nificantly lower than that of pure salt can be found such that reflectors remain roughly horizontal and can be correlated with reflectors beneath the adjacent continental rise. This suggests that another mechanism, possibly a submarine landslide or slumping incorporating significant quantities of salt, may be a better explanation of this feature.

Both lines SS-1 and SS-2 show a change in the number of good reflectors per unit depth in the continental rise–abyssal plain region. The lower portion of the section contains relatively fewer reflections than can be explained on the basis of the decreasing of signal-to-noise ratios with greater depth. Near the Sigsbee Scarp, the change occurs at about 2.5 seconds subbottom. A similar phenomenon was observed in single-channel reflection data north of DSDP hole 91. Worzel and Bryant (*10*) correlated the upper zone of numerous reflectors with a zone of interlayered turbidites and abyssal sediments found in DSDP cores. The zone consists of rocks of Pleistocene and Upper Pliocene age. Within the upper zone, our data show subzones in which the reflectors are wavy, anastomosing, and locally discontinuous. Structural discontinuities are limited to the subzones and do not extend into suprajacent or subjacent strata. We suggest that periods of alternating sea-floor erosion and deposition caused the irregularities in the reflectors.

Deep sediment-filled basins of the lower slope are of interest because of their possible hydrocarbon potential. In our area of investigation, these basins range up to 20 km long and are several kilometers wide. Refraction data (*7*, *8*) indicate that the velocities probably average between 2.0 and 2.5 km/sec. Hence, sediment thickness in the basins may locally range up to 7.5 km. We have not attempted to analyze the data for indications of natural gas accumulations (*11*), but cursory examination of the data suggests likely possibilities. The largest of the basins lies near the northern apex of our two CDP lines in less than 1.5 km of water. This is within the range of present-day commercial drilling technology.

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Porphyrin Triplet State Probing the Diffusion of Oxygen in Hemoglobin

Abstract. *Laser photolysis study of porphyrin-globin shows that the triplet state of the porphyrin is detectable by its light absorption and that it can be used to determine the rate of penetration of oxygen into the hemoglobin pocket in which the porphyrin is embedded. The oxygen penetration rate does not determine the binding rate of oxygen to iron in hemoglobin.*

The fixation of molecular oxygen to the heme in hemoglobin is expected to depend on the rate of diffusion of the ligand into the heme pocket. To study this diffusion we have made use of the well-known property of oxygen as a triplet-state quencher. The principle of the method was to populate the porphyrin triplet state by nanosecond laser excitation and follow the triplet decay by rapid kinetic spectrophotometry in the presence and absence of oxygen; the enhancement of the decay rate in the presence of oxygen gives a measure of the rate of oxygen diffusion into the heme pocket. Since the hemoglobin triplet is too short-lived for such a study, we investigated instead the corresponding iron-free porphyrin-globin, which has a relatively long triplet lifetime ($> 10^{-4}$ second). We studied the tetrameric porphyrin-globin obtained from human hemoglobin A (in aqueous solution, *p*H 7, 2°C, $10^{-4}M$ in porphyrin) and the corresponding free protoporphyrin IX (in dioxan at 23°C). The globin protecting effect on the porphyrin was expected to appear as a reduction of the triplet quenching constant of the porphyrin-globin compared to that of the free porphyrin.

Transient changes in absorbancy in the solutions irradiated by a laser pulse (*1*) (529 nm, 3×10^{-8} second width at half-maximum) were measured at selected wavelengths. Excitation of degassed solutions of the porphyrin-globin and the free porphyrin, respectively, produced a transient spectrum (Fig. 1), which was attributed to the porphyrin triplet state, populated by intersystem crossing from the excited singlet state. The broad absorption features of the spectrum are character-

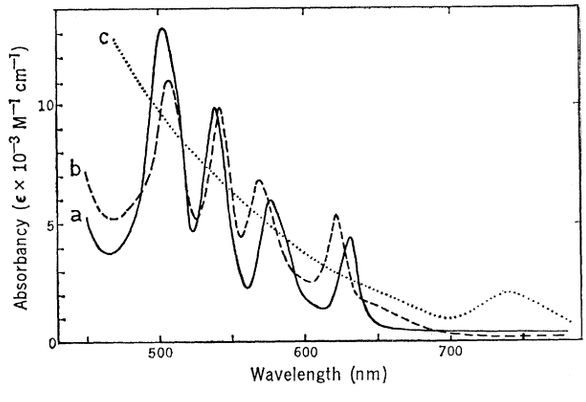

Fig. 1. Curve a: Absorption spectrum of free protoporphyrin IX in dioxan. Curve b: Spectrum of the porphyrin-globin corresponding to human hemoglobin A, in phosphate-buffered aqueous solution at *p*H 7. Curve c: The triplet spectrum obtained on laser excitation of the above compounds.

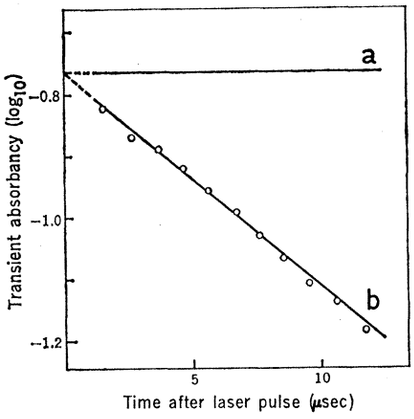

Fig. 2. Decay of triplet porphyrin-globin in deaerated (curve a) and aerated (curve b) solution, measured at 470 nm after laser excitation, at equal laser energy.

istic of porphyrin triplet absorptions already reported (*2, 3*). The decay of the transient was unnoticeable over a period of 2×10^{-5} second (Figs. 2 and 3). Saturation of the solutions by air did not affect the initial triplet population; however, the porphyrin triplet (^3P) decay was enhanced, indicating the occurrence of the oxygen quenching reaction:

$$^3P + {}^3O_2 \rightarrow P + {}^1O_2 \text{ (or } {}^3O_2) \quad (1)$$

($^3O_2$ is ground-state oxygen; $^1O_2$ is an excited singlet state). First-order decay plots (Figs. 2 and 3) gave straight lines at all the wavelengths studied, in agreement with Eq. 1; the first-order rate constant may then be set equal to $k_q[O_2]$. From known oxygen solubilities in aerated water and dioxan ($4.3 \times 10^{-4}M$ and $1.50 \times 10^{-3}M$, respectively), one obtains $k_q = 1.6 \times 10^9 M^{-1}$ sec^{-1} for the free porphyrin and $k_q = 1.8 \times 10^8$ for the porphyrin-globin. The former of these values is in the range normally encountered for diffusion-controlled triplet quenching by oxygen; the latter is an order of magnitude smaller. One may set $k_q = p_q k_{diff}$, where k_{diff} is the rate constant of diffusion-controlled reactant encounters and p_q is the probability of quenching during the encounters. (For the porphyrin-globin we define the encounter as the close approach of oxygen to the globin; the further diffusion into the heme pocket is included in the p_q factor.) The constant k_{diff} is directly proportional to the absolute temperature and inversely proportional to the viscosity of the solution. In a solution containing a macromolecular compound (such as the porphyrin-globin) the oxygen diffusion would be determined by the microscopic rather than by the macroscopic viscosity, that is, by the viscosity of the solvent [see (*4*)]. Using known values of the viscosities of water (1.67 centipoises) and dioxan (1.20 centipoises) we then obtain a ratio of 6 : 1 for the p_q value of free porphyrin to that of the porphyrin-globin. This result shows that in the latter compound the porphyrin is protected efficiently by the globin. Complementary runs on denatured globin with porphyrin adsorbed on the surface gave, on the contrary, the same p_q value ($k_q = 1 \times 10^9 M^{-1}$ sec^{-1}) as the free porphyrin. One may thus conclude that the protecting effect of the native globin is due to the insertion of the porphyrin in the heme pocket of the globin.

The results have a direct bearing on the oxygen diffusion in hemoglobin. Hemoglobin is known to exist in two distinct structural forms: the fully deoxygenated deoxy form and the at least partially oxygenated oxy form (*5*). The porphyrin-globins have the same molecular properties as the corresponding hemoglobin (*6*); it has been proposed that their tertiary and quaternary structures are those of the deoxy form (*7*). One may thus assume that the heme pockets of the deoxy hemoglobin and the porphyrin-globin are very similar to each other. The diffusion of oxygen into the porphyrin-globin pocket, producing triplet quenching, then should closely correspond to the rate of penetration of oxygen into the heme pocket of hemoglobin. It is interesting to note that the oxygen penetration rate apparently is not the rate-determining step in the binding of oxygen to iron in hemoglobin. Reported values of the binding rate constant (*8*) are indeed more than an order of magnitude smaller than that of the porphyrin-globin triplet quenching.

It has been shown (*8, 9*) that the fixation of oxygen to the deoxy form of hemoglobin enhances the rate of oxygenation of the remaining sites. This is attributed to the change in arrangement of the globin chains after the introduction of oxygen into the heme pockets of the α chains. The resulting structural transition would then make possible the oxygen introduction into the β chain pockets (*10*). However, we found that the porphyrin-globin triplet quenching by oxygen is strictly first-order. This result shows that the four different porphyrin sites (α or β chains) of the tetrameric molecule are equally accessible to oxygen.

A further important result is the observation of equal porphyrin triplet population of the porphyrin-globin in the presence and absence of oxygen at equal laser energy (Fig. 2). This result shows that the heme pockets do not trap the oxygen molecules. If an oxygen were trapped more or less permanently in the heme pocket, we would expect an extremely rapid quenching of the triplet porphyrin molecule occupying the same pocket, and we would, in our experimental conditions, have found an apparent reduction of the triplet yield.

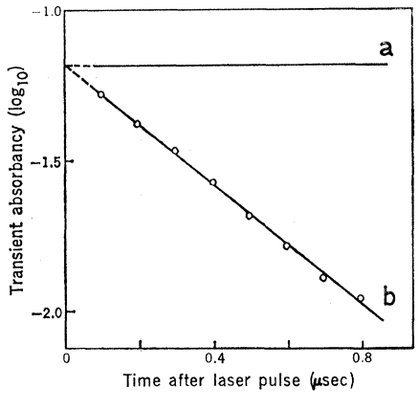

Fig. 3. Decay of triplet protoporphyrin IX in deaerated (curve a) and aerated (curve b) solution, measured at 470 nm after laser excitation, at equal laser energy.

Thus, triplet porphyrin probing of oxygen diffusion is a powerful means of studying the dynamics of oxygen fixation in hemoglobin.

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