HYDROGEN-EXCHANGE STUDY OF A NUCLEOSOME

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Received 10 September 1980

1. Introduction

Having established the hydrogen–deuterium exchange kinetics of a free DNA molecule [1], we now proceed to a study of DNA in chromatin. Here, we examine the deuteration kinetics of a nucleosome particle by stopped-flow ultraviolet absorption spectrophotometry. Hydrogen exchange is only slightly changed in nucleosomes as compared with free DNA.

2. Materials and methods

Nucleosomes were obtained from calf thymus nuclei by digestion with micrococcal nuclease and then by fractionation by sucrose gradient centrifugation [2,3]. Nuclei isolated from 5 g calf thymus were suspended in 10 ml 0.05 M sucrose + 1 mM $CaCl_2$ + 1 mM Tris (pH 8). This was digested with 150 units nuclease/ml at 37°C for 30 min. The digestion was stopped by adjusting ethylenediamine tetraacetic acid (EDTA) to 5 mM and by cooling on ice. The product was centrifuged for 10 min at 10 000 × g, and the supernatant layered onto a 5→20% sucrose gradient containing 0.1 M NaCl and 1 mM EDTA (pH 7). Centrifugation was in a Hitachi RPS 25 rotor at 22 500 rev./min for 17 h at 4°C. The major peak was collected and dialyzed against a phosphate buffer.

Calf thymus DNA was purchased from Sigma. This was dissolved into ^1H_2O by stirring overnight at 4°C, then sonicated at 0°C for 30 min. Spermidine was also purchased from Sigma.

Hydrogen–deuterium exchange kinetics was examined by the use of a Union Giken stopped-flow spectrophotometer RA-401 in combination with a Union Giken data-processor RA-450, a monitor-scope, and an X,Y-plotter. The method was that used in [1].

3. Results and discussion

Fig.1 (a, b) show typical reaction traces, obtained, respectively, for DNA and for nucleosome. A replot of such kinetic data, as illustrated in fig.2, 3, shows that the A_{285} decreases as at least 2 first-order processes both for DNA and for nucleosome. It is noticeable that the hydrogen-exchange rate constant of nucleosome (0.1 s^{-1}) is practically equal to that of the isolated double-helical DNA in the slower process. The rate constant of the faster process, on the other hand, is found to be slightly but appreciably (with sufficient reproducibility) lower (0.64 s^{-1}) in nucleosome than that in isolated double-helical DNA (0.95 s^{-1}). On the basis of the assignment of the slow reaction to the hydrogen involved in the base-pair and of the fast reaction to atoms not involved in the basepair [1,4–6], a simple interpretation is suggested:

> The DNA molecule in nucleosome is not greatly different in its structure and structural fluctuation from the free double-helical DNA, while its outer amino hydrogens are slightly disturbed by proteins in having access to the solvent.

The intramolecular and inter-molecular environments of the base-residues in nucleosome may have some similarity to those of the DNA–spermidine complex, because histones have a number of NH_3^+ groups like spermidine. The hydrogen-exchange reactions, however, are not similar to eaoh other. As is shown in fig.4, the DNA–spermidine complex also has two kinds of labile hydrogens whose deuteration is detectable by A_{285}. The fast deuteration process has an almost equal rate constant to that of free double-helical DNA, while the slow reaction is higher.

DNA in nucleosome may well be located on its surface [7–11] and hence must be well exposed to

Elsevier/North-Holland Biomedical Press

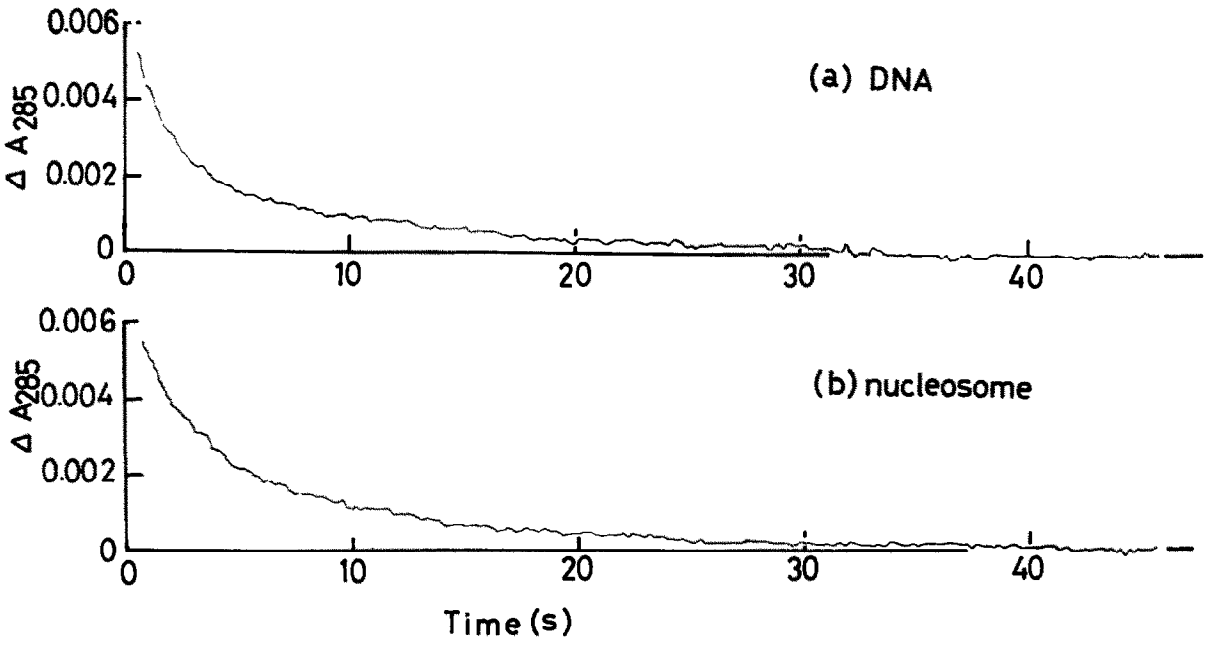

Fig.1. (a) The time dependence of the decrease in absorbance (ΔA_{285}) at 285 nm observed when DNA dissolved in ^1H_2O (5 mM Na-phosphate buffer + 1 mM EDTA (pH 7) was mixed with ^2H_2O (1:1, v/v; DNA final conc. was A_{259} 3.96). (b) The time dependence of the A_{285} decrease observed when nucleosome dissolved in ^1H_2O (solvent as in (a) (pH 7) 37°C) was mixed with ^2H_2O; nucleosome final conc. was A_{260} 3.60.

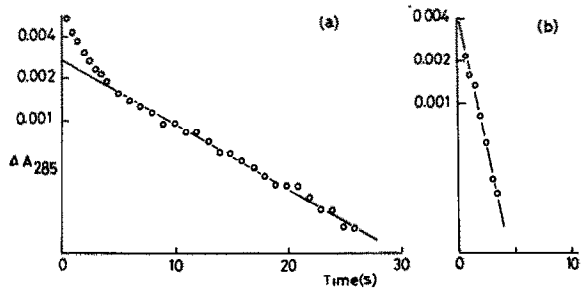

Fig.2. Semilogarithmic plot of the data for DNA given in fig.1(a). The plotted points for a longer incubation time gave a straight line (upper); the line was extrapolated into shorter incubation time; and the deviation of every plotted point in the ΔA_{285}-value from the above straight line was determined and replotted in semi-logarithmically (lower).

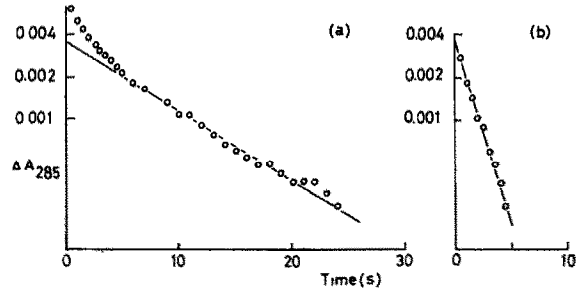

Fig.3. Semilogarithmic plot of the data for nucleosome given in fig.1(b). A analysis similar to that in the legend of fig.2 was made.

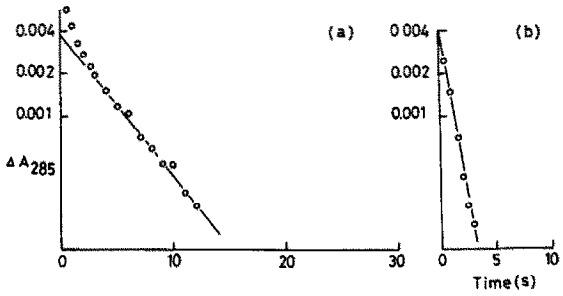

Fig.4. Semilogarithmic plot of the ΔA_{285}–time data for DNA + spermidine. DNA (0.6 mM) and spermidine (0.6 mM) were dissolved in ^1H_2O (5 mM Na-phosphate buffer + 1 mM EDTA (pH 7) 37°C) with ^2H_2O (1:1, v/v; final DNA final conc. 0.3 mM, spermidine final conc. 0.3 mM).

the solvent. Therefore, no great difference is expected for the complex from the free constituent in hydrogen exchange between the DNA moiety and the solvent. The rate of the slower exchange of DNA (assignable to the hydrogens involved in the basepairs) however, is considered to reflect the fluctuation amplitude (the chance of breakage of the inter-base hydrogen bonding) [4], and is sensitive to perturbation of DNA structure (by spermidine binding, for example). However, the DNA molecule must be subjected to

some perturbation on the nucleosome particle surface, as it is bound with some deformation in its structure [10,12,13]. Therefore, it is rather surprising that the rate has been found to be equal:

> We conclude that the amplitude of the structural fluctuation of DNA does not depend upon whether it is free in solution or packaged in nucleosomes.

In [14] the internal motion in double-helical DNA had a correlation time of $\sim 4 \times 10^{-10}$ s, both free in solution and bound to nucleosomal core proteins. Our hydrogen-exchange kinetic study cannot be related with such a correlation time, because the rate-determining step in the hydrogen exchange reaction is the chemical exchange rather than the conformational change. Instead, we can suggest that, not only the fluctuation rate, but also the fluctuation amplitude of the DNA helix remains unchanged on going from the free state in solution to the bound state in nucleosomes. We suggest that only the outer hydrogen atoms (that are not involved in the base-pairing) of DNA are involved in DNA–protein binding in nucleosomes, on the basis of our observation of the faster reaction class of hydrogens in DNA.

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