Horn, M. J., Jones, D. B., and Ringel, S. J. (1942b), *J. Biol. Chem. 144*, 93.
Hurlbert, R. B., Schmitz, H., Brumm, A. F., and Potter, V. R. (1954), *J. Biol. Chem. 209*, 23.
Katzin, L. I., and Gulyas, E. (1964), *J. Am. Chem. Soc. 86*, 1655.
Kondo, Y. (1959), *J. Sericult. Sci. Japan 28*, 1.
Kondo, Y. (1962), *J. Biochem. (Japan) 51*, 188.
Massey, V., Palmer, G., and Bennett, R. (1961), *Biochim. Biophys. Acta 48*, 1.
Piez, K. A., Irreverre, F., and Wolff, H. L. (1956), *J. Biol. Chem. 223*, 687.
Rajagopal Rao, D., Ennor, A. H., and Thorpe, B. (1966), *Biochem. Biophys. Res. Commun. 22*, 163.
Rosen, H. (1957), *Arch. Biochem. Biophys. 67*, 10.
Rosenberg, H., Ennor, A. H., Hagermann, D. D., and Sugai, S. (1962), *Biochem. J. 84*, 536.
Rothman, F., and Higa, A. (1962), *Anal. Biochem. 3*, 173.
Schöberl, A., and Wagner, A. (1947), *Chem. Ber. 80*, 379.
Schram, E. (1963), Organic Scintillation Detectors, Amsterdam, Elsevier.
Sloane, N. H., and Untch, K. G. (1966), *Biochemistry 5*, 2658.
Smith, I. (1960), Chromatographic and Electrophoretic Techniques, Vol. II, London, W. Heinemann, Ltd., p 98.
Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30*, 1190.
Stein, J. M. (1955), *Chem. Ind. (London)* 774.
Stein, J. M., and Kermack, W. O. (1959), *Biochem. J. 71*, 648.
Work, E. (1957), *Biochem. J. 67*, 416.
Wyatt, G. R. (1961), *Ann. Rev. Entomol. 6*, 75.

Studies on the Quantum Yields of the Photodissociation of Carbon Monoxide from Hemoglobin and Myoglobin*

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ABSTRACT: The photodissociation of carbon monoxide from normal and modified myoglobin, hemoglobin subunits, and hemoglobin has been studied. The quantum yield of this process was found to be dependent, even for monomeric proteins, on ionic strength, protein concentration, and primary structure. It has not been possible to establish definitely whether or not protein aggregation state can influence quantum yield.

Carbon monoxide liganded to a heme protein is dissociated by light with a very high quantum yield (Haldane and Smith, 1896; Warburg *et al.*, 1929). This very high quantum yield for the photodissociation of carbon monoxide hemoglobin is unique to this ligand. In fact all the other ferrohemoglobin–ligand complexes, although photodissociable (Gibson and Ainsworth, 1957), have a much lower quantum yield, irrespective of their free energy of binding. As a result of measurements of the quantum yield of this photodissociation process for horse myoglobin and horse hemoglobin, Bücher and Negelein (1942) suggested that the quantum yield of the reaction might be inversely proportional to the degree of polymerization of the molecule, and hence to the number of hemes per molecule. At 4° in 0.02 M pyrophosphate buffer (pH 8.2) at a protein concentration of approximately 0.1 mg/ml, they found that the quantum yield with myoglobin was very nearly unity (0.92) while with hemoglobin it was 0.27. When the hemoglobin solution was made 2.6 M in sodium chloride, to give an ionic strength at which hemoglobin is largely dissociated into dimers, the quantum yield increased to 0.37.

An inverse relation between polymerization and quantum yield would indicate some type of intramolecular energy transfer between subunits which would be of considerable interest. In addition, if there really were a simple correlation between the quantum yield and the number of subunits per molecule, quantum yield measurements could provide a very simple and elegant means of studying the dissociation of hemoglobin at very low concentrations. With a modification of the method of Bücher and Negelein, these measurements can be extended down to protein concentrations of 0.01 mg/ml.

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For these reasons we have studied the quantum yield of carbon monoxide dissociation from both hemoglobin and myoglobin in considerable detail. The measurements were made on solutions at different protein concentrations and ionic strengths. Similar measurements were also made on a number of modifications of both proteins as well as on the isolated α and β chains of hemoglobin.

Materials and Methods

Horse and human hemoglobin were both prepared from fresh blood by the usual ammonium sulfate procedure (Rossi-Fanelli *et al.*, 1961) or by the toluene method (Taylor and Hastings, 1939). No differences were found in the behavior of the material obtained by these two methods.

Horse ferrimyoglobin was prepared from skeletal muscles and crystallized by the method of Rossi-Fanelli (1949). Sperm whale ferrimyoglobin was a commercial preparation obtained from the Seravac Co. (batch no. 7). Ferromyoglobin solutions were obtained from ferrimyoglobin solutions by enzymatic reduction (Rossi-Fanelli and Antonini, 1958).

α_{PMB}[1] *and* β_{PMB} chains of human hemoglobin were prepared by the method of Bucci and Fronticelli (1965) and of Bucci *et al.* (1965). The removal of PMB from the cysteine residues to regenerate the SH groups was accomplished in a variety of ways. In the early phases of the work α_{PMB} chains were treated with a 40-fold excess of homocysteine at pH 7.5 for 1 hr and then dialyzed against water. β_{PMB} chains, for which the homocysteine was less effective, were first passed through a Dintzis (1952) column to remove excess PMB and were then treated for 1 hr with a 25-fold excess of thioglycolate at pH 7.5 followed by passage through a second Dintzis column and dialysis. This procedure was subsequently replaced by dialysis of the PMB chains, both α and β, for 12 hr, against 0.05% thioglycolate in pH 7.5 buffer containing 0.01 M NaCl and 5×10^{-4} M EDTA. The SH chains were then dialyzed against the same buffer without thioglycolate. SH chains prepared by the latter procedure were much more stable than those prepared by the earlier method. The EDTA prevents catalytic oxidation of the chains by trace amounts of heavy metal ions, and NaCl has a definite stabilizing effect on the α_{SH} chains. The PMB chains were also dialyzed against this same buffer.

SH groups were titrated by the method of Boyer (1954).

Carboxypeptidase A digested hemoglobin was kindly prepared by Dr. Romano Zito, by the method previously described (Zito *et al.*, 1964). Completion of digestion was checked by amino acid analysis; the oxygen equilibrium was found to be the same as that previously described ($n = 1$, $\log P_{1/2} = -0.2$ at 20° and pH 7) (Antonini *et al.*, 1961).

Acetylation of human hemoglobin and horse myoglobin was carried out following the procedure reported by Bucci *et al.* (1963). The extent of acetylation for both proteins was about 70%, as measured by the ninhydrin reaction (Moore and Stein, 1948).

Buffers. For all measurements at pH 7, a stock solution of 0.1 M phosphate buffer, ionic strength 0.22, was made up. Solutions of lower ionic strengths were obtained from this by diluting with distilled water; solutions of higher ionic strength, by the addition of sodium chloride. For measurements at pH 9.2, 0.2 and 0.02% borate solutions were used.

Quantum Yield Measurement. Quantum yields were obtained by the method Warburg (1949) from the rates of approach to equilibrium when the intensity of illumination on a heme protein solution containing a mixture of CO and O_2 is altered.

The reactions involved may be written as

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

$$FeO_2 \underset{k'}{\overset{k}{\rightleftharpoons}} Fe + O_2 \qquad (2)$$

The corresponding velocities are

$$-\frac{d(FeCO)}{dt} = l(FeCO) - l'P_{CO}(Fe) \qquad (3)$$

and

$$-\frac{d(FeO_2)}{dt} = k(FeO_2) - k'P_{O_2}(Fe) \qquad (4)$$

Since the quantum yield for oxygen is extremely low (0.8×10^{-2} in the case of hemoglobin) (Gibson and Ainsworth, 1957) at the light intensities used in this work, the only significant effect is on the rate constant (l) which can be written as

$$l = l_d + \phi i \epsilon \qquad (5)$$

where ϕ is the quantum yield for the displacement of CO by light, i is the light intensity, ϵ the molar extinction coefficient, and l_d the value of the constant at zero light intensity.

When such a system, initially at equilibrium in the dark, is illuminated, the equilibrium (or at least a steady state) will be disturbed because of the alteration in l. At ligand concentrations such that the concentration of (Fe) can be neglected and in systems where

$$l'P_{CO} \ll k'P_{O_2} \qquad (6)$$

[1] Abbreviations used: α_{SH} and β_{SH}, α and β chains of hemoglobin with the sulphydryl groups unreacted; α_{PMB} and β_{PMB}, hemoglobin chains in which the sulphydryl groups have been reacted with PMB; CPA, carboxypeptidase A; PMB, *p*-mercuribenzoate.

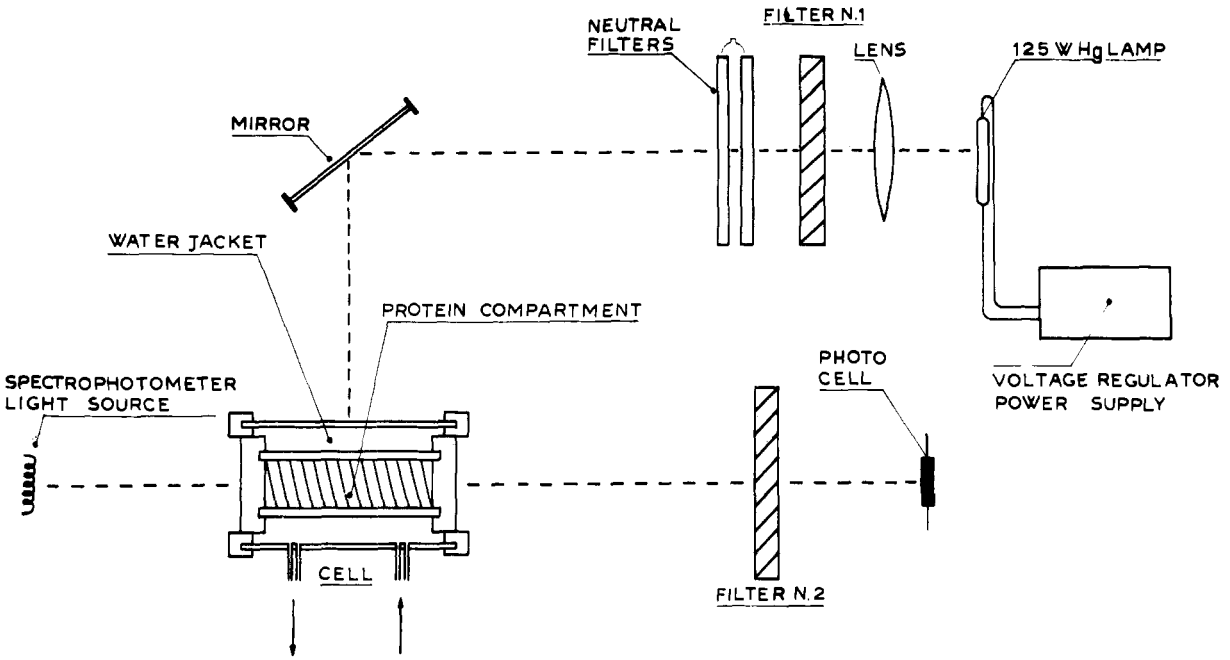

FIGURE 1: Apparatus used for quantum yield measurements.

the rate constant for attainment of the new equilibrium is

$$R_{\text{light}} = l + k\frac{l'P_{CO}}{k'P_{O_2}} \tag{7}$$

$$= l_d + \phi i\epsilon + k\frac{l'P_{CO}}{k'P_{O_2}} \tag{8}$$

When the light is removed the system will revert to the original state with a rate constant

$$R_{\text{dark}} = l_d + k\frac{l'P_{CO}}{k'P_{O_2}} \tag{9}$$

It is clear that

$$R_{\text{light}} = R_{\text{dark}} + \phi i\epsilon \tag{10}$$

Instead of measuring the rate constant for the displacement of CO by O_2 at a single light intensity, as was done by Bücher and Negelein, in this work the light intensity was varied by the use of calibrated neutral filters. By plotting R_{light} *vs.* light intensity one obtains a straight line whose intercept with the ordinate axis gives R_{dark}. The slope is, of course, proportional to $\phi\epsilon$, the quantum yield multiplied by the molar extinction coefficient at the irradiating wavelength.

The apparatus used is shown in Figure 1. The Lucite cell was cylindrical. The dimensions of the protein compartment were 50 × 7 mm in diameter. In order to increase the uniformity of illumination, the cell was covered with aluminium foil on the side opposite the lamp.

The lamp was an HPK 125-w mercury tube made by the Philips Co. (type 572038); it was powered by a voltage regulator (SERTI, Rome, Model "SCT," no. 6832) to provide a constant output. The cylindrical shape of the lamp proved to be very convenient, since the lamp image could be simply focussed on the length of the protein compartment of the cell to give a very intense uniform illumination.

The spectra of filters 1 and 2 are shown in Figure 2. Filter 1 allowed only the 546-mμ band of the irradiating mercury light to pass, while filter 2 completely excluded this band. This combination of filters prevented any of the light used for photodissociation from reaching the photocell.

At the highest protein concentrations (0.15 mg/ml) used in these experiments, calculations indicated that the intensity of the irradiating light varied by less than 10% between the periphery and center of the cell. Moreover, in going from the lowest to the highest protein concentration used, the *average* light intensity throughout the cell decreases by less than 3%.

The apparatus was installed in a Beckman DK 1 recording spectrophotometer. Kinetic curves were obtained with the time-constant switch set at 0.1 sec.

All measurements were made at 20°. Two wavelengths were used; the Soret peak of the CO form where a slit width of 0.8 mm was used and the region of optical density minimum at 378 mμ where a 1.2-mm slit width was found to be necessary.

Gas mixtures were prepared from tanks of purified oxygen and carbon monoxide. P_{O_2}:P_{CO} ratios were chosen so that the pseudo-first-order rate constants were always less than 1.0 sec^{-1}.

Light intensity was not measured directly; instead myoglobin was taken as a standard and assigned a

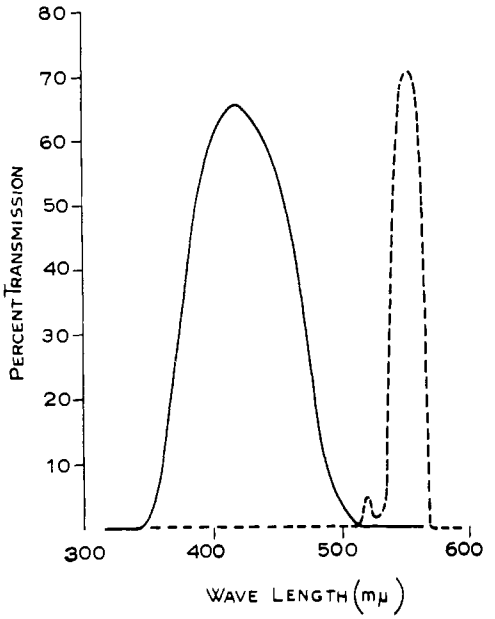

FIGURE 2: Transmission spectra of filters 1 (- - - -) and 2 (———).

quantum yield of unity. The constancy of the intensity of illumination was checked in each set of measurements by running a myoglobin standard at the beginning and at the end of the experiment. In no case did the two standards differ significantly. Bücher and Negelein actually reported an average value of 0.92 for the quantum yield for myoglobin; but since confidence limits of this value were not known and our prime interest in any case was in relative quantum yields, a value of unity was assumed. The reader may want to decrease the quantum yields reported in this paper by 8% to compare them with those of Bücher and Negelein.

Extinction coefficients at 546 mμ were determined by the pyridine hemochromogen method as reported by de Duve (1948). A value of 32×10^{-3} was taken as the molar extinction coefficient of pyridine hemochromogen. The extinction coefficients obtained for the optical density maxima were the same as those previously reported (Antonini, 1965). The ratio of the extinction coefficient of myoglobin to that of hemoglobin at 546 mμ was found to be 1.07.

Results and Discussion

Myoglobin. Since throughout this work the quantum yield of myoglobin, taken to be unity, was used as a standard to calibrate the intensity of the light source, it was important to show that in fact ϕ for myoglobin was invariant within the range of ionic strength and protein concentration used. As can be seen in Table I, which reports the values obtained under a variety of conditions, the quantum yield of myoglobin depends on both ionic strength and protein concentration. At a

TABLE I: ϕ for Myoglobin.[a]

Ionic Strength	Protein Concn (mg/ml)	ϕ
0.22	0.015	Defined as unity
0.22	0.15	1.0
0.22	0.03	1.0
0.011	0.015	1.0, 1.0
0.011	0.15	0.85, 0.86, 0.85, 0.85

[a] Reactions were at pH 7.0 and 20°.

protein concentration of 0.15 mg/ml, a decrease in ionic strength from 0.22 to 0.011 decreased the quantum yield from 1.0 to 0.85. On the other hand, at a constant ionic strength of 0.011, dilution of the protein from 0.15 to 0.015 mg/ml increases the quantum yield from 0.85 to unity. This effect of dilution on quantum yield is very surprising, since at these low protein concentrations one would not expect intermolecular energy transfer or quenching owing to the large distance between molecules.

The standard for measuring the light intensity described under Materials and Methods was a myoglobin solution of concentration 0.015 mg/ml and of ionic strength 0.22. In all of the experiments, sperm whale and horse myoglobin behaved identically. For this reason they were used interchangeably throughout this work.

Human Hemoglobin. The results of a typical experiment are shown in Figure 3. Here R, the rate constant for the approach to the steady state, is plotted as a function of light intensity for a solution containing 0.1 mg/ml of human hemoglobin at pH 7 and an ionic strength of 0.011, and for a solution containing 0.015 mg/ml of myoglobin at pH 7 and an ionic strength of 0.22. Assuming ϕ to be unity for myoglobin, the value calculated for hemoglobin under these conditions, from the slopes of these curves, is 0.37.

From such experiments as this, ϕ was determined for human hemoglobin solutions at several different ionic strengths and protein concentrations. The data are given in Table II. It will be seen that there is a clear tendency for ϕ to increase when the protein is diluted or the ionic strength is increased. At an ionic strength of 0.011 and a protein concentration of 0.1 mg/ml, ϕ_{av} is equal to 0.40. At an ionic strength of 1.35 and a protein concentration of 0.01 mg/ml, ϕ has increased to 0.81. This might be taken as evidence that the dissociation of hemoglobin results in an increase in the quantum yield, and this was the conclusion of Bücher and Negelein on the basis of similar data. However, similar dependence of quantum yield on ionic strength and protein concentration are found for myoglobin, making it impossible to deduce an aggregation–quantum yield dependency for hemoglobin.

Increasing the pH at low ionic strength and at a

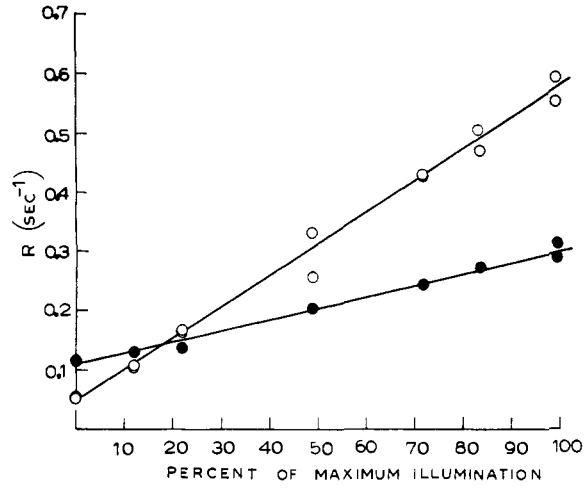

FIGURE 3: R plotted as a function of light intensity (per cent of maximum illumination) for a 0.015 mg/ml of myoglobin solution in 0.22 ionic strength, pH 7 buffer (O—O) and for a 0.1 mg/ml of human hemoglobin solution in 0.011 ionic strength, pH 7 buffer (●—●). The quantum yield for the hemoglobin is equal to the slope of the hemoglobin plot divided by the slope of the myoglobin plot and the result multiplied by 1.07, which is the ratio of the myoglobin to the hemoglobin extinction coefficient at the irradiating wavelength (546 mμ).

TABLE II: ϕ for Normal Human Hemoglobin.[a]

Ionic Strength	Protein Concn (mg/ml)	pH	ϕ
0.011	0.16	7.0	0.37
0.011	0.1	7.0	0.37
0.011	0.1	7.0	0.41
0.011	0.1	7.0	0.44
0.011	0.016	7.0	0.59
0.011	0.01	7.0	0.49
0.11	0.1	7.0	0.53
0.11	0.01	7.0	0.67
0.22	0.015	7.0	0.71
1.35	0.1	7.0	0.56
1.35	0.01	7.0	0.81
0.2% borate	0.1	9.2	0.37

[a] Reactions were at 20°.

protein concentration of 0.1 mg/ml has very little effect on ϕ. This is contrary to the data obtained for horse hemoglobin by Bücher and Negelein. It is worth noting that the P_{O_2}:P_{CO} ratio was varied from 8 to 25 without any effect on the value of ϕ obtained.

Horse Hemoglobin. In their original studies on the quantum yields of heme proteins, Bücher and Negelein used horse hemoglobin. The major part of the work reported in this paper was carried out on human hemoglobin. However, in order to compare our results with these earlier ones, some measurements were made on horse hemoglobin. At an ionic strength of 0.011 in pH 7.0 phosphate, a 0.01-mg/ml solution of horse hemoglobin gave a quantum yield of 0.35. The same quantum yield was found for 0.08 mg/ml of horse hemoglobin solutions in 0.2 and 0.02% borate, pH 9.2. Bücher and Negelein reported quantum yields for 0.1 mg/ml of horse hemoglobin solutions in 0.01 M pyrophosphate buffer as being 0.30 at pH 7. 6 and 0.27 at pH 8.1. In 0.01 M borate buffer at pH 9.1 the quantum yield reported was 0.24. The discrepancies in the two sets of data may be due to the differences in temperature (Bücher and Negelein worked at 4° while the work reported in this paper was done at 20°.) and to a possible difference in the behavior of the system in phosphate and pyrophosphate buffers.

Acetylated Hemoglobin and Myoglobin. It has been shown by Bucci *et al.* (1963) that acetylation of hemoglobin causes it to dissociate into dimers, in which form it is stable over a wide range of concentration. Acetylation, therefore, appeared to offer an ideal test of the relationship between degree of aggregation and quantum yields. A comparison between normal and acetylated hemoglobin was made at a protein concentration and ionic strength favorable to tetramer formation in normal hemoglobin.

All of the measurements reported in Table III were made on the same day and under identical conditions. Acetylated hemoglobin does appear to have a higher quantum yield than normal hemoglobin; ϕ for normal hemoglobin is 0.41 and for acetylated hemoglobin it is 0.51. This 25% difference is outside the estimated error of the method under the conditions of these experiments. On the other hand, the quantum yield of acetylated myoglobin, which was also measured, was found to be unity. Here again, however, it is not possible to conclude that dissociation alone produces the quantum yield change, since the myo-

TABLE III: ϕ of Acetylated Hemoglobin and Acetylated Myoglobin.[a]

Sample	Protein Concn (mg/ml)	Ionic Strength	ϕ
Hb-acetyl	0.1	0.011	0.51
Hb	0.1	0.011	0.41
Mb-acetyl	0.01	0.22	1.0

[a] Reactions were at pH 7.0 and 20°.

globin control is not adequate. If the quantum yield increase found for hemoglobin were due only to the chemical alteration, then the quantum yield of acetylated myoglobin would not change, since a value greater than unity is not possible.

There is another effect of acetylation in these proteins. This is on the value of R_{dark}, the rate constant of approach to equilibrium in the absence of light. As described under Methods, this rate constant is given by

$$R_{dark} = l_d + k\frac{l'P_{CO}}{k'P_{O_2}}$$

At a given value of P_{CO}/P_{O_2}, R_{dark} for acetylated myoglobin is two times that of normal myoglobin and for acetylated hemoglobin it is 1.5 times that of normal hemoglobin.

The α and β Chains of Hemoglobin. The availability of the isolated chains of hemoglobin (as a result of the work of Bucci and Fronticelli, 1965) offered another system with which to study the effect of various parameters on quantum yield. Prior to the separation of the chains, the sulphydryl groups of hemoglobin are reacted with PMB to yield the PMB derivatives. After separation the sulphydryl groups are regenerated by a variety of methods. Because of this the chains are available in both the PMB and the SH forms. It has been shown (Bucci *et al.*, 1965) that the α chains (both SH and PMB) exist as monomers, while the β chains tend to aggregate, β_{SH} very strongly and β_{PMB} less strongly.

In Table IV are presented the results that were obtained. Because of the importance of these results for the issues at stake, several measurements were made on the different preparations and the data were analyzed statistically. The limits of error given in Table IV are the 95% confidence limits determined from the "Student's *t*" distribution (Mood, 1960).

It is clear that the values of ϕ for α_{PMB} and α_{SH} are *not* unity. This is true even though the measurements were made under conditions that should promote dissociation, *i.e.*, an ionic strength of 0.22 and protein concentrations of 0.015 mg/ml. This establishes very clearly that two monomeric heme proteins, hemoglobin α chains and myoglobin, under exactly the same conditions of pH, solvent composition and protein concentration, have different quantum yields. At the low protein concentrations used, β_{PMB} is also a monomer (Bucci *et al.*, 1965) and has a quantum yield which differs both from that of the α chains and from that of myoglobin. The quantum yield must also depend on protein composition.

A comparison of the quantum yields of the isolated chains with those of mixtures of the chains, $\alpha_{PMB} + \beta_{PMB}$ and hemoglobin A, may also be of interest. Under identical conditions of protein concentration (0.015 mg/ml) and ionic strength (0.22), the data indicate that the quantum yield for the isolated SH chains (0.68 for α_{SH} and 0.60 for β_{SH}) differs very little from that of hemoglobin A (0.67), and show only a slight decrease for the mixture of the PMB chains (0.65) in comparison to the PMB chains (0.71 for α_{PMB} and 0.85 for β_{PMB}), which, however, is based on only one experiment on the mixture. However, the interpretation is made difficult by the lack of knowledge of the exact state of aggregation of hemoglobin at these low protein concentrations.

It should be noted that for a given P_{CO}/P_{O_2}, R_{dark} for the α_{PMB}, α_{SH}, and β_{SH} chains differs very little. In contrast, R_{dark} of β_{PMB} chains is always 2–2.5 times as great as for the other chains. These alterations in R_{dark} for α_{PMB} chains are mentioned here only for completeness and are dealt with more fully in a separate communication (Brunori *et al.*, 1966).

CPA-Treated Hemoglobin. Since the degree of association of the heme protein subunits does not appear to be the only or even the principal determinant of the quantum yield of the photochemical CO dissociation, it is interesting to consider the possible effect of other parameters. In particular it was asked if the cooperative effect or heme–heme interactions in hemoglobin might affect the quantum yield.

It has been shown (Antonini *et al.*, 1961) that CPA cleaves the terminal two amino acids from the carboxyl end of the β chains of hemoglobin A to yield an altered hemoglobin, which exhibits no heme–heme interactions. The quantum yields of hemoglobin A and hemoglobin A (CPA) were compared under a set of conditions.

The data are presented in Table V. Each line in the table gives the data from a single experiment in which ϕ for hemoglobin and hemoglobin (CPA) was measured. As will be seen, within experimental error no differences were found in the quantum yields of these two proteins, which shows that there is no apparent relationship between quantum yield and heme–heme interaction.

TABLE IV: ϕ of the Chains of Hemoglobin.[a]

Sample	Protein Concn	Ionic Strength	ϕ
Mb	0.015	0.22	Defined as unity
α_{PMB}	0.015	0.22	0.71 ± 0.06
α_{SH}	0.015	0.22	0.68 ± 0.03
β_{PMB}	0.015	0.22	0.85 ± 0.16
β_{SH}	0.015	0.22	0.60 ± 0.08
$\alpha_{PMB} + \beta_{PMB}$	0.015	0.22	0.65

[a] Reactions were at pH 7.0 and 20°.

Conclusions

The data clearly show that differences in the quantum yield of CO photodissociation cannot be ascribed solely to changes in extent of asociation of the protein. Although the data of Bücher and Negelein could be explained simply by a dependence of quantum yield on the number of subunits in a molecule, the more extended data in this paper clearly establish that several other

TABLE V: Comparison of ϕ for Normal and CPA-Treated Hemoglobin.[a]

Ionic Strength	Protein Concn (mg/ml)	ϕ for Hb	ϕ for Hb(CPA)
0.011	0.1	0.44	0.44
0.22	0.015	0.67, 0.74	0.64
0.22	0.015	0.73	0.69, 0.75
0.22	0.015	0.69	0.75

[a] Reactions were at pH 7.0 and 20°.

factors affect the quantum yield.

The data for myoglobin reported in Table I show that significant changes in the quantum yield can be produced by alterations in ionic strength or protein concentration. In these cases the modification of the quantum yield occurs without changes in the state of aggregation of the protein. Therefore, similar changes in the quantum yield of hemoglobin are not adequate to establish a relationship between quantum yield and protein dissociation. The results obtained for the isolated chains of hemoglobin establish that the quantum yield is dependent on at least one other variable. In addition to ionic strength and protein concentration, the composition of the protein is an important factor, since various monomeric proteins under the same experimental conditions have different quantum yields.

The effect of ionic strength and protein composition on energy quenching, which is indicated by an alteration in quantum yield, is not surprising in view of the effects that solvent and protein composition are known to have on such things as fluorescence quenching (Weber and Teale, 1965). However, the discovery that the concentration of a non aggregating protein can affect the quantum yield of CO photodissociation is most unexpected, since it would appear to indicate some type of intermolecular interaction at very low protein concentrations.

References

Antonini, E. (1965), *Physiol. Rev. 45*, 123.

Antonini, E., Wyman, J., Zito, R., Rossi Fanelli, A., and Caputo, A. (1961), *J. Biol. Chem. 236*, PC60.

Boyer, P. D. (1954), *J. Am. Chem. Soc. 76*, 4331.

Brunori, M., Noble, R., Antonini, E., and Wyman, J. (1966), *J. Biol. Chem. 241*, 5238.

Bucci, E., and Fronticelli, C. (1965), *J. Biol. Chem. 240*, PC551.

Bucci, E., Fronticelli, C., Bellelli, L., Antonini, E., Wyman, J., and Rossi Fanelli, A. (1963), *Arch. Biochem. Biophys. 100*, 364.

Bucci, E., Fronticelli, C., Chiancone, E., Wyman, J., Antonini, E., and Rossi Fanelli, A. (1965), *J. Mol. Biol. 12*, 183.

Bücher, T., and Negelein, E. (1942), *Biochem. Z. 311*, 163.

Dintzis, H. (1952), Ph.D. Thesis, Harvard University, Cambridge, Mass.

de Duve, C. (1948), *Acta Chem. Scand. 2*, 264.

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

Haldane, J., and Smith, J. L. (1896), *J. Physiol. 20*, 497.

Mood, A. M. (1960), Introduction to the Theory of Statistics, New York, N. Y., McGraw-Hill.

Moore, S., and Stein, W. H. (1948), *J. Biol. Chem. 176*, 367.

Rossi-Fanelli, A. (1949), Haemoglobin Symposium, Cambridge, 1948, p 115.

Rossi-Fanelli, A., and Antonini, E. (1958), *Arch. Biochem. Biophys. 11*, 478.

Rossi Fanelli, A., Antonini, E., and Caputo, A. (1961), *J. Biol. Chem. 236*, 391.

Taylor, J. P., and Hastings, A. B. (1939), *J. Biol. Chem. 131*, 649.

Warburg, O. (1949), Heavy Metal Prosthetic Groups and Enzyme Action, Oxford, Clarendon.

Warburg, O., Nagelein, E., and Christian, W. (1929), *Biochem. Z. 214*, 26.

Weber, G., and Teale, F. W. J. (1965), *Proteins 3*, 445.

Zito, R., Antonini, E., and Wyman, J. (1964), *J. Biol. Chem. 239*, 1804.

CORRECTION

In the paper "Binding of Proflavine to α-Chymotrypsin and Trypsin and Its Displacement by Avian Ovomucoids," by Gad Feinstein and Robert E. Feeney, Volume 6, March 1967, page 749, in line 17 of the abstract the word *faster* should read *slower*. The corrected sentence should read "It was found that turkey ovomucoid reacted slower with chymotrypsin than with trypsin."