Moon, R. B., and Richards, J. H. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2193.
Sasisekharan, V. (1959), *Acta Crystallogr. 12*, 903.
Schaefer, J. (1972), *Macromolecules 5*, 427.
Solomon, I. (1955), *Phys. Rev. 99*, 559.
Torchia, D. A. (1972), *Macromolecules 5*, 566.
Veis, A. (1964), The Macromolecular Chemistry of Gelatin, New York, N. Y., Academic Press, p 4.
von Hippel, P. H., and Schleich, T. (1969), *Accounts Chem. Res. 2*, 257.
von Hippel, P. H., and Wong, K.-Y. (1962), *Biochemistry 1*, 664.
von Hippel, P. H., and Wong, K.-Y. (1963a), *Biochemistry 2*, 1387.
von Hippel, P. H., and Wong, K.-Y. (1963b), *Biochemistry 2*, 1399.
Wallach, D. (1967), *J. Chem. Phys. 47*, 5258.
Woessner, D. E. (1962), *J. Chem. Phys. 36*, 1.
Yguerabide, J., Epstein, H. F., and Stryer, L. (1960), *J. Mol. Biol. 51*, 573.

Carbon Monoxide Binding by Simple Heme Proteins under Photodissociating Conditions†

Celia Bonaventura,*‡ Joseph Bonaventura,‡ Eraldo Antonini, Maurizio Brunori, and Jeffries Wyman

ABSTRACT: This paper reports the binding of CO in the presence of photodissociating light by several simple heme proteins: sperm-whale myoglobin, *Aplysia* myoglobin, horseradish peroxidase, *Chironomus* hemoglobin, isolated α and β chains of human hemoglobin A, and *Gastrophilus* hemoglobin. In the presence of light, the CO binding curve maintains the shape of the simple (noncooperative) titration observed in the "dark." The apparent dissociation constant, given by $c_{1/2}$, shifts as a linear function of light intensity. Unfiltered light from the 150-W xenon arc increases the value of $c_{1/2}$ by about 1000-fold for sperm-whale myoglobin. The kinetics of approach to the steady state when the light is turned on and the return to equilibrium when it is turned off conforms to a simple one-step process. In relaxation experiments involving small perturbations of the steady state, the relaxation is linear in the sum of the concentrations of the reactants ($[\overline{Fe}]$ and $[\overline{CO}]$) for any given light intensity, which agrees with the idea that the CO combination rate constant (l') is unaffected by light. The value of $1\tau/\text{at}$ $([\overline{Fe}] + [\overline{CO}]) = 0$ is a linear function of light intensity, as expected on simple grounds. Steady-state and kinetic measurements permit the calculation of the quantum yield for CO photodissociation of the various hemoproteins (relative to sperm-whale myoglobin). Such calculations show that the quantum yield is temperature independent. Studies of the effect of temperature on the steady state and kinetics of the process are fully consistent with the hypothesis that photodissociation acts in addition to, and independently of, the dissociation process which occurs in the dark (l). Thus, the effective dissociation rate constant in the presence of light is given by $l + \omega I$, where ωI denotes the photochemical dissociation.

The photosensitivity of the carbon monoxide derivative of hemoglobin was originally reported by Haldane and Lorrain-Smith in 1895. The observations were extended to other hemoproteins by Warburg and collaborators (1949), who were the first to put the phenomenon on a quantitative basis. Since then it has been the subject of experiments by a variety of authors (Bucher and Kaspers, 1947; Gibson, 1956; Ainsworth and Gibson, 1957; Noble *et al.*, 1967; Brunori *et al.*, 1972). In such studies, values of the quantum yield were obtained by one of three types of measurements: (i) determination of the amount of ligand liberated by a pulse of light of known intensity; (ii) comparison of the relaxation time for the transition from equilibrium to the steady state produced by light with the relaxation time for the reverse process, either in the presence of one ligand (*e.g.*, CO) or of two ligands with different quantum yields (*e.g.*, CO and O_2); (iii) observations of the displacement of the ligand binding curve under the influence of a steady light.

The present study represents an extension of previously reported experiments (Brunori *et al.*, 1972) to the case of several "simple," one-site hemoproteins, namely: sperm-whale myoglobin, *Aplysia* myoglobin, horseradish peroxidase, *Chironomus* hemoglobin, isolated α and β chains of human hemoglobin A, and *Gastrophilus* hemoglobin. In these experiments we have made use of methods ii and iii, and have included an investigation of the effect of temperature.

The action of light is interpreted as affecting only the dissociation velocity constant in accordance with the simple scheme

$$\mathrm{Fe} + \mathrm{CO} \underset{l+\omega I}{\overset{l'}{\rightleftharpoons}} \mathrm{FeCO} \qquad (1)$$

where l' and l are the combination and dissociation velocity constants operative in the dark, I is the light intensity, and ω is a proportionality constant. According to this, the pseudo-equilibrium constant, L, for the steady state produced by light

† From the Center of Molecular Biology of the C.N.R., Institutes of Biochemistry of the Universities of Rome and Camerino, and the Regina Elena Institute, Rome, Italy. *Received November 22, 1972.* A grant from the National Science Foundation to J. W. is gratefully acknowledged. J. B. was supported by American Cancer Society Grant PF-627, C. B. by an EMBO Long Term Fellowship.

‡ Present address: Duke University, Marine Laboratory, Beaufort, N. C. 28516.

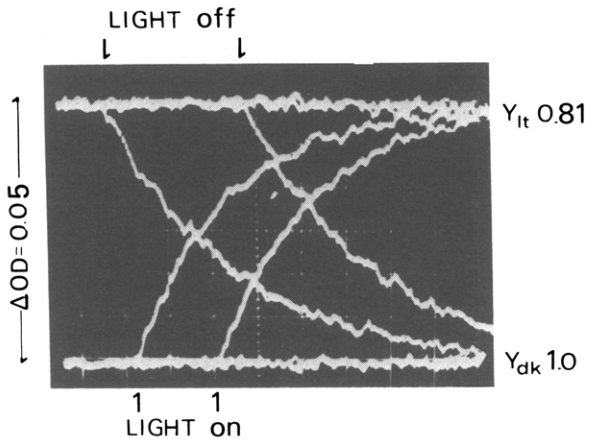

FIGURE 1: Oscilloscope traces showing the transition to a new steady level of CO binding by a 2.5 μM solution of sperm-whale myoglobin after turning light on (upward arrows) and the slower transition to the dark equilibrium level that occurs after turning light off (downward arrows). The optical density change (0.05) was monitored at 436 nm; temperature, 20°; 0.2 M phosphate buffer, pH 7.0. The free CO concentration in the dark was 10^{-5} M.

is related to the CO concentration at half-saturation ($c_{1/2}$) as follows.

$$L = 1/C_{1/2} = \frac{l'}{l + \omega I} = \frac{[FeCO]}{[Fe][CO]} \qquad (2)$$

Materials and Methods

Sperm-whale myoglobin from Seravac was repurified by ammonium sulfate precipitation. *Aplysia* myoglobin was prepared from the buccal muscles by the method of Rossi-Fanelli and Antonini (1957). Horseradish peroxidase, type VI, was obtained from Sigma. *Chironomus* hemoglobin was a gift from Dr. R. Huber. Human hemoglobin α and β chains were prepared by the PMB method of Bucci and Fronticelli (1965). The SH chains were regenerated from the PMB chains by the method of De Renzo *et al.* (1967). *Gastrophilus* hemoglobin was a gift from Dr. C. Phelps. Experiments were usually performed with the protein in 0.2 M phosphate buffer, pH 7.0. Protein concentrations were determined spectrophotometrically by using published extinction coefficients (Antonini and Brunori, 1971).

Measurements of light-induced spectral changes were made by using a cross-illumination apparatus equipped with a steady source and a flash lamp. The photolyzing light was the total or visible (λ >490 nm) output of a 150-W dc-operated xenon arc. The flash lamp provided enough intensity to induce complete photodissociation. The infrared and ultraviolet arc outputs were automatically filtered out as a consequence of the lucite and water that surrounded the sample cell. The beam completely illuminated 2 ml of solution in the cell. Its path length was 1 cm; that of the monitoring light, which was at right angles to it, was 2 cm. Variation of the intensity was achieved by interposing neutral density filters in the path of the photolyzing beam. The temperature in the thermostated sample cell was determined by the use of an indicator (Phenol Red in 0.05 M Tris, pH 8) and comparison with a static calibration. The errors involved were, of course, greatest far from room temperature, and above 30° they were estimated to be about ±1°.

Solutions of hemoproteins containing different CO concentrations were prepared, in the presence of dithionite (1–2 mg/ml) by appropriate additions to each syringe of a hemoprotein solution that was 10^{-4} M in CO. The fractional saturation in the light, Y_{lt}, was determined from the relation

$$\bar{Y}_{lt} = \bar{Y}_{dk} \frac{\Delta OD \text{ due to light}}{\Delta OD \text{ between Hb and HbCO}} \qquad (3)$$

The ligand saturation in the dark, $\bar{Y}_{dk}$, was measured either in a Cary spectrophotometer or directly in the sample cell. In the latter case, the difference in optical density between the sample and the deoxy form was measured in each case by flashing off all the CO in the sample. These two methods of measuring $\bar{Y}_{dk}$ gave consistent results, with differences never exceeding 5%.

In view of the high binding constants of the proteins used, a plot of $\bar{Y}_{dk}$ *vs.* the calculated concentration of CO provided a check on the amount of ligand in the solution.

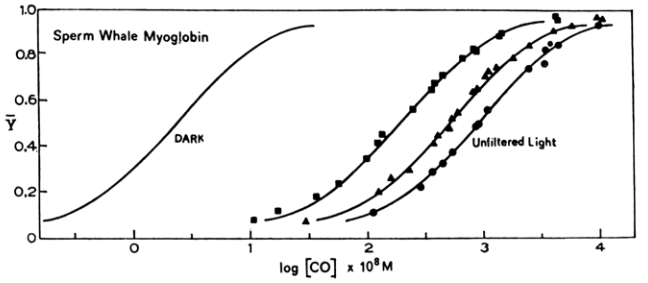

FIGURE 2: Carbon monoxide binding curves for sperm-whale myoglobin. The solid lines correspond to theoretical binding curves with a Hill constant of $n = 1$. The value $c_{1/2}$ in the absence of light is taken from Antonini and Brunori (1971). Experimental points are from several experiments using myoglobin concentrations of 1.9–4.9 μM (in heme) exposed to relative light intensities of 1.0 (●), 0.5 (▲), and 0.21 (■).

Results

The oscilloscope trace of a typical experiment is shown in Figure 1. The extent of photodissociation and the time course of the spectral change during the two relaxation processes (dark → light and light → dark) depends on the CO concentration, the quantum yield, the intensity of the light, and the velocity constants (l' and l) for the CO combination and dissociation in the dark.

Figure 2 illustrates the fact that for sperm-whale myoglobin the CO binding curve maintains its characteristic shape, with Hill's constant $n = 1$, over a wide range of light intensities, sufficient to cause a nearly 1000-fold increase in $c_{1/2}$. Over this whole range, the increase is strictly linear in light intensity (I). Similar results were obtained for all the other proteins studied.

When perturbation induced by light is sufficiently small, relaxation conditions apply (Eigen and De Maeyer, 1963), and for the simple cases considered here there is only one relaxation time (τ) given by

$$1/\tau_{dk} = l'([\overline{Fe}] + [\overline{CO}]) + l \qquad (4)$$

and

$$1/\tau_{lt} = l'([\overline{Fe}] + [\overline{CO}]) + (l + \omega I) \qquad (5)$$

where the term ωI, which has the dimension of reciprocal seconds, can be interpreted as a supplement to the dissociation constant l arising from the action of the light and where $[\overline{Fe}]$

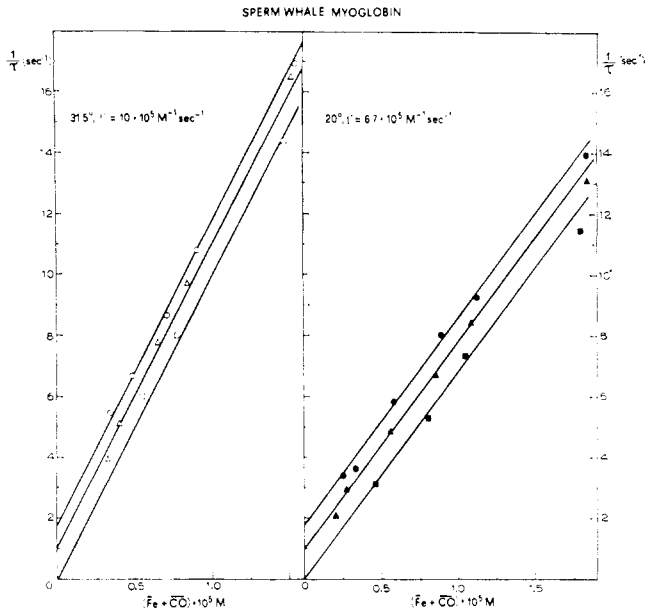

FIGURE 3: Rate constants for the approach to light or dark states for myoglobin at 20° (right) and 31.5° (left). Relaxation times observed after turning light *on* are represented by circles (relative light intensity = 1.0) and triangles (relative light intensity = 0.5). Relaxation times observed after turning light *off* are represented by squares. The straight lines have slopes equal to the combination velocity constants at the two temperatures (determined by flash photolysis at higher CO concentrations).

and [$\overline{CO}$] represent the equilibrium concentrations of free sites and free ligand. In this simple treatment the rates of approach to the steady state in the light and to equilibrium in the dark should be linearly dependent on ([$\overline{Fe}$] + [$\overline{CO}$]) and exhibit the same slopes (l'). The results of Figure 3 show this to be the case for sperm-whale myoglobin at both 20 and 31.5°. In both cases the rate of dissociation in the dark (0.02 sec^{-1} at 20°) (Antonini and Brunori, 1971) is negligible in comparison with that in the light. The intercept of these curves with the ordinate axis, which represents ($l + \omega I$) in Figure 3, is linearly dependent on light intensity and independent of temperature within the range examined.

Values for ($l + \omega I$), determined by kinetic method ii, can also be obtained from the steady-state measurements (method iii) as the product of $c_{1/2}$ and l' (eq 2). Table I shows that for sperm-whale myoglobin the two methods give the same results, which is a check on the simple scheme represented in eq 1 and 2. From these data it is possible to determine the relative values of the quantum yield (ϕ) for

TABLE I: Static and Kinetic Determinations of the Effective CO Dissociation Velocity Constant ($1 + \omega I$) for Sperm-Whale Myoglobin.[a]

	Intensity = 0.5		Intensity = 1.0	
Temp, °C	Static (sec^{-1})	Kinetic (sec^{-1})	Static (sec^{-1})	Kinetic (sec^{-1})
20.0	0.97	1.0	1.81	1.75
31.5	0.93	1.08	1.78	1.71

[a] Kinetic values from intercept of $1/\tau$ *vs.* ([$\overline{Fe}$] + [$\overline{CO}$]); static values from ($c_{1/2} \times l'$). Relative intensities are given.

TABLE II: Relative Quantum Yields for Photodissociation of CO from Different Hemoproteins.[a]

Protein	$10^{-5}\ l'$ ($M^{-1}\ sec^{-1}$)	l (sec^{-1})	ϕ Relative	ϕ Reported
Sperm-whale myoglobin	6.7	0.02	1.0	1.0
Aplysia myoglobin	5.4	0.02	1.0	
Hemoglobin A chains				
β-PMB	24	0.027	0.85	0.85
β-SH	45	0.008	0.51	0.60
α-PMB	39	0.016	0.67	0.71
α-SH	40	0.013	0.65	0.68
Horseradish peroxidase	0.034		0.7	
Gastrophilus hemoglobin	10.4	0.11	0.76	
Chironomus hemoglobin	200		0.75	

[a] Values of combination velocity constants from flash photolysis, Antonini and Brunori (1971), or Amiconi *et al.* (1972). Dissociation velocity constants and reported ϕ values from Antonini and Brunori (1971). ϕ relative lists quantum yields relative to sperm whale myoglobin at 20°.

different proteins, since ω may be written as the product of ϕ and an overall absorption coefficient ($\bar{E}$).

The calculation for the steady-state case is as follows. If we distinguish two different proteins by subscripts 1 and 2, then, for $l \ll \omega I$, we have the relationship

$$\frac{\phi_2}{\phi_1} = \frac{(c_{1/2})_2 l_2'}{\bar{E}_2 I_2} \frac{\bar{E}_1 I_1}{(c_{1/2})_1 l_1'} \tag{6}$$

Since for sperm-whale myoglobin at 20° the quantum yield for CO is unity (Warburg, 1949; Bucher and Kaspers, 1947), it is possible to assign values of ϕ for other proteins. Table II shows that, so far as they go, such determinations are in excellent agreement with values obtained by other methods. The photochemical dissociation term, ωI, is independent of temperature. For sperm-whale myoglobin this is shown by the data in Table I; similar results were obtained for the two other proteins in which temperature effects were studied, namely, *Aplysia* myoglobin and horseradish peroxidase. Confirmation of the assumption that light only affects the dissociation velocity constant is given by the results shown in Figure 3. The values of l' obtained from the kinetic data are independent of light intensity at both temperatures. From this one would deduce, as shown in the Discussion, that under high intensity illumination the apparent enthalpy change for CO binding, which is known to be negative in the dark (Antonini and Brunori, 1971), would become positive. Table III shows that this is indeed the case for all hemoproteins studied here, and this positive value approximates the activation enthalpy of the "on" process (ΔH_{on}).

Discussion

In the present experiments light is used as a perturbing agent in the same way as temperature or pressure is used in

TABLE III: Comparison of (a) Apparent Enthalpy for CO Binding by Various Hemoproteins as Determined under Photodissociating Conditions (ΔH_{app}) with (b) Activation Enthalpy for the "On" Process (ΔH_{on}) for the Same Hemoproteins.[a]

Protein	ΔH_{app} in the Light[b] (kcal/mol)	ΔH_{on}[c] (kcal/mol)
Sperm-whale myoglobin	+7.7	+6.5
Aplysia myoglobin	+6.6	+7.8
Horseradish peroxidase	+13.1	+14.1

[a] The three proteins listed have a negative enthalpy of ligand binding in the absence of light (Antonini and Brunori, 1971). [b] Determined from van't Hoff plot of log $c_{1/2}$ *vs.* $1/T$. [c] Determined from an Arrhenius plot of log l' *vs.* $1/T$.

ordinary relaxation experiments; the only difference is that in the light experiments the relaxation is to a steady state rather than to a true equilibrium. In spite of this the classical relaxation equations serve to describe the data satisfactorily. The effective "off" constant at any given light intensity can be determined from the value of $1/\tau$ extrapolated to $([\overline{Fe}] + [\overline{CO}]) = 0$, or from the value of $c_{1/2}l'$, at the same light intensity (since $c_{1/2} = l/l'$). Both methods show that the effective "off" constant is a linear function of light intensity. This is illustrated by Figure 3 and Table I. The action of the light can accordingly be described as in eq 1 and 2 by the addition of a rate term ωI to the off constant l which applies to the reaction in the dark. A multiplicative formulation with (ωIl) as the effective "off" constant is inconsistent with the experimental observations. This is shown by a study of the effect of temperature on the value of $c_{1/2}$. The "off" constant, l, is known to have a large dependence on temperature (Antonini and Brunori, 1971), and if a multiplicative formulation were correct the effective dissociation velocity constant in the presence of the light should be temperature dependent. This is contrary to the observations, which show that the effective "off" constants determined as outlined above are independent of temperature (see Figure 3 and Table I).

This is clarified by a consideration of the activation energy profiles shown in Figure 4, which are associated with ligand binding and release when photodissociation is negligible ($\omega I = 0$) or dominant ($\omega I \gg l$). Under the influence of intense illumination only the heat of activation of the "on" process is operative. It follows that at moderate intensity the enthalpy of CO binding must depend on the relative contributions of l and ωI to the overall "off" constant. The following equations describe this. The apparent (or pseudo) equilibrium constant L depends on the activation energy for ligand binding and release in accordance with the expression

$$L = \frac{l'}{l + \omega I} = \frac{a_1 e^{-\Delta E'/RT}}{a_2 e^{-\Delta E/RT} + \omega I} \tag{7}$$

It follows that the apparent enthalpy ΔH_s calculated for the steady state is given by

$$-\Delta H_s = \frac{R\ \mathrm{d}\ln L}{\mathrm{d}(1/T)} = \frac{R\ \mathrm{d}\ln l'}{\mathrm{d}(1/T)} - \frac{R\ \mathrm{d}\ln (l + \omega I)}{\mathrm{d}(1/T)} \tag{8}$$

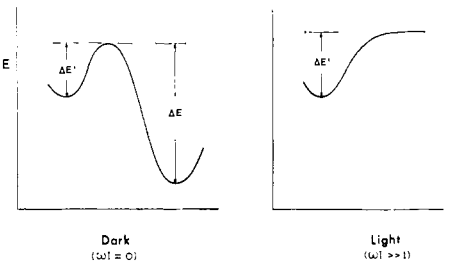

FIGURE 4: Schematic illustration of the differences in activation energies for ligand binding and release when photodissociation is negligible ($\omega I = 0$) or dominant ($\omega I \gg l$). $\Delta E'$ is the activation energy associated with ligand combination and ΔE is the activation energy associated with dissociation. The dissociation velocity constant in the dark is l and ωI represents the additional dissociation term induced by light.

Since we observe $\mathrm{d}(\omega I)/\mathrm{d}(1/T) = 0$, this gives

$$\frac{R\ \mathrm{d}\ln L}{\mathrm{d}(1/T)} = \frac{R\ \mathrm{d}\ln l'}{\mathrm{d}(1/T)} - \frac{Rl}{(l + \omega I)} \frac{\mathrm{d}\ln l}{\mathrm{d}(1/T)} \tag{9}$$

or, as an equivalent

$$\Delta H_s = \Delta H_{on} - \frac{l}{l + \omega I} \Delta H_{off} \tag{10}$$

Thus, in these simple systems the transition between the enthalpy changes observed in the dark and the ΔH_s should take the form of a hyperbola, with an asymptote when $I \rightarrow \infty$ of $\Delta H_s = \Delta H_{on}$. As shown in Table III, the value of ΔH_s under high intensity illumination approximates ΔH_{on} for all of the hemoproteins so far examined. This fact, along with the kinetic data, is conclusive evidence that photodissociation *adds* to the normal process of dissociation and does not increase the normal "off" rate by a multiplicative factor.

Examination of the steady-state situation of the CO binding curves under photodissociating conditions shows that all the simple hemoproteins investigated can be described by the simple one-step process represented by eq 1. There is no kinetic or spectral evidence of the transient excited state which should precede photodissociation. Similar behavior for CO binding under photodissociating conditions may be expected for other molecules with single binding sites.

We have shown in this paper that in the case of simple hemoproteins light affects only the "off" rates and that this is the result of an additive (and not a multiplicative) factor. This provides the basis for photochemical studies of more complex molecules, like tetrameric hemoglobins. We have suggested that this method may be used in an investigation of the relative roles of "on" and "off" processes in determining homotropic interaction effects and perhaps the heterotropic interactions as well. We have recently reported results which show that, under photodissociating conditions, both the Bohr effect and the cooperativity of CO binding by human hemoglobin are invariant (Brunori *et al.*, 1972). Greater understanding of such behavior may be anticipated from studies, now in progress, concerning the effect of light on abnormal or chemically modified hemoglobins that are "frozen" in high or low affinity conformations.

References

Ainsworth, S., and Gibson, Q. H. (1957), *Nature (London) 180*, 1416.

Amiconi, G., Antonini, E., Brunori, M., Formanek, H., and Huber, H. (1972), *Eur. J. Biochem. 31*, 52.
Antonini, E., and Brunori, M. (1971), Hemoglobin and Myoglobin in Their Reactions with Ligands, Amsterdam, North-Holland Publishing Co.
Brunori, M., Bonaventura, J., Bonaventura, C., Antonini, E., and Wyman, J. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 868.
Bucci, E., and Fronticelli, E. (1965), *J. Biol. Chem. 240*, PC551.
Bucher, T., and Kaspers, J. (1947), *Biochim. Biophys. Acta 1*, 21.
De Renzo, E. C., Ioppolo, C., Amiconi, G., Antonini, E., and Wyman, J. (1967), *J. Biol. Chem. 242*, 4850.
Eigen, M., and De Maeyer, L. (1963), *Tech. Org. Chem. 8*, 895.
Gibson, Q. H. (1956), *J. Physiol. 134*, 112.
Haldane, J., and Lorrain-Smith, J. (1895), *J. Physiol. 20*, 497.
Noble, R. W., Brunori, M., Wyman, J., and Antonini, E. (1967), *Biochemistry 6*, 1216.
Rossi-Fanelli, A., and Antonini, E. (1957), *Biochimica 22*, 335.
Warburg, O. (1949), Heavy Metal Prosthetic Groups and Enzyme Action, Oxford, Clarendon Press.

Determination of Rotational Mobilities of Backbone and Side-Chain Carbons of Poly(γ-benzyl L-glutamate) in the Helical and Random-Coil States from Measurements of Carbon-13 Relaxation Times and Nuclear Overhauser Enhancements†

Adam Allerhand* and Eric Oldfield

ABSTRACT: We have determined the ^{13}C spin–lattice relaxation times (T_1) of the various types of carbons in helical and random-coil poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$) samples of molecular weight 7,000, 17,000, and 46,000 in $CDCl_3$–CF_3-COOH mixtures, by means of proton-decoupled natural-abundance ^{13}C partially relaxed Fourier transform nuclear magnetic resonance (nmr) spectra at 14.2 kG. We have also measured the nuclear Overhauser enhancement (NOE) of the various carbons by means of integrated intensities in decoupled and undecoupled ^{13}C spectra. The T_1 values of C^α change by less than a factor of 2 when going from helical to random-coil (BzlGlu)$_n$. However, the NOE changes from about 1.1 (10% intensity enhancement) to more than 2 (100% intensity enhancement). The very low NOE for the α-carbons of helical (BzlGlu)$_n$ is evidence for a rotational correlation time too slow to satisfy the extreme narrowing condition. The combination of T_1 and NOE values yields effective rotational correlation times (τ_{eff}) for C^α of 24–32 nsec for the helix and only about 0.8 nsec for the random coil. These results suggest that τ_{eff} of C^α of the helical species is dominated by overall rotation with little or no contribution from segmental motion. When going to the random-coil species, τ_{eff} of C^α decreases by a factor of about 30 as a result of the onset of rapid segmental motion. The α-carbon T_1 values of helix and coil differ only slightly because of their particular positions on different sides of the minimum in the functional relation between the spin–lattice relaxation time and the correlation time. In both the helical and random-coil molecules, there is a progressive increase in T_1 of the protonated carbons of the side chains when going away from the polymer backbone, as a result of fast internal rotations. In the helical molecules, there is a large increase in NOE when going from C^α to C^β, because τ_{eff} acquires contributions from internal motions with rates that satisfy the extreme narrowing condition. In contrast, in random-coil (BzlGlu)$_n$ τ_{eff} of C^α is already in the extreme narrowing limit, and changes in NOE along the side chains are small.

Proton nuclear magnetic resonance (nmr) has been used extensively for studying the helix–coil transition of homopolymeric polypeptides (Bovey, 1972; Bradbury *et al.*, 1973). Recently, ^{13}C nmr has also been used for this purpose (Paolillo *et al.*, 1972; Boccalon *et al.*, 1972). The usual approach has been to observe chemical shift variations between the helical and random-coil species. However, theoretical calculations of differences in chemical shifts between helical and random-coil environments would be extremely difficult. Thus, the observed chemical shifts must first be *empirically* assigned to the helical and random-coil environments on the basis of other measurements.

We show here that measurements of ^{13}C spin–lattice relaxation times (T_1), spin–spin relaxation times (T_2), and nuclear Overhauser enhancements (NOE) of α-carbons provide a probe for studying transitions from rigid to flexible forms of a polymer without *a priori* knowledge about conformations. The ^{13}C relaxation and NOE measurements should distinguish the relatively immobile C^α–H^α groups of a helical polypeptide from the mobile C^α–H^α groups of a random-coil polymer undergoing fast segmental motion. We present ^{13}C T_1, T_2, and NOE measurements for the α-carbons of poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$ Figure 1) of various molecular

† Contribution No. 2209 from the Department of Chemistry, Indiana University, Bloomington, Indiana 47401. *Received April 25, 1973.* This research was supported by the National Institutes of Health (Grant NS-10977-01), the National Science Foundation (Grant GP-17966), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and by Eli Lilly and Co. One of us (E. O.) thanks the European Molecular Biology Organization and the Gilbert Foyle Trust of Great Britian for partial support.