appear, in the main, to increase the proportion of molecules which are in the thermally unfolded state.

Carboxyhemoglobin dimers and tetramers, as well as oxyhemoglobin tetramers, had hydrogen exchange kinetics which followed Eq. 1 except that no thermal unfolding terms were experimentally discernible. The distribution functions are shown in Fig. 2. The effect of abolishing interdimer contacts in HbCO is to shift the distribution to the left, while preserving the shape of the distribution. Most simply, the dimer differs from the tetramer in that all exchangeable hydrogens are uniformly less masked in the dimer than in the tetramer. The nature of the ligand bound to the heme group seems to have more profound effects. Replacing the tightly bound CO with more loosely bound O_2 apparently moves the peak of the distribution to the left, but broadens the width of the distribution. Although HbCO and HbO_2 are crystallographically isomorphous, such that their static structures are very similar, these results apparently indicate that, in solution, the forms have important differences in their dynamical structures.

These results for lysozyme and hemoglobin illustrate the utility of the hydrogen exchange distribution function in separating and describing effects of various interactions which may occur within a protein and between a protein and constituents of its environment.

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HYDROGEN-DEUTERIUM EXCHANGE STUDIES OF PROTEINS AND NUCLEIC ACIDS

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A kinetic study of the hydrogen exchange reaction of a protein or a nucleic acid can provide useful information on the structure and fluctuation of such a biological macromolecule (1–3). However, reactions faster than 10 s are not easily traced by the methods used in earlier studies (2, 3). Recent introduction of stopped-flow ultraviolet absorption and emission spectrophotometry into the hydrogen exchange studies of proteins and nucleic acids promises great development in this field (4–11). By this new technique we can now explore the millisecond region of the time-scale of hydrogen exchange kinetics. In this discussion we demonstrate some new aspects of the dynamic properties of proteins and nucleic acids on the basis of our results obtained by the "stopped flow hydrogen exchange" method.

RESULTS AND DISCUSSION

A time-dependent ultraviolet absorption or emission intensity has been examined after rapid transfer of a sample from a light water medium into a heavy water medium. The samples

used were various tyrosine and tryptophan derivatives. It was shown that the deuteration rate of phenol OH of a tyrosine residue could be determined by both ultraviolet absorption and emission measurements. The deuteration rate of indole NH of a tryptophan residue, on the other hand, could be traced only by an absorption method. The deuteration of indole NH does not cause any change in the fluorescence intensity of tryptophan. By taking advantage of these facts, we can determine the deuteration rates of the tyrosine OH and tryptophan NH independently of each other even of a protein that has both tryptophan and tyrosine residues; tyrosine by fluorescence and tryptophan by absorption.

We have applied these methods to the hydrogen exchange studies of tyrosine and tryptophan residues of several proteins, including hen egg-white lysozyme (6), bovine pancreatic ribonuclease A (7), yeast 3-phosphoglycerate kinase (9), streptomyces subtilisin inhibitor, erabutoxin *b*, and heavy meromyosin.

Erabutoxin *b* is a neurotoxic protein containing 62 amino acids. Its "functionally invariant" tryptophan 29 was found to be exposed to the solvent ($k = 4.3\ s^{-1}$ at pH 6.4 and 34°C), while "structurally invariant" tyrosine 25 is buried in the molecule; its deuteration rate ($80\ s^{-1}$ at pH 6.3 and 33°C) is only 1/20 times as high as that of completely exposed tyrosine (in collaboration with Drs. N. Tamiya and C. Takasaki). In our hydrogen exchange study of tryptophan residues of heavy meromyosin and subfragment-1, it was suggested that the conformational change took place at the (S-1)-(S-2) hinge region in the course of the ATP hydrolysis (in collaboration with Drs. T. Yamada and H. Shimizu).

Hydrogen exchange reactions of nucleic acids can also be traced by a method similar to that described. We examined the exchange rate of several polynucleotides in their double helical and single-stranded forms and compared them with those of mononucleotides. We concluded that every double-helical polynucleotide has two types of structural fluctuation. One of them is predominant in the higher temperature range (close to its melting temperature) and the other in a lower temperature region. In the former a number of base-pairs are broken simultaneously and then reformed cooperatively ("overall" fluctuation), whereas in the latter only one or two base-pairs are occasionally broken and reformed ("localized" fluctuation).

Lastly, the deuteration of the base residues in calf thymus DNA, chromatin, and nucleosome were examined. In nucleosome the deuteration rate was found to be nearly equal to that of free DNA. Thus, the proteins in nucleosome do not seem to affect the fluctuation amplitude of DNA. This was found to be the case also for DNA plus helix-destabilizing protein (12).

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

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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.