STOPPED-FLOW ULTRAVIOLET SPECTROSCOPY FOR HYDROGEN-EXCHANGE STUDIES OF NUCLEIC ACIDS

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1. Introduction

A kinetic study of the hydrogen exchange reaction of a protein or a nucleic acid in aqueous solution can provide a useful information on the structure and its fluctuation of such a biological macromolecule [1–4]. For nucleic acids, however, the hydrogen exchange reactions have so far been followed almost only by the method of the Sephadex column chromatography at lower temperatures (0°C or so) [4–8]. At room temperatures, the reaction takes place so fast, that a new method should be developed for following it. Cross showed recently that the exchange of protons on adenosine with solvent ^2H_2O can be observed by a stopped-flow and ultraviolet spectroscopy [9]. This method can be applied to a fast exchange reaction which takes place in a few milliseconds, and therefore seems to be suitable for monitoring the hydrogen exchange reactions of nucleic acids in general. We have recently examined native and heat-denatured calf thymus deoxyribonucleic acids (DNA) by this method. The results will be given below.

2. Materials and methods

A sample of calf thymus DNA was purchased from Sigma Chemical Co. This was used after a sonication and a dialysis. This sample is called 'native DNA'; in the standard saline solution (0.15 M NaCl + 0.015 M sodium citrate) its melting temperature was 86°C and the hypochromicity at 260 nm was 38%. A 'heat-denatured DNA' sample was obtained by incubating the native DNA solution at 100°C for 5 min and by rapid cooling. ^2H_2O (99.75 atom%) was purchased from Merck and was used after distillation. The hydrogen–deuterium exchange reaction of DNA was traced by the use of a Union Giken Stopped-Flow Spectrophotometer RA-401. This consists of a rapid-mixing device with a dead time of 0.5 ms and an ultraviolet spectrometer with sensitivity 0.0004 (in absorbance) and response time 1 μs. This was connected with a Union Giken Data-Processor RA-450, a monitor-scope, and an XY-plotter.

3. Results and discussion

Figure 1 shows a typical reaction trace, obtained by our present setup for native DNA at 290 nm. Figure 2 shows a typical one for heat-denatured DNA. A great difference of a native (mostly double-helical) and a heat-denatured (single-stranded in large part) DNAs in the rate of hydrogen–deuterium exchange reaction is readily seen in these figures. It may also be pointed out here that the signal-to-noise ratio of our kinetic measurement is greater than 10, not only

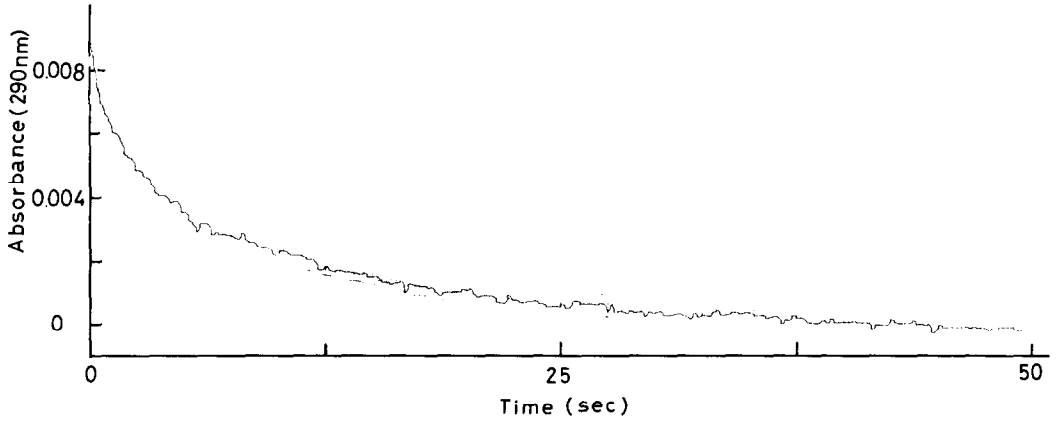

Fig.1. A recorded curve in a stopped-flow ultraviolet absorption study of the $^1H \rightarrow ^2H$ exchange reaction of native DNA from calf thymus. DNA (2.98×10^{-4} M nucleotide pair) in ^1H_2O (0.1 M NaCl) was rapidly mixed with ^2H_2O in 1:1 v/v, so that the final concentration of DNA is 1.49×10^{-4} M and the final concentration of NaCl is 0.05 M, pH 6.8. Then a time-dependent decrease in absorbance at 290 nm was examined, and the data were stored. The same experiment was repeated for 10 times, the absorbance versus time data were accumulated, and then recorded. The temperature of the solutions were kept at 25°C.

for the slower reaction (fig.1) but also for the fast one (fig.2).

A re-plot of such a kinetic data for native DNA, as illustrated in fig.3, shows that the absorbance decrease at 290 nm takes place as two first order processes. The rate constant of the faster process is found to be 0.70 s^{-1} and that of the slower one 0.077 s^{-1} 25°C, pH 6.8 (pH meter reading) and NaCl 0.05 M. The slower process is assignable to the hydrogen atoms involved in the base–base hydrogen bonding, whereas the faster one to those free from such hydrogen bonding. The two first order processes were also found in our similar experiments at different temperatures. The rate constants thus determined for the faster and slower processes are plotted in fig.4. This figure involves data at 0°C obtained by Englander et al. [4]. They examined tritium–hydrogen exchange reaction of a native calf thymus DNA, at pH 7, with 0.1 M NaCl at 0°C, and found two first-order processes. As may be seen in the Arrhenius plots (fig.4), it is probable that our faster and slower processes are the same, respectively, with the faster

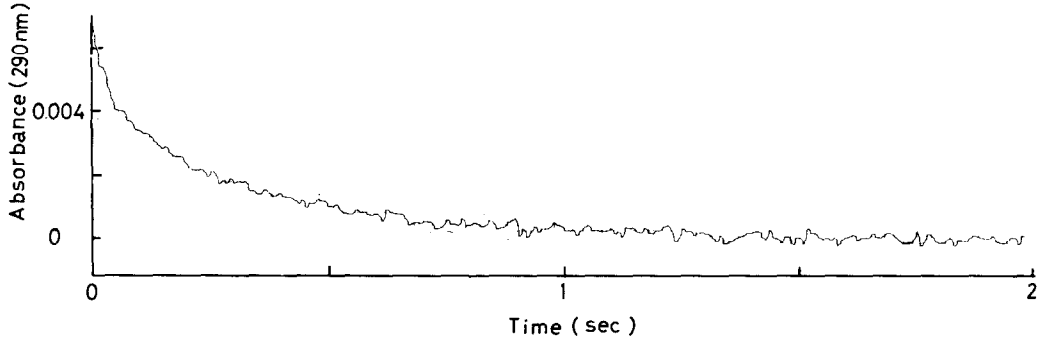

Fig.2. A recorded curve, as an output of a similar examination to what is described in the legend of fig.1, except that the sample here is heat-denatured DNA (at 25°C, pH 6.8; examined by a beam of 290 nm).

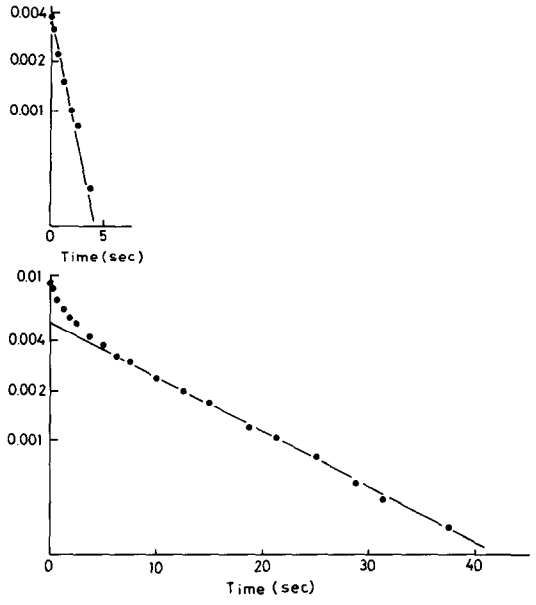

Fig.3. Semi-logarithmic plot of the absorbance versus time data obtained in fig.1. The plot gives a straight line in the range of longer time. From the inclination of this line, the rate constant of a slower process is obtained. Next, in the range of shorter time, the deviation of every plotted point in the absorbance value from the straight line just mentioned is replotted in a semi-logarithmic manner. From the inclination of the straight line thus obtained, the rate constant of a faster process is geven.

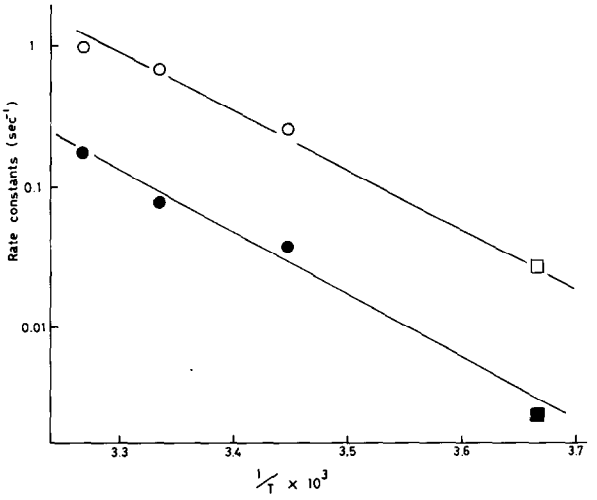

Fig.4. The rate constants of the hydrogen–deuterium exchange reactions of native DNA plotted on a logarithmic scale against reciprocal absolute temperature. (○) and (●) : for fast and slow processes, respectively, determined by stopped-flow ultraviolet spectroscopy. (□) and (■) : for fast and slow processes, respectively, determined by Sephadex column chromatography (Englander et al. [4]).

and slower processes found by Englander et al. [4]. Thus, for a native DNA, the stopped-flow ultraviolet spectroscopy seems to provide an equivalent type of information to that obtained by the Sephadex column chromatography. The experimental procedure in the former, however, can be completed within a much shorter time than that in the latter. In addition, the stopped-flow ultraviolet spectroscopy has another advantage: by the use of the ultraviolet beams of different wavelengths, the hydrogen–deuterium exchange reactions on the adenine–thymine base pair are distinguishable from those on the guanine–cytosine base pair.

The hydrogen exchange rate of a single-stranded DNA is so high that only the stopped-flow ultraviolet spectroscopy can follow the progress. Re-plotting of the kinetic data for heat-denatured DNA (fig.2) has been made in a similar way to what was done for native DNA (fig.3). The absorbance change was found to take place as two first order processes with the rate constants 27 s^{-1} and 2.8 s^{-1} (at 25°C, pH 6.8). The faster process is assignable to base residues fully exposed to the solvent, because its rate (27 s^{-1}) has the same order of magnitude to that of the hydrogen exchange reactions of nucleotide monomers. The hydrogen exchange rate constants of GMP (0.043 mM) and CMP (0.26 mM), for example, are found to be 43 s^{-1} and 13 s^{-1} (at 25°C, pH 6.8), respectively. The rate constant (2.8 s^{-1}) of the slower process of the heat-denatured DNA is lower by one order of magnitude than those of mononucleotides, but still four times as great as the rate constant (0.77 s^{-1}) of the hydrogen atoms free from the hydrogen bond in native DNA. We suggest to assign this process to hydrogen atoms on base residues buried in a randomly coiled DNA chain but often exposed to the solvent through a fluctuation of the structure.

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