A Novel Approach to Water Proton Relaxation in Paramagnetic Ion–Macromolecule Complexes†

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ABSTRACT: The use of the proton relaxation enhancement (PRE) technique in solutions of macromolecules and paramagnetic ions to obtain information on molecular motion and ion-hydration numbers can be hazardous with the possibility of large errors in the derived parameters. Here we describe a method based on solvent proton–deuteron comparative relaxation measurements at a limited number of different magnetic fields for obtaining, reasonably accurately, rapidly, and unambiguously, certain of the parameters normally obtained from a PRE study. In the non-immune rabbit immunoglobulin G(IgG)·Gd(III) system, we are able to obtain values for the ion hydration number (q), the residence lifetime of water molecules in the hydration sphere of the ion (τ_M) and a correlation time of 5 ns, which can be associated to a large part with a rotational correlation time (τ_R) for the Fc portion (C-terminal half of heavy-chain dimer) of the IgG molecule. This shows that there is rapid internal motion in the Fc region. The limitations and possible applications of the method are discussed.

Since its introduction in the early sixties (Eisinger et al., 1962, Cohn and Leigh, 1962) the proton relaxation enhancement (PRE[1]) technique—essentially the observation of an enhanced relaxation of solvent water protons in the hydration sphere of a paramagnetic ion as a result of the ion binding to a macromolecule—has received much attention from NMR spectroscopists working within biology (Dwek, 1973). The high concentration of water molecules in aqueous solution (55.6 M) makes the method an attractive starting point for the study of macromolecules. However, while the use of the method as a titration indicator to obtain binding data is straightforward (Dower et al., 1975), this cannot be said of its application in the field of macromolecular dynamics. Here the full potential of the method has undoubtedly not been realized. This is because analysis of relaxation data obtained at various frequencies and temperatures generally includes a best-fitting procedure involving a large number of variable parameters (at least six) so that errors in deduced values, although often not quoted, are expected to be large. In the specific case of the IgG·Gd(III) system, we have found large errors in many of the variable parameters; e.g., the metal ion coordination number, q, can take values between 2 and 8 with only small effect on the error of best-fit (Burton et al.,1977). Only under certain favorable conditions (Lanir et al., 1975) can the method be reliably applied to obtaining an ion-hydration number.

The weakness of the method in attempting to obtain information on molecular motion of water in the metal ion site, hydration number, etc. is, in simple terms, that it tries for too much: poorly defined values are obtained for a large number of parameters. Furthermore, some of the parameters are, for most purposes, of much greater interest than others. Clearly, what is required is a method that aims for better definition of a few carefully chosen parameters.

With this in mind, we have developed a method for investigating water-proton relaxation in paramagnetic ion macromolecule systems in a H_2O–D_2O solvent involving proton–deuteron comparative relaxation measurements. The idea of such measurements, in order to show fast exchange conditions for water protons in aqueous Gd(III) solutions, was originally proposed by Reuben (1975). Here we show how such measurements at a limited number of different magnetic fields can yield quite accurate values for the key parameters normally obtained from PRE studies. In the system chosen, the IgG·Gd(III) system, the molecular motion parameters cannot be well characterized by conventional PRE methods for the reasons discussed above. However, using the method described here, we are able to obtain accurate values for the ion coordination number (q), the proton exchange rate between bound site and bulk solvent (τ_M^{-1}), and a high-frequency correlation time (τ_c). The latter can be identified with a rotational correlation time, the magnitude of which shows that there is considerable rotational freedom in the Fc region of the immunoglobulin molecule. This may have important consequences for the mechanism by which the complement system of proteins is triggered.

Materials and Methods

Preparation of Solutions. The buffer used in all cases was 0.15 MNaCl, 0.05 M Pipes in 50% doubly distilled H_2O–50% D_2O adjusted to pH 5.5 with NaOH. Non-immune rabbit IgG was prepared as described previously (Dower et al., 1975). The concentration of IgG in solution was determined by both weighing and absorbance measurements ($\epsilon_{1cm}^{1mg/ml} = 1.4$ at 280 nm). The preparation of Gd(III) solutions and the use of silicone-treated NMR tubes with such solutions have been previously described (Dower et al., 1975). The concentration of Gd(III) in standard solutions was determined by weighing and checked by relaxation measurements as described (Burton et al., 1977). Protein and protein–Gd(III) solutions were frozen between measurements. La(III) solutions were prepared by simply dissolving $LaCl_3$ in Pipes buffer.

Relaxation Measurements. Proton and deuteron longitu-

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[1] Abbreviations used are: IgG, immunoglobulin G; Fc, C-terminal half of heavy-chain dimer; PRE, proton relaxation enhancement; NMR, nuclear magnetic resonance; Pipes, 1,4-piperazinediethanesulfonic acid.

dinal (T_1) and transverse (T_2) relaxation times were measured on a Bruker B-KR 322 s pulsed NMR spectrometer using homebuilt probes. T_1 measurements were made using a 180°-τ-90° pulse sequence and T_2 measurements by means of the Meiboom-Gill modification of the Carr-Purcell sequence. The temperature was controlled at 19 ± 1 °C. Proton and deuteron measurements were made on *the same solutions.*

Theory

The theory of water proton relaxation for aqueous solutions of paramagnetic ions and macromolecules has been extensively discussed (Dwek, 1973). For deuterons in similar solutions in D_2O there is the added possibility of quadrupolar relaxation. However, the theory for the paramagnetic contributions to the observed relaxation rates in the two cases is essentially the same. Only the theory necessary for an understanding of the significance of proton-deuteron comparative measurements is presented below.

The normalized paramagnetic contribution, $1/P_M T_{iB}$, to the observed water proton relaxation rates for water in the first hydration sphere of a paramagnetic ion bound to a macromolecule is given by (Dwek, 1973):

$$\frac{1}{P_M T_{iB}} = \frac{q}{T_{iM} + \tau_M} \quad i = 1,2 \tag{1}$$

where P_M is a normalizing factor = N/55.6 (N is the molar concentration of paramagnetic ion: 55.6 the molar concentration of water); q is the hydration number of the paramagnetic ion (more correctly, the number of water molecules exchanging between the ion and bulk solvent sufficiently quickly to be detectable on the NMR time scale); and τ_M is the lifetime of water molecules in the bound site.

Considering longitudinal relaxation rates and using a simplified Solomon-Bloembergen expression for T_{1M} (Burton et al., to be published; Dwek, 1973) eq 1 can be written as:

$$P_M T_{1B} = \frac{15}{6}\left(\frac{r^6}{q\gamma_I^2 g^2 \beta^2 S(S+1)}\right)\left(\frac{1+\omega^2\tau_c^2}{\omega^2\tau_c}\right) B^2 + \frac{\tau_M}{q} \tag{2}$$

where r is the ion-proton distance; ω is the proton Larmor precession frequency; τ_c is a correlation time characterizing the dipole-dipole interaction between paramagnetic ion electron and water proton spins, B is the magnetic field; γ_I is the nuclear magnetogyric ratio; g is the electronic g factor; S is the electron-spin quantum number, and β is the Bohr magneton. The simplified expression for T_{1M} assumes that the terms arising from the modulation of the scalar interaction and those terms involving the transverse electron-spin relaxation time can be neglected. These assumptions are justified specifically for this system elsewhere (Burton et al., 1977) but it is expected they will hold in the great majority of paramagnetic ion-macromolecule systems.

Equation 2 also yields the paramagnetic contribution to deuteron relaxation rates for solutions in D_2O, instead of H_2O. For proton and deuteron paramagnetic rates at the same magnetic field, the only factor differing in eq 2 in the two cases is ω. Determination of both proton and deuteron paramagnetic contribution to the relaxation rates at the same field (B_1) thus involves two equations and three unknowns, namely, q, τ_c, and τ_M (it is assumed that a value of r is available, e.g., from crystallography). If we suppose that the value of τ_c varies with magnetic field, then the determination of both proton and deuteron rates at a different field (B_2) can only introduce one new unknown—a new τ_c—while introducing two new equations. The resulting set of four simultaneous equations can then be solved to yield values for the four unknowns q, τ_M, $\tau_c(B_1)$, and $\tau_c(B_2)$. In certain limiting cases, not all of these parameters can be obtained. These will be discussed later.

Practically, the problem is then one of determining the paramagnetic contribution to the observed relaxation rates. For proton rates, this problem has been discussed elsewhere (Dwek, 1973) and only the necessary equations are presented here without comment:

$$\frac{1}{T_{1P}} = \frac{1 - X_B}{T_{1F}} + \frac{X_B}{T_{1B}} \tag{3}$$

$$\frac{1}{T_{1P}} = \frac{1}{T_{1(obsd)}^*} - \frac{1}{T_{1(0)}^*} \tag{4}$$

$$\frac{1}{T_{1F}} = \frac{1}{T_{1(obsd)}} - \frac{1}{T_{1(0)}} \tag{5}$$

where $1/T_{1P}$ is the overall paramagnetic contribution to the water proton relaxation rate, X_B is the fraction of metal ion bound to the protein, $1/T_{1F}$ the contribution from water bound to the free metal ion, $1/T_{1(obsd)}^*$ and $1/T_{1(obsd)}$ the observed relaxation rates for metal ion-macromolecule and metal ion solutions, respectively, and, finally, $1/T_{1(0)}^*$ and $1/T_{1(0)}$ the corresponding rates in the absence of metal ion.

For deuterons, which possess a quadrupole moment, there is the possibility of quadrupolar relaxation in the hydration sphere of the metal ion. $1/T_{1(0)}^*$ should thus be obtained from a solution containing macromolecule and a suitable nonparamagnetic metal ion, so that a similar quantity of macromolecule-metal ion complex exists in solution as in the paramagnetic ion case. For Gd(III), the obvious choice is La(III) but, as will be seen later, the macromolecule on its own is, in fact, a sufficient blank solution. A contribution from quadrupolar relaxation to ^2H relaxation rates in simple metal ion solutions is not expected as extreme narrowing ($\omega_I^2\tau_c^2 \ll 1$) conditions are then satisfied.

A similar analysis to the above is also possible using transverse relaxation rates, although the analogous equation to eq 2 is then more complex and not always soluble. This plus the greater inherent error present in T_2 measurements mean that the method is far better adapted to the use of T_1 data.

Results and Discussion

It has been shown (Dower et al., 1975) that IgG has six Gd(III) binding sites: two equivalent tightly binding sites on the Fc portion of the molecule (dissociation constant $K_d \sim 6\mu M$) and four weak sites on the Fab arms of the molecule ($K_d \sim 140\mu M$). Experimental conditions could thus be arranged in this study so that in IgG-Gd(III) solutions approximately 90% of the Gd(III) was bound to IgG and the binding to the weak sites was negligible in comparison to that at the tight sites. Hence, information deduced from experimental data pertains to the tight sites of the Fc region.

Before collecting data, it was necessary to carry out two control experiments. In the first, ^1H and ^2H relaxation times for a solution of IgG and Gd(III) were measured at the same field (1.45 T) varying the ratio of H_2O to D_2O in the buffer. Only small variations in T_1 values (<10%) were observed: large variations would clearly subject the whole method to question. A H_2O-D_2O composition of 1:1 was eventually chosen for all experiments, as an increased D_2O content appeared to have adverse effects on IgG solubility. In the second control experiment, ^2H relaxation times for a solution of IgG were measured at 9.36 MHz as La(III) up to 10 mM was titrated

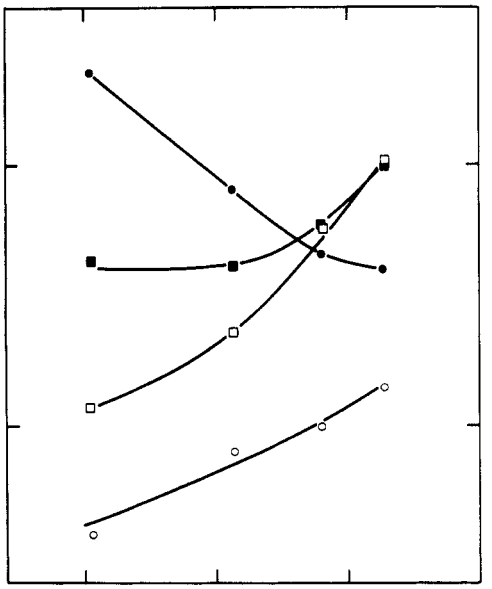

FIGURE 1: The magnetic-field dependence of the normalized solvent proton and deuteron relaxation rates in the IgG·Gd(III) complex. $1/P_M T_{1B}$ values are denoted by closed symbols: (●) ($1/P_M T_{1B}$) proton; (■) ($1/P_M T_{1B}$) deuteron. $1/P_M T_{2B}$ values are denoted by open symbols: (○) ($1/P_M T_{2B}$) proton; (□) ($1/P_M T_{2B}$) deuteron. The continuous lines are drawn simply to make trends more obvious: no further significance should be attached to them. The experimental conditions were: IgG concentration, 0.28 mM; Gd(III) concentration, 0.49 mM; NaCl concentration, 0.15 M; Pipes buffer, 0.05 M in D_2O–H_2O in ratio 1:1; pH 5.5; temperature, 19 ± 1 °C.

TABLE I: Parameters for Water Protons in the Hydration Sphere of the IgG·Gd(III) Complex.[a]

	τ_c (84 MHz) (ns)	τ_c (61 MHz) (ns)	τ_c (47 MHz) (ns)	τ_c (37 MHz) (ns)	τ_M (ns)	q
solution 1	4.3	2.4			463	4.11
	4.5		1.2		389	3.94
	4.6			0.60	359	3.88
		3.9	1.9		455	4.81
		3.9		1.0	428	4.70
			3.6	1.8	654	7.25
solution 2	4.2	2.2			494	4.22
	4.4		1.1		406	4.02
	4.7			0.50	305	3.80
		3.7	1.8		463	4.79
		4.2		0.9	330	4.22
			4.5	2.0	383	5.24
Conventional PRE	5.6	4.3	3.3	2.5	230	4.5

[a] Each set of parameters was obtained by combining proton and deuteron T_1 measurements at two different fields as reflected in the inclusion of two τ_c's in each instance. The τ_c's are referred to proton resonance frequencies. The results from two solutions are shown, prepared from different IgG batches and Gd(III) solutions. Solution 1 is the one represented in Figure 1. r was taken as 0.309 nm (Reuben, 1971). Also shown for comparison are the best-fit values obtained from a PRE analysis—the errors in these values are, however, expected to be large (Burton et al., to be published).

into the solution (compare Gd(III) concentration of 0.5 mM used in all experiments). No change in the relaxation times was observed. Even allowing for possible weaker binding of La(III) as compared to Gd(III), it is clear that quadrupole relaxation in the hydration sphere of the metal ion does not contribute significantly to the observed relaxation rates. Hence, IgG on its own is a sufficient "blank" solution, as discussed earlier (and see eq 4).

The results of proton and deuteron longitudinal and transverse relaxation measurements at four different magnetic field strengths are presented in Figure 1. The sets of T_1 measurements then yield the values for q, τ_c, and τ_M presented in Table I.

The field dependence of solvent proton relaxation rates in the IgG·Gd(III) system is discussed in detail elsewhere (Burton et al., 1977) and the parameters given in Table I largely confirm the interpretation presented in that reference. However, before discussing in detail the derived parameters, it is instructive to consider the main features of Figure 1, especially in terms of a proton–deuteron relaxation behavior comparison.

The deuteron rates are, generally, roughly an order of magnitude less than the corresponding proton rates, an observation which can be traced back to the lower value of ω for ^2H as compared to ^1H at the same magnetic field (see eq 2). From the magnitude of τ_M in Table I, it can be deduced that the leveling off in the proton transverse relaxation rate at high field arises from this rate passing into slow exchange conditions ($\tau_M \sim T_{2M}$, compare eq 1). For the corresponding deuteron rates, a large experimental error is unfortunately expected (~20%) so that care is necessary in describing any trends. However, it seems likely that the rate increases with increasing field in roughly the manner expected for fast-exchange conditions for the deuteron rates. From the magnitude of τ_M, fast-exchange conditions ($\tau_M \ll T_{1M}$) are also anticipated for both proton and deuteron longitudinal relaxation rates. These have an opposite field dependence because for the proton rates $\omega_I^2\tau_c^2 > 1$ over the magnetic field range studied, whereas for the deuteron rates $\omega_I^2\tau_c^2 < 1$. In both cases, although at different ends of the range studies, a leveling off in the rates is observed as the region $\omega_I^2\tau_c^2 \sim 1$ is approached.

The above interpretation is neatly confirmed by the temperature dependence at constant field presented in Figure 2. This can be understood from page 201 of Dwek (1973). Briefly, both proton and deuteron longitudinal relaxation rates are in fast exchange but have opposite temperature dependences, since $\omega_H^2\tau_c^2 > 1$, whereas $\omega_D^2\tau_c^2 < 1$ at this field. The proton transverse relaxation rate has a negative temperature dependence because of a dominant slow-exchange contribution ($\tau_M \sim T_{2M}$), whereas the corresponding deuteron rate has the opposite dependence, since fast exchange conditions ($\tau_M \ll T_{2M}$) are then prevalent.

Table I shows good consistency in the derived parameters except for the lower frequency τ_c values and all values obtained by combining the data at the lowest fields. It can be easily shown that values derived by *combining* the lower-field data are much more sensitive to error in the measured relaxation times (where indeed such error is expected to be largest) and that this is a consequence of a dominant slow-exchange contribution to the proton rates at the lower-proton frequencies. The best-defined correlation time is τ_c (84 MHz) with a value of approximately 4–5 ns. We have adopted two approaches for estimating q and τ_M. If the data from the lowest field is excluded, then q is obtained to be 4.4 ± 0.2 and τ_M 455 ± 30 ns. Alternatively, if data obtained from the highest field is combined with that from the lowest and errors of 5% produced in the measured proton rates and 10% in the deuteron rates (experimental errors are expected to be of this order) in such a way that error in the derived parameters is maximized then q is

obtained as 4.1 ± 0.4 and τ_M as 384 ± 60 ns. τ_c (84 MHz) can be similarly obtained as 4.5 ± 0.8 ns. Uncertainty within the crystallographic limits (Reuben, 1971) produces further error in q of the order of ±10%. Such procedures, of course, give only a rough estimate of the errors involved but do succeed in showing that these are relatively small.

A detailed PRE analysis yields a best-fit value for τ_R in this system of 9 ns (Burton et al., 1977), indicating the possibility of considerable rotational motion in the Fc region of the molecule, since a value of 168 ns has been obtained for rotation of the whole IgG molecule (Yguerabide et al., 1970). However, care must be taken in drawing firm conclusions from the PRE analysis as large errors in the value of τ_R could arise from the interdependence of the many parameters. Thus, we have shown that, although the *best* fit value of τ_R is 9 ns, the error estimations only allow a definition of τ_R in the range 0.4 to 40 ns (Burton et al., 1977). A coupling of the conventional analysis with the results presented here considerably reduces this uncertainty in τ_R. At 84 MHz, the best-fit fractional contribution of τ_R to τ_c is 0.65 (Burton et al., 1977)—the remaining contribution coming from the electron-spin relaxation rate τ_S. Using this fractional contribution with the present results for τ_c at 84 MHz then gives τ_R as 7 ns. The good definition of τ_M, q, and τ_c obtained from this method when compared with the data from a conventional PRE analysis then allows a much better estimation of τ_R as within the range of 5–10 ns. (The range arises from uncertainty in the electron-spin lattice relaxation time, τ_S, which has a best-fit value of 15 ns at 84 MHz but could be as low as 9 ns (Burton et al., 1977).) We are thus able to support the findings of Huber et al. (1976) from X-ray crystallographic studies of IgG and provide direct evidence for the existence of rapid internal motion of the Fc position of the IgG molecule in solution.

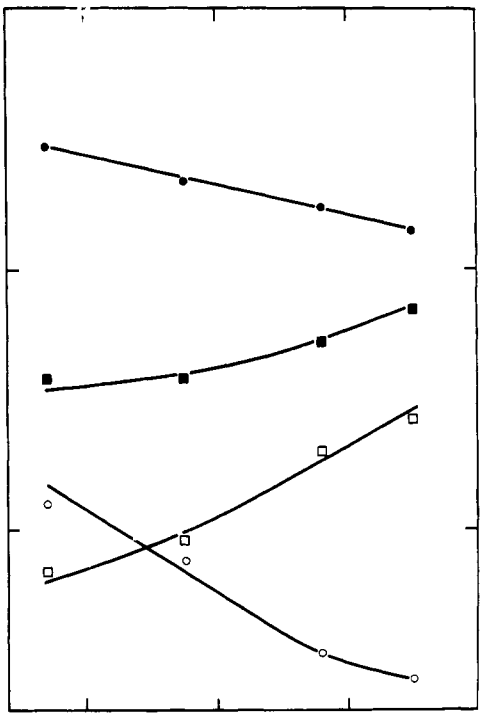

FIGURE 2: The temperature dependence of the normalized solvent proton and deuteron relaxation rates in the IgG·Gd(III) complex at a magnetic field of 1.97 T. Designations and conditions as in Figure 1.

Conclusion

In this work, we have clearly demonstrated how proton-deuteron comparative relaxation measurements can simply yield values for ion hydration number and molecular motion parameters in paramagnetic ion macromolecule solutions. As compared to the conventional application of PRE, the method is much simpler to apply (the equations can be solved exactly instead of the use of a multiparameter best-fitting procedure), the errors in the derived parameters are generally expected to be much smaller, and the amount of data required for unambiguous interpretation is much less with a consequent saving in time and energy. Where it can be applied, the method should thus be one of choice with the possibility of further PRE investigation if information on such parameters as the electron-spin relaxation time of the paramagnetic ion (τ_S) is required. A possible exception here is the determination of q, if fast exchange conditions are known to apply to the observed relaxation rates when the method of Navon (1970) can give an accurate estimate of this parameter. It is then appropriate to consider the situations in which the method cannot be applied or in which severe limitations are placed on it.

As was seen earlier, a very dominant slow exchange contribution to one of the relaxation rates (proton) can lead to large uncertainties in the derived parameters. In the case of no slow exchange contribution to any of the rates, no information on the value of τ_M can be obtained (apart from a lower limit). Generally, the condition $\omega_D^2\tau_c^2 \ll 1$ will apply for the frequencies at which deuteron rates are measured on a normal pulsed NMR spectrometer, whereas $\omega_H^2\tau_c^2$ can be greater or less than one. If $\omega_H^2\tau_c^2 \ll 1$ for all measured proton rates, then ω will disappear from eq 2 and again large uncertainties in the derived parameters can arise.

A practical limitation of the method arises out of the fact that it might be necessary to use quite high concentrations of paramagnetic ion before a measurable paramagnetic contribution to the ^2H rates is obtained. If this condition, coupled with the limited solubility of the macromolecule, leads to experimental conditions in which only a very small amount of the total metal ion in solution is bound to the macromolecule, then uncertainties in K_d for the metal can lead to large errors in T_{1B} values (see eq 3). This problem is more acute for ^2H than ^1H rates because of the higher intrinsic relaxation rate of D_2O compared to H_2O and the lower paramagnetic rates expected generally for D_2O compared to H_2O.

It should also be noted that for the metal ion used in this study, Gd(III), it is known that the theoretically expected ratio of proton–deuteron rates is observed in aqueous Gd(III) solutions (Reuben, 1975). If the method is to be applied to other paramagnetic ions, then their behavior in aqueous solution without the presence of macromolecule should first be investigated.

The analysis presented here assumes that a number of variables are left unchanged when the water protons are replaced by deuterons. Solid-state studies indicate that isotopic substitution should have a negligible effect on r (Hamilton and Ibers, 1968). No isotope effect is anticipated if whole H_2O, D_2O, or HDO molecules are involved in exchange from the metal ion to the bulk medium. Problems could arise if protons or deuterons rather than H_2O, D_2O, or HDO molecules are involved in the rate-determining step for the transfer of magnetization from the metal ion coordination sphere to the bulk medium. This would seem unlikely, however, as water is known to bind comparatively loosely to the Gd(III) ion (Reuben, 1975; Eigen and De Maeyer, 1963). Measurements using ^{17}O nmr would help to confirm this point. The additional effect of the quadrupole moment of ^2H has already been discussed.

Finally, we stress that using the method described we have been able to obtain simply and quickly, reliable molecular-motion parameters for the IgG·Gd(III) complex and thereby provide evidence for rapid motion in the Fc portion of the IgG molecule. The proposal of Huber et al. (1976) that the inherently flexible antibody molecules become rigid upon interaction can now be investigated by this method in an appropriate antibody–antigen system. This could be the "trigger" for complement activation and clearly a definitive answer to this must come from a technique monitoring changes in solution—as is the case here.

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Role of Hydrophobic Forces in Membrane Protein Asymmetry†

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ABSTRACT: M13 virus coat protein is an integral cytoplasmic membrane protein at all stages of viral infection. The pure virus coat protein can also be incorporated into synthetic lecithin vesicles near the lipid-phase transition temperature (T_m), spanning the bilayer with its N terminus exposed on the outside and its C-terminus inside (Wickner, W. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 1159–1163). The assembly of coat protein into vesicles in this asymmetric fashion has a sharp maximum near the phase-transition temperature of the lipid fatty acyl chains. At temperatures well below the T_m, coat protein assembles into lecithin vesicles with both termini exposed on the exterior vesicle surface. In contrast to this important role of lipid physical state, asymmetry of assembly is unaffected by parameters which govern polar interactions such as pH, ionic strength, lipid polar head group, or prior proteolytic removal of either the N or C terminus. It is proposed that the orientation of this membrane protein, and perhaps others, is determined by interactions of the hydrophobic portion of the protein with the lipid hydrocarbon core.

Asymmetric orientation is a characteristic feature of membrane proteins (Singer, 1974). The question of how this is established is at the heart of understanding membrane assembly. Once established, asymmetry is maintained by the low rates of diffusion of protein polar faces across the hydrocarbon core. This fact underscores a second question: How can proteins be assembled into a conformation which spans the bilayer?

The (gene 8) coat protein of the filamentous coliphage M13 offers several advantages for studying these problems. It is composed of fifty amino acid residues (Asbeck et al., 1969; Nakashima and Konigsberg, 1974) with a central hydrophobic region. Both the coat protein from infecting virus and that newly synthesized by the infected cell are integral components of the cytoplasmic membrane (Trenkner et al., 1967; Smilowitz et al., 1972; Marco et al., 1974; Smilowitz, 1974); both coat the progeny DNA as it extrudes through the membrane (Smilowitz, 1974). During infection, coat protein accounts for up to a third of the membrane protein synthesis (Smilowitz et al., 1972) and each coat protein molecule has its acidic N terminus exposed on the membrane outer surface (Wickner, 1975; Wickner, 1976). It has been proposed that the basic C terminus of coat protein is on the inner surface of the cytoplasmic membrane and is involved in conducting DNA through the lipid bilayer (Marvin and Wachtel, 1975).

In a previous communication (Wickner, 1976), coat protein was found to assemble into lecithin vesicles prepared by the cholate-dilution technique of Racker et al. (1975). In these experiments, clear, nonsedimentable solutions of cholate, lipid,

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