PHYSICS AND RADIATION BIOLOGY

In-111 Transferrin Labeling Studied by Perturbed Angular Correlations

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The technique of perturbed angular correlations (P.A.C.) was used to study transferrin labeled with radioactive indium. Three forms of the labeled system were studied: a) the In-111 was bound to transferrin with no iron present at either of the binding sites; b) the In-111 probe was used to label transferrin that was 33% saturated with iron; and c) the label was attached to saturated Fe-transferrin. The measured perturbation factors, $G_{22}(t)$, indicate that the three binding states are clearly differentiated. Cases (a) and (b) exhibit characteristic features of time-independent quadrupole interactions, and the average quadrupole interaction frequencies, ω_Q, were determined in these cases.

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The method of perturbed angular correlations (P.A.C.) has been demonstrated as a labeling technique in the study of biologic macromolecules (*1,2*). In these studies, the coincidence counting rate, $W(\theta)$, of two gamma rays emitted in cascade from the attached radioactive probe is measured as a function of the angle θ between their directions of propagation. Following emission of the first gamma, the angular correlation, $W(\theta)$, may be strongly perturbed during the intermediate-state lifetime, τ_N, by the interaction of external fields with the nucleus. For example, the electric quadrupole moment Q of the intermediate state may interact with electric field gradients at the site of the nucleus. Measurements may be of the time-integral form, $W(\theta,\infty)$, in which the resolving time (2τ) of the coincidence circuit is much longer ($2\tau >> \tau_N$) than that of the intermediate-state lifetime (*3*), or they may be of the time-differential form, $W(\theta,t)$, for which $2\tau << \tau_N$ (*1,2*). The latter is the more powerful technique and can yield information on rotational correlation times, internal motions, and conformational changes of the macromolecules to which the probe is attached.

In the present studies, the time-differential technique was used to study the In-111-transferrin system, measurements being made on the 173 keV–247 keV cascade in Cd-111 which proceeds through an intermediate state of spin 5/2 and mean lifetime 122 nsec ($T_{1/2}$ = 85 ns). The perturbed angular correlation in this case can be shown (*4*) to be

$$W(\theta) = \frac{e^{-t/\tau_N}}{\tau_N}[1 + A_{22}G_{22}(t)P_2(\cos\theta)], \quad (1)$$

where $P_2(\cos\theta) = \frac{1}{2}(3\cos^2\theta - 1)$ is the Legendre polynomial, and $A_{22} = -0.18$. The perturbation factor $G_{22}(t)$, where t is the time interval between the emission of the two gamma photons, contains the information of interest, and in the unperturbed case $G_{22}(t) = 1$.

The form of $G_{22}(t)$ depends on the nature of the perturbing interactions and on the rate of molecular motion. Quadrupole interactions are expected to dominate in liquids, and for the case of rapid molecular motion (*4*), the result is

$$G_{22}(t) = e^{-\lambda_2 t}. \quad (2)$$

For the case of a nuclear spin I = 5/2 and an axially symmetric electric field gradient,

$$\lambda_2 = 2.8\,\omega_0^2\tau_c, \quad (3)$$

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where τ_c is the molecular rotational correlation time and ω_0 is related to the quadrupole interaction frequency, ω_Q, by $\omega_o = 6\omega_Q$ (*6*).

In the limit of slow molecular motion (*7*), $G_{22}(t)$ shows simple periodic behavior modified by an exponential,

$$G_{22}(t) = e^{-t/\tau_c} \sum_{n=0}^{3} a_n \cos(n\omega_0 t), \qquad (4)$$

where the coefficients a_n are given in reference (*7*).

Transferrin is a glyco-protein of molecular weight 77,000. It has two iron-binding sites, which are very similar chemically, but possibly not identical (*9*). These sites are not fully saturated with iron in normal blood. Transferrin binds Fe^{3+} very strongly in these sites, and our spectrophometric studies indicate that both In^{3+} and the In-111 daughter, Cd^{2+}, do in fact bind also at these sites. Both of the isotopes In-113m and In-111 have been used in studies of blood-pool (*10*) and tumor (*11*) scans.

The present measurements were undertaken in order to demonstrate the usefulness of P.A.C. in determining the nature of the probe's environment, or changes in the latter, in radioactive labeling studies. In addition, there was also interest as to which of the theoretical descriptions (*2,7*) suitably applied.

MATERIALS AND METHODS

The In-111 was obtained commercially as carrier-free $^{111}In\ Cl_3$; human apotransferrin was also obtained commercially. The transferrin samples were prepared in such a manner that predominantly three forms of the labeled system would occur, each differing in the nature of the binding sites of the In-111 probe.

All the samples were prepared in Tris-chloride buffer (0.1 *M*, pH 7.8) which contained 0.015 *M* bicarbonate. In the first sample (a), In-111 was added in the form of its citrate complex to apotransferrin. In this case binding at the vacant Fe sites was expected to be predominant. In the second sample (b), In-111 was added to unsaturated Fe-transferrin containing 33% Fe, the latter being close to the normal Fe content (~30%) of transferrin. In this case a mixture of In-Fe-transferrin/In-transferrin sites would be expected approximately in the ratio 2:1 (*9*). Here interest centered on whether the angular correlation would be significantly affected by the presence of the iron. Thirdly (c), a sample was prepared by adding In to transferrin saturated with Fe. In this case, the In binding is nonspecific; presumably it binds to carboxyl and other amino acid groups on the exterior of the molecule. While some degree of nonspecific binding might occur in cases (a) and (b), specific binding would be expected to predominate.

The determination of the perturbation factors $G_{22}(t)$ was accomplished by simultaneous measurement of the $W(\pi/2,t)$ and $W(\pi,t)$ correlations using the relationship

$$G_{22}(t) = \frac{2}{A_2} \frac{W(\pi,t) - W(\pi/2,t)}{W(\pi,t) + 2W(\pi/2,t)}.$$

A fast-slow coincidence system comprising three NaI(Tl) detectors (2- × 2-in. diam.) was used to record the $W(\pi/2,t)$ and $W(\pi,t)$ correlations in separate halves of a multichannel analyzer memory. The time resolution of this system with Co-60 gammas was 1.3 ns. Source strengths were typically of the order of 30 μCi, and individual runs about 12 hr in duration.

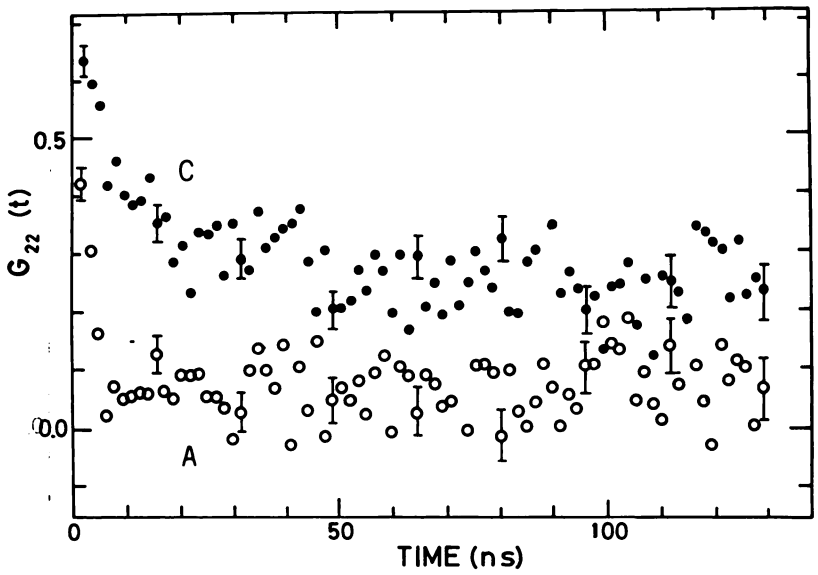

FIG. 1. Perturbation factors $G_{22}(t)$, for cases (a) (open circles) and (c) (nonspecific binding).

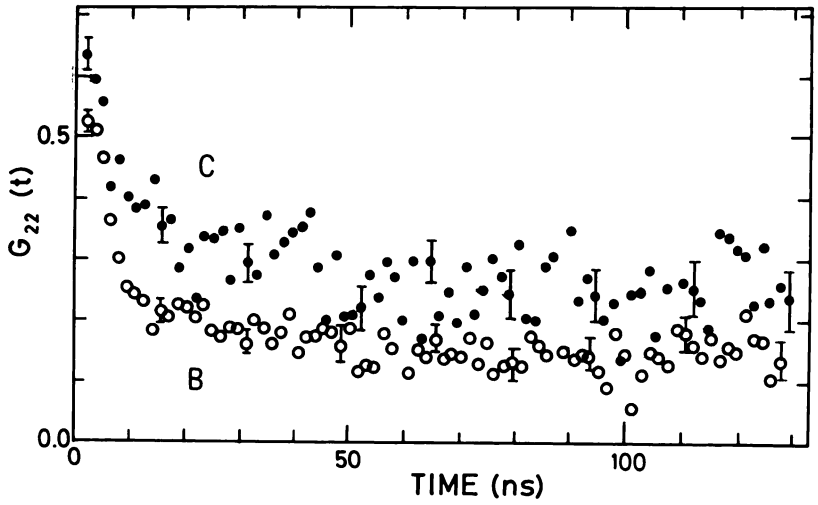

FIG. 2. Perturbation factors $G_{22}(t)$, for cases (b) (open circles) and (c) (nonspecific binding).

RESULTS AND DISCUSSION

The results comparing the three In-111-transferrin samples are shown in Figs. 1, 2, and 3. It can be seen that the correlations are differentiated according to the nature of the binding. In case (a) the angular correlation is more strongly perturbed, indicating a larger quadrupole coupling constant. The form of $G_{22}(t)$, particularly for case (a), is similar to that obtained in solids, or frozen solutions, and suggests that quasistatic quadrupole interactions (in which, however, $G_{22}(t)$ is *itself* time-dependent) are mainly involved.

After correction for the finite resolving time of the coincidence system, a theoretical fit to the measured perturbation factors was made using a function of the form

$$G_{22}(t)_{TH} = A + B\, G_{22}(t,\omega_o,\tau_c), \qquad (5)$$

where $G_{22}(t,\omega_o,\tau_c)$ is defined in Eq. 4. The value of chi-squared was minimized using A, B, ω_o and τ_c as variable parameters with the CERN program "MINUIT" (*12*). Figure 3 shows these theoretical fits to the data for cases (a) and (b) over the region of the time scale where $G_{22}(t)$ in Eq. 4 falls to a characteristic minimum followed by a secondary maximum. The latter is smeared out presumably by inhomogeneities in V_{zz} or by additional contributions from nonspecifically bound In-111. As shown in Figs. 1 and 2, the effects of smearing are particularly emphasized in the nonspecific case (c).

The fitted values obtained for ω_o and τ_c for each of the cases (a) and (b) were 46.3 ± 3.1 MHz, 10.0 ± 1.5 nsec, and 17.8 ± 1.0 MHz, 177^{+99}_{-134} ns, respectively. It should be remembered, however, that particularly in the case of (b), these represent averages over several interactions. With resolving times

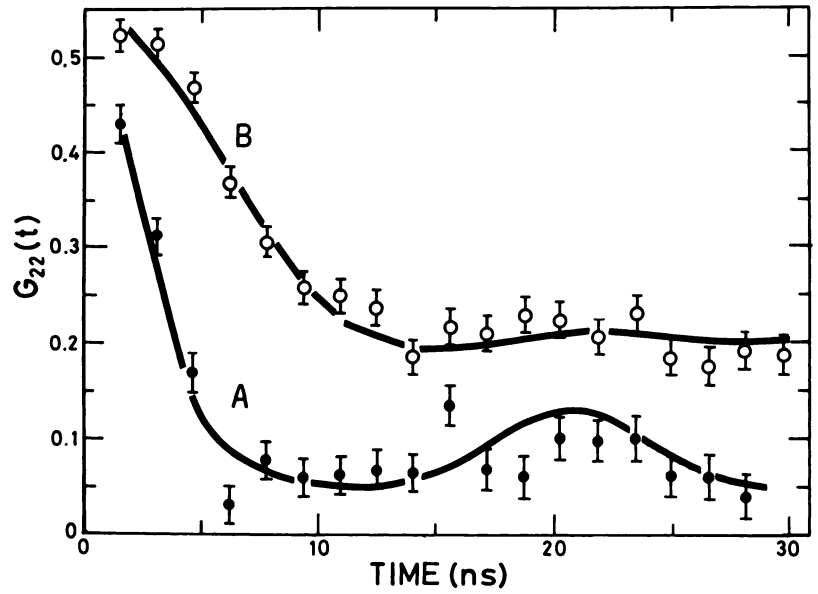

FIG. 3. Theoretical fits to cases (a) (lower curve) and (b). Fitted parameters are: for (a), $\omega_o = 46.3 \pm 3.1$ MHz, $\tau_c = 10.0 \pm 1.5$ ns; and for (b), $\omega_o = 17.8 \pm 1.0$ MHz, $\tau_c = 117 \pm^{99}_{134}$ nsec.

close to 1 nsec, it should be possible to determine quadrupole frequencies in the range 1 MHz $< \omega_0 <$ 1,000 MHz, whereas in selecting alternative labels, random coincidence rates place a practical upper limit of about 10 μsec for the intermediate-state lifetime.

It was clear that a theoretical curve of the type represented by Eq. 2 could not give a satisfactory fit to these data. It would be interesting to test the validity of Eq. 4 with well-defined specific binding over a wide range of correlation times and including parameters in the calculations to account for the effects of multiple interactions and of nonaxial symmetry in V_{zz}. With such a theoretical treatment it should be possible to determine quantitatively the percentages of the various interactions present.

P.A.C. has been shown here to be a viable method for determining the nature of the indium binding to the three types of transferrin, and because of the inherent high sensitivity (10^{-12} M) should prove a useful technique for examining other systems of biochemical or physiologic interest.

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REFERENCES

1. LEIPERT TK, BALDESCHWIELER JD, SHIRLEY DA: Applications of gamma ray angular correlations to the study of biological macromolecules in solution. *Nature* 220: 907–909, 1968

2. MEARES CF, BRYANT RG, BALDESCHWIELER JD, et al: Study of carbonic anhydrase using perturbed angular correlations of gamma radiation. *Proc Natl Acad Sci USA* 64: 1155–1161, 1969

3. GOODWIN DA, DIAMANTI CI, MEARES CF, et al: Simple modifications of the Anger positron camera for angular correlation measurements. *J Nucl Med* 15: 495, 1974 (Abst)

4. STEFFEN RM: Influence of the time-dependent quadrupole interaction on the directional correlation of the Cd^{111} gamma rays. *Phys Rev* 13: 116–125, 1956

5. ABRAGAM A, POUND RV: Influence of electric and magnetic fields on angular correlations. *Phys Rev* 92: 943–962, 1953

6. STEFFEN RM, FRAUNFELDER H: Influence of extranuclear fields on angular correlations. In *Perturbed Angular Correlations*, Karlsson E, Matthias E, Siegbahn K, eds. Amsterdam, North Holland Publishing Co, 1964, p 1

7. MARSHALL AG, MEARES CF: Effect of slow rotational diffusion on angular correlations. *J Chem Phys* 56: 1226–1229, 1972

8. DEBYE P: *Polar Molecules*. New York, Dover Publications, 1945, p 101

9. HARRIS DC: Different metal-binding properties of the two sites of human transferrin. *Biochemistry* 16: 560–564, 1977

10. STERN HS, GOODWIN DN, SCHEFFEL U, et al: In^{113m} for blood-pool and brain scanning. *Nucleonics* 25: 62–68, 1967

11. FARRER PA, SAHA GB, SHIBATA HN: Evaluation of ^{111}In-transferrin as a tumor scanning agent in humans. *J Nucl Med* 13: 429, 1972 (Abst)

12. JAMES F, ROOS M: Minuit—A system for function minimization analysis of the parameter errors and correlations. *Computer Physics Communications* 10: 343–367, 1975