrum of the same DNA denatured by alkali at pH 12.5. In the first case two spectra were measured in succession, that of the DNA sample at 88° C and that of the corresponding blank at the same temperature. Both were read against the same blank (automatically compensated over the range 3200 Å–1830 Å) at room temperature. The difference between the two former spectra gives the true absorption spectrum of the DNA at 88° C. This method was adopted because in our spectrophotometer the temperature of the reference compartement cannot easily be varied. In the second case, the DNA sample at pH 12.5 was directly compared with the corresponding blank at the same pH.

The two spectra of the denatured DNA are shown in fig. 1, together with the absorption spectrum of T2 DNA in the native state in 0.1M NaCl. The last was also measured in order to be able to calculate the hyperchromic effect at different wavelengths. These results are in agreement with those of Voet et al. [1] and Falk [2] and with those of Felsenfeld [3], with regard

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(***) Nella seduta del 13 gennaio 1968.

to the thermal hyperchromic effect. No evidence was obtained for the existence of a second absorption peak near 2100 Å as suggested by Das Gupta *et al.* [4–5]. However, this question was not examined in detail and in our measurements the decrease of the absorption coefficient may be masked by the absorption peaks of residual oxygen.

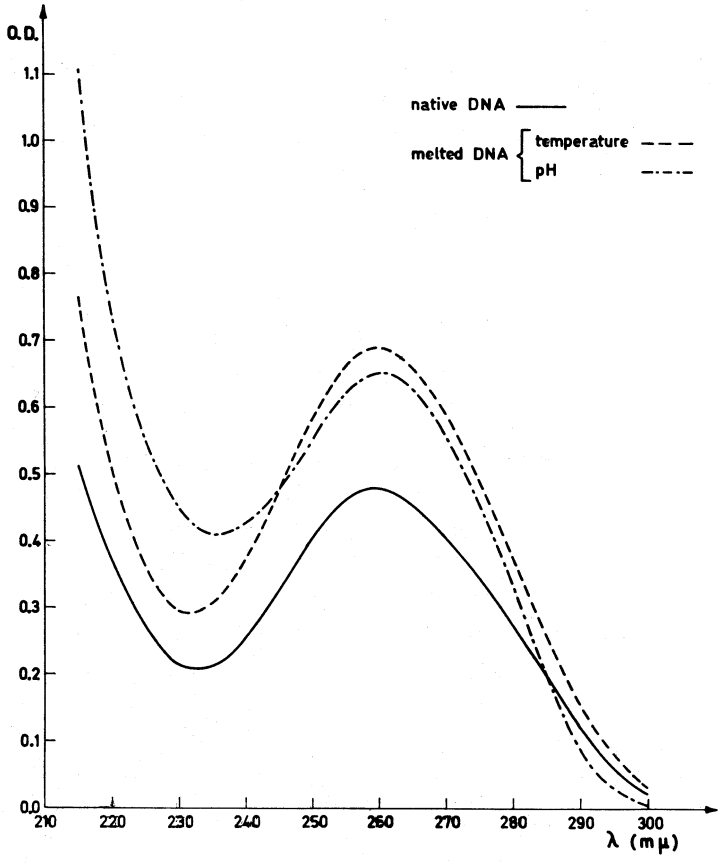

Fig. 1.

In fig. 2, the hyperchromic effect was calculated as a function of wave length from the two spectra, and compared with the residual hyperchromic effect of the two denatured samples, brought again to room temperature or neutral pH. In both melted DNA samples the molecules were certainly unwound and no hydrogen bond could remain.

The two samples had the same residual hyperchromic effect at all wavelengths, and this fact demonstrates that their final state is the same. The hyperchromic effects at 88° C and at pH 12.5, however, are strikingly different, most of all for the shorter and longer wavelengths. Thus it was concluded that the two intermediate states to which DNA molecules are brought by thermal and by alkaline denaturation are different.

This conclusion agrees fairly well with the results of our previous studies on the process of alkaline denaturation of DNA [6–7–8]. On those grounds, a simple model was proposed for that process. The source of the energy spent in the unwinding process was derived from the capture of the protons of the hydrogen bridges, between the bases of each pair of nucleotides, by the hydroxylions of the solution. The two separated strands of each DNA

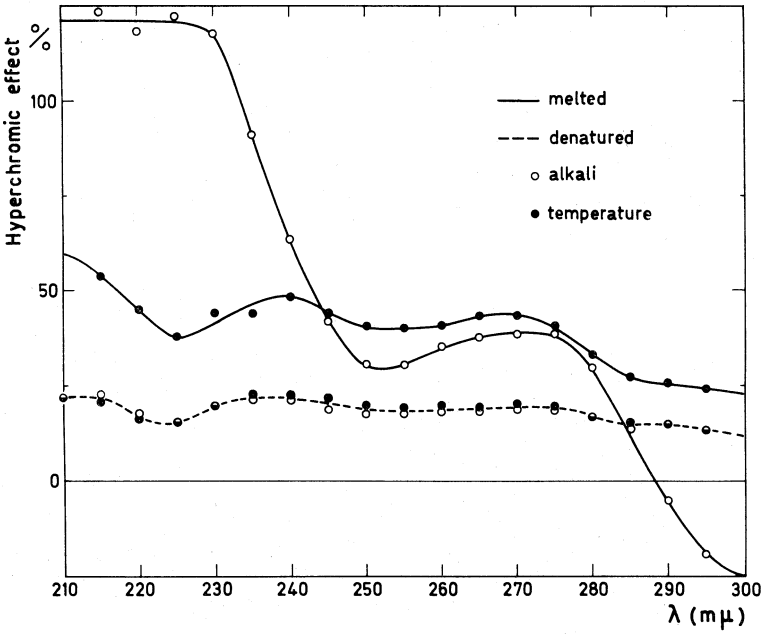

Fig. 2.

molecule at pH 12.5 are thus thought to be deprived of one or two protons per base pair and, if so, their absorption spectrum must be different from that of the separated strands in a DNA solution heated to 88° C. When the solution is brought back to pH 7, the missing protons are recovered and the absorption spectrum turns out to be identical with that of heat-denatured, rapidly cooled, DNA.

The results described above seem to support this model.

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