# STRUCTURE DYNAMICS OF PROTEINS BY HYDROGEN EXCHANGE METHODS

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## INTRODUCTION

Hydrogen exchange data can monitor the kinetic behavior of all but the most rapidly exchanging protons. Our experiments with lysozyme showed that the rate constants could be characterized by the function

$$H(t) = b(1+at)^{-n}\exp(-ct), \quad (1)$$

where $H(t)$=hydrogens remaining per molecule at time $t$, and $b$ = maximum number of observable hydrogens. The power law term, $(1+at)^{-n}$, describes exchange from the native state of the protein, while the term $\exp(-ct)$ accounts for exchange from the thermably unfolded state. Eq. 1 can be transformed to yield a distribution function:

$$G(k) = ba^{-n}\exp(-(k-c)/a)k(k-c)^{n-1}/\Gamma(n), \quad (2)$$

which describes the probability of observing $H(t)$ governed by rate constants over the interval of $\log k$ to $\log k + d\log k$. As seen in Fig. 1, $G(k)$ is bimodal with the asymptotic behavior attributable to thermal unfolding.

In this communication we shall show the utility of such a distribution function.

## METHODS

Hydrogen exchange of lysozyme at 25°C was carried out as described earlier (1, 2). Hydrogen exchange experiments with hemoglobin were also carried out as in earlier publications (3, 4). Details of experimental conditions are given in the figure legends.

## RESULTS AND DISCUSSION

First, we shall report some results of lysozyme outexchange in the presence of trichloroethanol (TCE) or at 3,000 atm of pressure (Fig. 1). Addition of perturbant TCE and application of pressure to lysozyme both shift the rate at which thermal unfolding occurs and thus increase the importance of thermal unfolding pathway in outexchange. Cosolvent and pressure have much lesser effects on the native state of lysozyme. TCE broadens the distribution while pressure sharpens it. Nevertheless, the dynamic characteristics of the native state of lysozyme are essentially preserved in the presence of TCE or under high pressure. These perturbants

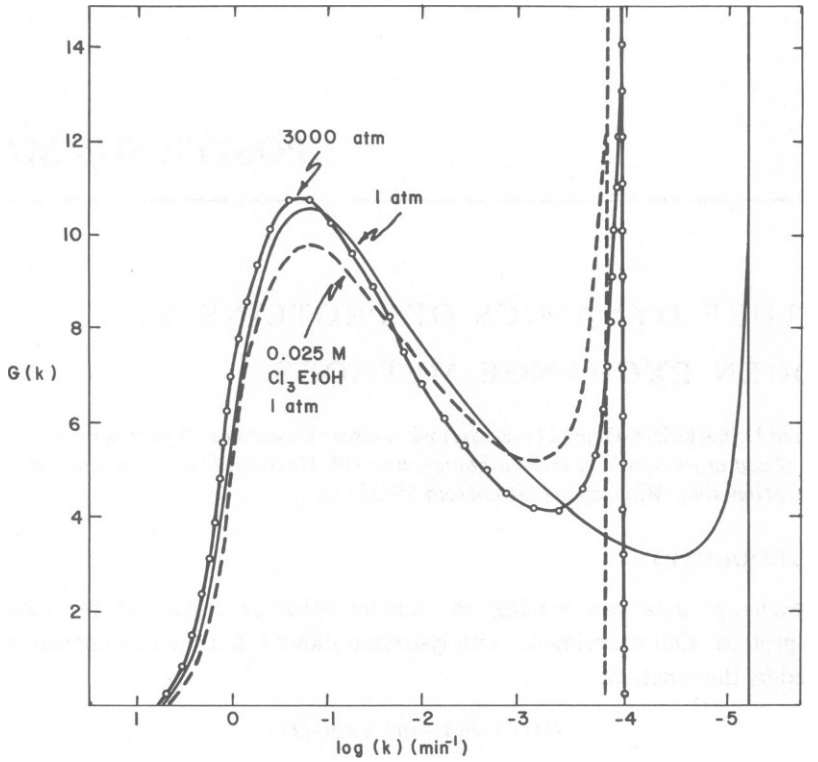


Figure 1 Hydrogen exchange distribution functions for lysozyme at 25°C, and pH 7.0. Lysozyme in 0.15 M NaCl (——) and in 0.15 M NaCl + 0.025 M trichloroethanol (- - - -). Data from reference 1. Lysozyme in 0.15 M NaCl at 3,000 atm pressure (-.-.-.). Data taken by Knox by methods described in reference 2.

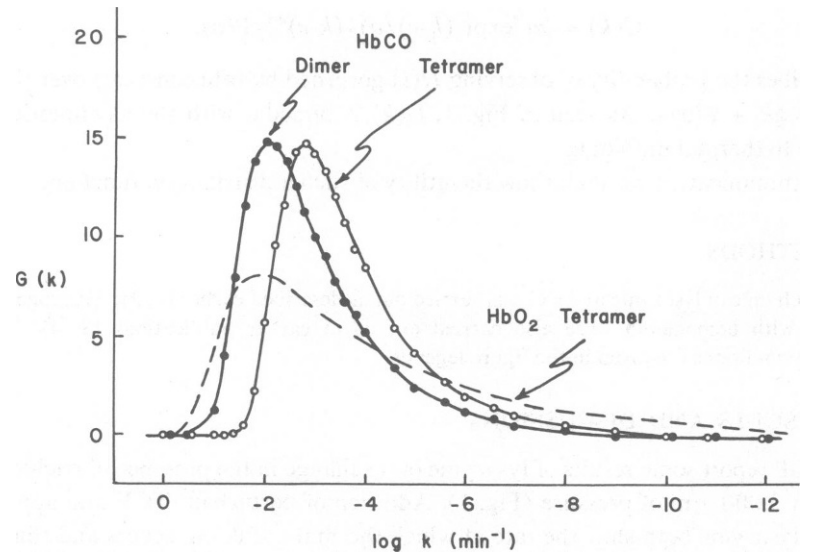


Figure 2 Hydrogen exchange distribution functions for human hemoglobin at 5°C, pH 6.5, in 0.1 M Na phosphate. Carboxyhemoglobin A at $5 \times 10^{-4}$ M (tetramer basis) (-0-0) and $1 \times 10^{-8}$ M (tetramer basis) (-●-●-). As documented in reference 3, at higher concentration, HbCO is >95% tetramer; at lower concentration >95% dimer. Data taken by methods in reference 3. Oxyhemoglobin at $1 \times 10^{-4}$ M (tetramer basis) (- - -), where $HbO_2$ is >90% tetrameric. Data from reference 4.

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.

## REFERENCES


1. Knox, D. G., and A. Rosenberg. 1980. Distribution of rate constants in hydrogen exchange kinetics of proteins. *Biopolymers.* **19**:1049–1057.
2. Carter, J. V., D. G. Knox, and A. Rosenberg. 1978. Pressure effects on folded proteins in solution. *J. Biol. Chem.* **253**:1947–1953.
3. Barksdale, A. D., and A. Rosenberg. 1978. Thermodynamic characterization of subunit association in liganded ferrohemoglobin. *J. Biol. Chem.* **253**:4881–4885.
4. Hedlund, B. E., P. E. Hallaway, B. E. Hallaway, E. S. Benson, and A. Rosenberg. 1978. Hydrogen exchange kinetics of human hemoglobins. *J. Biol. Chem.* **253**:3702–3707.


# 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