temperature and shows there is a sharp structural transition in the protein that occurs at 27°C. When the TNS binding is repeated at 30°C, $K_{diss} = 1.4 \times 10^{-4}$ M for E while all other binding parameters are the same as at 25°C.

These results are consistent with a picture of a mobile bilobed protein with an apolar active site region between two flexibly connected domains. Further, it is suggested that TST participates in catalytically obligatory conformational changes which include an "open-close" equilibrium between the two domains.

It is further proposed that the comparison between x-ray structural predictions and solution behavior can serve as a diagnostic for structural flexibility, when these comparisons are based on a number of experimental approaches.

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TIME DIFFERENTIAL PERTURBED ANGULAR CORRELATION MEASUREMENTS IN METAL ION COMPLEXED ATP

F. A. Smith and M. E. Phillips, *Physics Department, St. Bartholomew's Medical College, University of London, Charterhouse Square, London EC1M 6BQ, England*

INTRODUCTION

The perturbed angular correlation technique is being increasingly used to study the motion, conformation, or binding properties of biologically relevant molecules (1–3). It does, however, suffer because, except in a few cases, the P.A.C. nuclides are not directly involved in biological processes and the results therefore must be treated with caution. Nevertheless, it has been argued, for example, that a study of the binding of In ATP to 3-phosphoglycerate kinase (4) is not adversely affected by the replacement of Fe^{3+} by In^{3+}. In this work we wish to point out that whether or not a metal ion replacement is valid from a biological viewpoint, the interpretation of the results can be frustrated by the particular radiochemical properties of the probe nuclide. Our work has used ^{181}Hf and ^{57}Co bound to ATP in an attempt to separate effects due on the one hand to the rotational diffusion of the metal ion-ATP unit as a whole, and on the other to the aftereffects of the nuclear decay and any possible static quadrupole effects at the metal ion binding site. To do this successfully, complementary data also has to be obtained. Because of the difficulties associated with the interpretation of viscosity data on large molecules, i.e., the use of Debye theory to give the rotational correlation time and the measurement of viscosity itself in nonnewtonian fluids, we have used dielectric relaxation measurements to provide additional information on molecular rotation.

METHOD

A fast-slow coincidence apparatus was used with two Pilot U plastic scintillator detectors, having a time resolution of 1 ns and an energy resolution of ~25%. Time differential P.A.C. measurements were made on two metal ion-ATP systems at room temperature. The sample volumes were generally ~2 ml (which contained several hundred microcuries), and the shielded detectors were some 5 cm from the source. The finite source volume and the large solid angles subtended at the detectors made it necessary to correct the angular correlation coefficients for the effects of intrasource scattering and absorption (5). The aqueous samples were prepared by adding a 1.4-M solution of $HfOCl_2$ (Kodak ICN Pharmaceuticals Inc., Cleveland, Ohio) to a 0.5-M solution of disodium ATP (Grade 1, Sigma Chemical Co., St. Louis, Mo.) and adjusting the pH, using 1 N NaOH and 1 N HCl until the clear complex solutions appeared. Solutions with increased viscosity were obtained by adding polyacrylamide, and a PAA gel was obtained by using acrylamide monomer, bis-acrylamide, riboflavin and exposing it to intense ultraviolet light. The concentration ratio of metal ion to ATP was 1:1.7, ensuring that no free metal was available. Time domain dielectric relaxation measurements were made on similar samples over the frequency range 10–1000 MHz.

RESULTS

Due to the poor energy resolution of the plastic detectors, mixing of several γ-γ cascades occurred within the energy windows of the discriminators. In the case of the 133:482-keV cascade in ^{181}Ta after the β^- decay of ^{181}Hf, this mixing reduced the second-order angular correlation coefficient from -0.277 to -0.20. For the simpler 122:14-keV cascade in ^{57}Fe after the β^+ decay of ^{57}Co, the full theoretical coefficent, -0.0176, could be used. Because of the smaller angular correlation coefficient, the results obtained using ^{57}Co are less reliable than those with ^{181}Hf. However, for both Co and Hf in the presence of varying amounts of HCl, more attenuation is seen in the presence of low acid concentration than at high concentrations, and when bound to ATP, both metals show significant attenuation. By using the slow relaxation model of Marshall and Meares (6), it is possible to define limits within which the rotational diffusion time must lie, irrespective of the quadrupole interaction strengths, for a given experimental attenuation coefficient G. These limits are given in Table I and show that as the pH of the Hf ATP solution is increased from 7 to 9, the correlation times decrease. The

TABLE I

P.A.C. ATTENTUATION COEFFICIENTS IN ^{181}Hf AND ^{57}Co SOURCES

Source	$A_2(0)$	τ	A_2G_2	G_2	Range of τ_c
	(ns)		(∞)	(∞)	
$HfOCl_2$ in 9.6 M HCl	-0.202 ± 0.009	12.4 ± 0.8	-0.085	0.42	—
$HfOCl_2$ in 0.5 M HCl	-0.079 ± 0.025	7.6 ± 3.0	-0.023	0.11	—
$CoCl_2$ in 10 M HCl	—	—	-0.024	1.36	—
$CoCl_2$ in 0.1 M HCl	—	—	-0.003	0.16	—
Hf ATP pH 7	-0.028 ± 0.009	0.005 ± 0.008	-0.022	0.11	$2.10^{-9} - 2.10^{-8}$
pH 8	-0.019 ± 0.026	0.045 ± 0.065	-0.004	0.02	$3.10^{-10} - 2.10^{-9}$
pH 9	-0.001 ± 0.003	0 ± 0.04	-0.003	0.01	$2.10^{-10} - 1.10^{-9}$
Co ATP pH 7	—	—	-0.01	0.57	$>10^{-7}$
Hf ATP pH 8					
+ 1% PAA	—	—	0	—	—
+ 4% PAA	—	—	0	—	—
+ Gel	—	—	0	—	—

order of magnitude of these calculated times is confirmed by the dielectric relaxation data, which gives relaxation frequencies at ~200 and 700 MHz for Hf ATP at pH 9. If the attenuation of the angular correlation is due solely to time-dependent processes, the change with pH could imply either faster rotational diffusion or a speedier electronic rearrangement after the β-decay. On the addition of the viscosity increasing agents, up to 4% PAA and also a PAA gel, the attenuation coefficient goes to zero within the accuracy of the measurements. This result is incompatible with the expected change in the rotational motion when the viscosity is increased, and suggests that the strong attenuation is due to nuclear decay aftereffects. Dielectric relaxation data, however, show no significant change in the observed relaxation frequencies for Hf ATP at pH 9 upon the addition of 2% PAA; this might suggest the importance of group motion within the molecule. Angular correlation and dielectric relaxation measurements are continuing in these systems.

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DIFFRACTION ANALYSIS OF MOTION IN PROTEINS

Wayne A. Hendrickson and John H. Konnert, *Laboratory for the Structure of Matter, Naval Research Laboratory, Code 6030, Washington, D. C. 20375 U.S.A.*

BACKGROUND

Analysis of the x-ray or neutron diffraction from crystals gives only an average picture of the contents of a crystallographic unit cell, the average being over a long time (usually many hours) and the entire lattice. However, this picture can rather accurately define not only the mean atomic positions but also the distribution of displacements from rest positions. These displacements may include components from three main sources: (*a*) thermal vibration, both of individual atoms and of rigid groups, (*b*) dynamic disordering among thermally accessible conformational states, and (*c*) static variations among the structures within different unit cells, either due to lattice imperfections or because of conformational heterogeneity that is frozen in at the experimental temperature. Thus the accumulated spoor of atomic motions is accessible to the diffraction experiment even though the dynamic pathways are not.