11. Mandel, C., N. R. Kallenbach, and S. W. Englander. *J. Mol. Biol.*. In press.
12. Shigematsu, T., T. Enomoto, M. Yamada, M. Nakanishi, and M. Tsuboi. 1978. *Proc. Natl. Acad. Sci. U.S.A.* 75:4690–4693.

FLUCTUATIONAL OPENING-CLOSING REACTIONS IN DNA AND MONONUCLEOSOME CORES OF CHROMATIN PROBED BY H-EXCHANGE AND LIGAND BINDING REACTIONS

C. Mandal, S. W. Englander, P. R. Young, and N. R. Kallenbach, *Departments of Biology, Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104 U.S.A.*

The DNA double helix represents a dynamic structure in solution that undergoes a spectrum of conformational opening and closing reactions under conditions remote from onset of denaturation. Two kinds of opening reactions of the double helix are manifested by (*a*) the ability of base and solvent protons to exchange, and (*b*) the access of the duplex interior to intercalating agents such as ethidium bromide.

Exchange behavior of base ring and amino N-H hydrogens defines a significant transient open-state of the double helix (1, 2, 3) according to the following evidence. The ring N_3-H proton of uridine, seemingly the least accessible proton in an A·U base pair, is the fastest to exchange, while the two NH_2 amino protons of A are both slower and exchange from the poly (A)·poly(U) double helix at identical rates (only one is H-bonded). This behavior proves to result from the fact that exchange occurs only from transiently opened base pairs for which rates are determined by the intrinsic exchange chemistry of UN_3-H and A-NH_2 protons rather than by their relative exposure in the native, structured form (1, 2, 3).

The UN_3-H proton is easily removed directly by OH^- or by general base acceptors, it therefore displays rapid exchange. By contrast A-NH_2 protons exchange via a more complex, slower pathway involving an initial pre-protonation at AN_1 of the ring. This step acts to reduce the normally unfavorable pK of A-NH_2 and thus permit transfer to OH^- or other base catalysts. Since this pathway involves both H^+ and OH^- or H^+ and general base, the exchange rate appears pH-independent for the $H^+ \times OH^-$-mechanism or proportional to the concentration of buffer base for the $H^+ \times$ buffer base mechanism.

The different exchange chemistries of these protons have made it possible to distinguish their individual contributions to the overall H-exchange curves of a variety of homopolynucleotide duplexes. For example the 2 slower protons in the poly(rA)·poly(rU) H-exchange curve obviously represent the A-NH_2 protons, since they display catalysis by amine buffers that is proportional to the concentration of the acid form of the buffer catalyst and quantitatively similar to that found for the two protons per base in the acid poly(rA) structure (necessarily from A-NH_2) (1). The experimental results also make it evident that some kind of base-pair opening is necessary for any exchange to occur. The chemistry just described for A-NH_2 exchange involves protonation at the adenosine ring N(1) which is not possible in the normally base-paired condition. Also, the faster exchange of the internal proton points to opening.

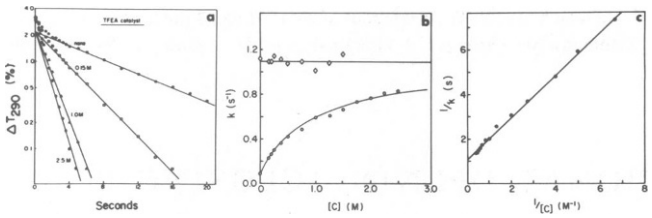

FIGURE 1

Several aspects of double helix dynamics are illustrated in Fig. 1. Fig. 1 *a* shows the time course of changes in optical absorbance that result when the A·U double helix in D_2O buffer is mixed into H_2O buffer containing varying amounts of the exchange catalyst trifluorethylamine (TFEA). The absorbance changes monitor H-D exchange of the nucleotide protons. The dependence of the slow phase (A-NH_2) on buffer concentration in hyperbolic, as demanded by an opening dependent exchange process (Fig. 1 *b*). The data are redrawn as a double reciprocal plot in Fig. 1 *c* according to a Lineweaver-Burke-like equation derived from an opening-dependent model (3).

Evidently all exchange is from the same opened form. The results in Fig. 1 show that the slower (A-NH_2) protons are catalyzed as TFEA is increased and approach the faster phase (U-NH) rate as a limit. Under all conditions, U-NH exchanges at what is apparently the opening-limiting rate, while A-NH_2 exchange follows a pre-equilibrium opening until it is catalyzed so strongly that it too approaches the identical opening-determined limit.

What is this open-state? The facts are these: (*a*) $\Delta G^\circ = +2$ kcal/mol, $\Delta H^\circ = +4$ to $+6$ kcal/mol, $\Delta S^\circ = +7$ to $+12$ e.u., (*b*) The opening rate is 1 s^{-1} at 20°C, $\Delta H^+ = +15$ kcal, (*c*) The closing rate is 20 s^{-1} at 20°C, $\Delta H^+ = +9$ kcal. These properties can be reconciled with a minimal localized swinging-out of one base pair which does not disrupt part of the stacking free energy of the helix. We believe the activation energies are high because of the difficulty of breaking hydrogen bonds without reforming them immediately to solvent.

The absorbance change accompanying H-D exchange of nucleobase protons has a maximum near 290 nm. H-exchange of aromatic amino acids in proteins can also be monitored at this wavelength; however, in mononucleosome core particles isolated from calf thymus nuclei the content of aromatic amino acids is negligible relative to the concentration of nucleotides. Thus the signal at 290 nm resulting from mixing cores in D_2O into H_2O reflects exchange of DNA protons. We have characterized the rate of exchange of the DNA bases within cores, as well as the catalysis by buffer base of this rate. Cores were prepared by micrococcal nuclease digestion of long chromatin following Lutter's procedure (4). The fastest (conformational) rate seen in the case of chromatin is faster by a factor of two than in free DNA, implying that the effect of folding the DNA within cores is to enhance the opening rate, k_{op}, in the same direction as would be expected on binding DNA to an unwinding protein (T. Schleich, personal communication). Catalysis of the amino hydrogens of DNA in the core will be described at the meeting; these data bear on the magnitude of k_{eq} and accessibility of the base to buffer-base species or OH^-.

The interaction between intercalating dye molecules and DNA defines a second kind of opening reaction of the double helix either in the free state or in the presence of histones. From analysis of the binding isotherms of ethidium bromide (EB) with DNA in high salt and in mononucleosome cores, it can be shown that a positively cooperative process exists in cores in contrast to free DNA. Kinetics of the EB interaction in high salt have been measured using

the absorbance changes of the drug itself to monitor binding and also by using H-D exchange of amino protons in EB, since binding to DNA inhibits this exchange. For the strong binding mode, the simplest model:

$$EB + DNA_{site} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} DNA\ (EB)$$

is consistent with data from both kinds of experiments; k_{-1} is 30 s^{-1} at 20°C 1 M NaCl, pH 7; k_1 is 10^6 M^{-1} s^{-1} where DNA sites are measured in units of 5P/site. The pseudo first order rate of occupying sites exceeds the hydrogen exchange opening rate by factors of 10 or more under typical experimental conditions. Thus intercalative opening does not require H-bond breakage. The change in intercalative opening caused by association of DNA in mononucleosome cores will be described at the meeting.

Supported by grants HL 21757 and CA 24101-01 from the National Institutes of Health and PCM 77-26740 from the National Science Foundation.

Received for publication 29 December 1979.

REFERENCES

1. Teitelbaum, H., and S. W. Englander. 1975. *J. Mol. Biol.* **92**:55–78.
2. Teitelbaum, H., and S. W. Englander. 1975. *J. Mol. Biol.* **92**:79–92.
3. Mandal, C., N. R. Kallenbach, and S. W. Englander. 1979. *J. Mol. Biol.* In press.
4. Lutter, L. 1978. *J. Mol. Biol.* **124**:391–420.

CONFORMATIONS AND CONFORMATIONAL DYNAMICS OF PROTEINS IN SOLUTION STUDIED BY NUCLEAR MAGNETIC DOUBLE RESONANCE

C. M. Dobson, J. C. Hoch, E. T. Olejniczak, and F. M. Poulsen, *Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138 U.S.A.*

Saturation of single resonances in the proton nuclear magnetic resonance (nmr) spectrum of a protein in solution results in changes in intensities of other resonances (Redfield and Gupta, 1971). These changes come about either through nuclear Overhauser effects which arise because of dipolar coupling between the protons in the molecule, or through cross-saturation effects which arise when the protein fluctuates between different well defined conformations. For lysozyme from hen egg white (mol. wt. ~14,500) the method reveals details of the protein structure and of individual atom fluctuations.

In Fig. 1 *a* a difference spectrum shows intensity changes of methyl group resonances which result from the saturation of the C(5)H resonance of Trp 28 in lysozyme. The resonances showing large effects have been assigned (Dobson, 1977; Poulsen, et al., 1980) and are from groups within 7Å of the Trp 28 C(5)H proton in the crystal structure. These observations show that the solution structure of lysozyme in the region of Trp 28 is close to that of the crystal structure because the magnitude of an Overhauser effect depends on the distance r_{ij} of a nucleus i from the saturated nucleus j.

Many similar Overhauser effects have been measured in the hydrophobic box region of