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A Study of the Properties of Two Porphyringlobin Species Formed in the Reaction of Protoporphyrin IX with Human Globin

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Globin was prepared from the main (A_0) component of human haemoglobin and reacted with protoporphyrin IX; the product, when subjected to chromatography on CM-Sephadex, separated into fast- and slow-moving species. These were isolated for examination. The dissociation constant for the tetramer–dimer equilibrium of fast-moving porphyringlobin was determined at 2.8×10^{-6}M; this is to be compared with values of 2.2×10^{-6}M and 8×10^{-8}M determined for oxyhaemoglobin and the slow-moving porphyringlobin respectively. It was also shown that the thiol groups of fast-moving porphyringlobin react with 4,4'dithiodipyridine at an identical rate with those of oxyhaemoglobin; in comparison, the rates of reaction of deoxyhaemoglobin and porphyringlobin are much slower but are again identical with one another. The quenching of porphyringlobin fluorescence by I^- ions was also studied. The quenching could not be represented by a simple Stern–Volmer relationship (whereas that of porphyrin–apomyoglobin is), but was represented by a model in which the fluorescence of fast-moving porphyringlobin was more accessible to the quencher than that of the slow-moving component. Similarly, fast-moving porphyringlobin was photodecomposed more rapidly by oxygen than the slow-moving species.

Previous studies of the properties of porphyringlobin have emphasized a comparison with the properties of haemoglobin with the object of determining the contribution that the iron atom of haem makes to the structure and function of haemoglobin. Thus Sebring & Steinhardt (1970) have shown that porphyringlobin is almost as stable to acid denaturation as methaemoglobin and have concluded that stability is achieved in both compounds without the contribution of a bond to the iron atom. More recently Noble *et al.* (1972) have considered the question whether the structural differences between deoxyhaemoglobin and oxyhaemoglobin derive from the corresponding change in the state of the iron from a penta- to hexa-co-ordinate structure or from the introduction of a bulky ligand. They state that if the latter alternative is true, porphyringlobin should have the structure of deoxyhaemoglobin, whereas if the former alternative holds, porphyringlobin should have a very different structure. As a result of demonstrating that several reactions of porphyringlobin resemble those of deoxyhaemoglobin more than those of liganded haemoglobin, Noble *et al.* (1972) conclude that the insertion of a bulky ligand into deoxyhaemoglobin is a necessary pre-requisite for the generation of the liganded structure. Parkhurst *et al.* (1970) also found that porphyrin bound to the β chains of a hybrid haemoglobin appears to induce a deoxy-like structure in the hybrid.

The arguments of Noble *et al.* (1972) particularly require the assumption that porphyringlobin possesses a unique structure. This assumption is not tenable, for the work of Sebring & Steinhardt (1970) has already shown that porphyringlobin is not homogeneous. Our own studies (Treffry & Ainsworth, 1974*a*) have confirmed that porphyringlobin is always heterogeneous, but have shown that, under defined conditions of reconstitution of the pigment, two main species can be identified and isolated as stable compounds. From the conditions required to separate the species by gel electrophoresis or chromatography on CM-Sephadex, it is evident that one species (the fast-moving one) is a stronger acid than the other. Defining the two species as fast-moving and slow-moving, we also showed that, although they remained stable when isolated, in admixture the slow-moving derivative was the more stable and grew in proportion as the other diminished. On these grounds we concluded that conformational change could be transmitted between fast- and slow-moving subunits when present in a hybrid porphyringlobin formed by dimer exchange.

In the present paper we report a comparative study of the properties of fast- and slow-moving porphyringlobin. The results we shall describe indicate that fast- and slow-moving porphyringlobin differ in some senses as oxy- and deoxy-haemoglobin respectively.

Experimental

Materials

4,4'-Dithiodipyridine was obtained from R. N. Emanuel Ltd., Wembley, Middx., U.K. Gases were

supplied by the British Oxygen Co. Ltd., Rotherham, Yorks., U.K. All other materials used were obtained from sources given by Treffry & Ainsworth (1974*a*).

Methods

Preparation of porphyringlobin. The preparation of haemoglobin, globin, porphyringlobin and the separation of fast- and slow-moving porphyringlobin were performed by methods described by Treffry & Ainsworth (1974*a*).

Photodecomposition of porphyringlobin. Samples of porphyringlobin undergoing photodecomposition were contained in a glass cuvette fused on to the bottom of a tonometer and equilibrated with the chosen gas mixture contained therein. The cuvette was immersed in a water bath kept at 25°C which had fused silica windows. The sample was uniformly illuminated, through a blue filter (CS 5-57, Corning Glassworks, Corning, N.Y., U.S.A.), by a 500W d.c. xenon arc and Köhler optical system provided by the input lenses of a 250mm focal length monochromator (Bausch and Lomb Optical Co., Rochester, N.Y., U.S.A.). A portion of the light passing through the solution was dispersed by a D292 grating monochromator (Hilger and Watts Ltd., London NW1, U.K.) set at 403nm with 0.2mm slits (dispersion 7nm/mm). Light passing the monochromator was detected by a photomultiplier and recorded to provide the initial rate of photodecomposition. The intensity of light, in ergs·cm^{-2}·s^{-1}, incident on the sample was measured with a thermopile (Hilger–Schwarz, model FT.10, Hilger and Watts Ltd.), and was varied by circular stops placed at the second lens of the optical system. The frequency distribution of quanta in the photolysing light was measured by using a Rhodamine B quantum counter (Weber & Teale, 1957) taking into account the various transmissions of the dispersing monochromator as a function of frequency (Parker & Rees, 1960). With this information and the absorption spectrum of the sample, the light absorbed by the sample was calculated in einsteins·litre^{-1}·s^{-1} (1 einstein = 6.03 × 10^{23} quanta).

Fluorescence measurements. Light from a 500W xenon arc was dispersed by a grating monochromator (Bausch and Lomb Optical Co.) set at 403nm with 0.25mm slits (dispersion 6.6nm/mm). Light passing the monochromator was chopped at 840Hz and focused on the sample contained in a 1cm silica cuvette. The cuvette was mounted in a thermostatically controlled copper block kept at 20°C. Fluorescence emitted by the sample was observed at 90° to the exciting light. After passing a filter (CS 3-66, Corning Glassworks), the fluorescence was detected by an E.M.I. 9558 QC photomultiplier (E.M.I. Electronics Ltd., Hayes, Middx., U.K.); the resulting signal was demodulated and recorded as described by Ainsworth & Winter (1964). The samples examined had a maximum absorbance at 403nm of 0.14 units.

Thiol group reactivity. The rates of reaction of the thiol groups of oxyhaemoglobin, deoxyhaemoglobin and fast- and slow-moving porphyringlobin with 4,4′-dithiodipyridine (4-Py-S-S-4-Py) in 0.1M-potassium phosphate buffer at 25°C were measured spectrophotometrically at 324nm as described by Ampulski *et al.* (1969). The reaction with deoxyhaemoglobin was studied by bubbling N_2 through a 4-Py-S-S-4-Py solution for 30min before adding a small volume of concentrated oxyhaemoglobin.

Ultracentrifugation. Measurements of the sedimentation velocities of fast- and slow-moving porphyringlobin were made by using a Beckman model E analytical ultracentrifuge with u.v. optics. The experiments were conducted at 20°C and at 60000rev./min in the A-ND rotor. The protein concentration was 35μg/ml in 0.1M-potassium phosphate buffer, pH7.0. The sedimentation of oxyhaemoglobin was studied at a protein concentration of 25μg/ml in 0.1M-Tris–HCl buffer, pH7.0, containing 0.09M-NaCl and 1mM-EDTA (Kellett, 1971). Photographs were taken after fixed-time-intervals and the boundary position estimated at one-half the plateau concentration.

Results

Dissociation constants for dimer–tetramer equilibria

Fig. 1 shows data for the sedimentation of fast- and slow-moving porphyringlobin and oxyhaemoglobin. From least-squares fits to the lines sedimentation coefficients, $s_{20,w}$, of 2.76×10^{-13}s and 3.94×10^{-13}s were obtained for fast- and slow-moving porphyringlobin respectively, which may be compared with the value of 2.72×10^{-13}s obtained from oxyhaemoglobin.

Assuming that the partial specific volume is 0.749ml/g and that the diffusion coefficient is 6.9×10^{-7}cm^2·s^{-1} (Tanford, 1961) the molecular weights of fast- and slow-moving porphyringlobin are 38779 and 54964 respectively: oxyhaemoglobin by comparison has a molecular weight of 38613. In turn, if a tetrameric molecular weight of 64500 is assumed, the measured molecular weights indicate that the dissociation constants for the dimer–tetramer equilibrium are 2.16×10^{-6}M, 2.8×10^{-6}M and 8×10^{-8}M for oxyhaemoglobin, fast- and slow-moving porphyringlobin respectively, when calculated with concentration defined by single chains. The dissociation constant for oxyhaemoglobin may be compared with a value of 2.9×10^{-6}M obtained by Kellett (1971) under the same experimental conditions and a value of 2.5×10^{-6}M obtained by sedimentation-velocity and gel-filtration experiments by Chiancone *et al.* (1968). The dissociation constant for fast-

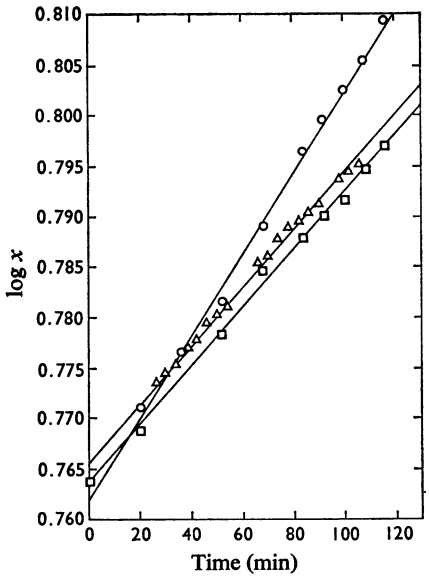

Fig. 1. *Time-course of sedimentation of oxyhaemoglobin* (△), *fast-moving porphyringlobin* (□) *and slow-moving porphyringlobin* (○)

x is the distance in cm of the sedimenting boundary from the axis of rotation. Other details are given under 'Methods'.

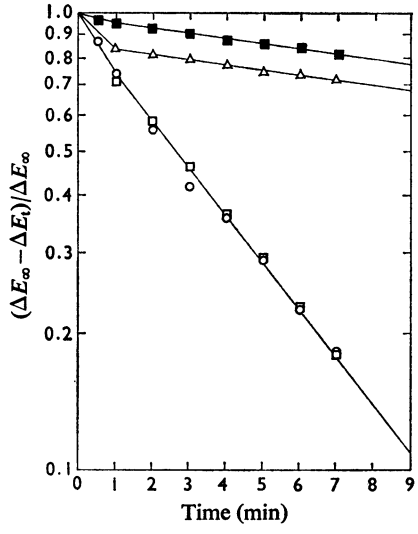

Fig. 2. *Time-course of reaction of 4-Py-S-S-4-Py* (0.230mM) *with slow-moving porphyringlobin* (■; 24.0μM), *deoxyhaemoglobin* (△; 28.4μM), *fast-moving porphyringlobin* (○; 28.3μM) *and oxyhaemoglobin* (□; 28.4μM) *in* 0.05M-*potassium phosphate buffer, pH* 7.0, 25°C

The reaction was monitored spectrophotometrically at 324nm.

moving porphyringlobin is very similar to that found for oxyhaemoglobin. In contrast, the dissociation constant for slow-moving porphyringlobin is much smaller and is similar to values quoted for deoxyhaemoglobin by Antonini & Brunori (1970); it is, however, much larger than the more recent values given for the deoxyhaemoglobin dissociation constant by Kellett (1971) and by Thomas & Edelstein (1972).

Reactivity of the thiol groups of porphyringlobin

The large difference between the reactivity of the thiol groups of oxy- and deoxy-haemoglobin has been used as a diagnostic of their structure (Antonini & Brunori, 1969; Maeda & Ohnishi, 1971; Gibson, 1973). Fig. 2 shows progress curves for the reaction of oxy- and deoxy-haemoglobin, and fast- and slow-moving porphyringlobin with 4-Py-S-S-4-Py in 0.1M-potassium phosphate buffer, pH7.0 (Ampulski *et al.*, 1969). The reactions are first-order with respect to thiol groups and at the completion of the reaction with oxyhaemoglobin and fast-moving porphyringlobin 2.4 cysteine residues had reacted with 4-Py-S-S-4-Py: a similar result was obtained with oxyhaemoglobin by Ampulski *et al.* (1969). The obvious feature of the curves, however, is the marked similarity in the behaviour of fast-moving porphyringlobin and oxyhaemoglobin; and the equally marked similarity of deoxyhaemoglobin and slow-moving porphyringlobin. (The initial fast reaction of deoxyhaemoglobin probably represents a small contamination by oxyhaemoglobin.)

Fig. 3 shows progress curves for the reaction of 4-Py-S-S-4-Py with different samples of fast- and slow-moving porphyringlobin before and immediately after mixing. It will be observed that the reaction of the mixture is slower than the weighted average of the two pigments taken in isolation. This experiment confirms the conclusion reached by Treffry & Ainsworth (1974*a*), that the two species can form mixed hybrids in which the more-stable slow-moving porphyringlobin forces the fast-moving porphyringlobin subunits to become more like itself. A similar conclusion can be drawn from the behaviour of mixtures of fast- and slow-moving porphyringlobin with haemoglobin derivatives (Treffry & Ainsworth, 1974*b*).

Photodecomposition of porphyringlobin

Treffry & Ainsworth (1974*a*) have demonstrated that porphyringlobin is decomposed by light, that more than one absorbing product arises in the decomposition of the chromophore and that several

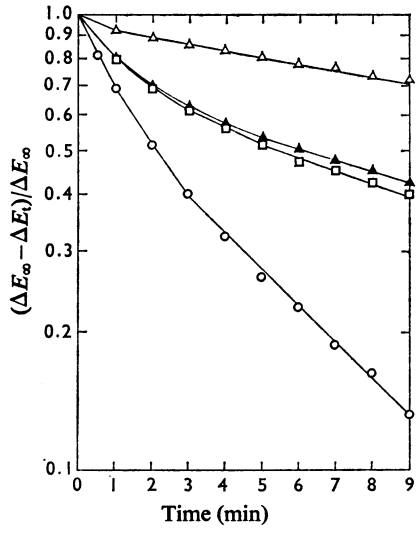

Fig. 3. *Time-course of reaction of* 4-*Py*-*S*-*S*-4-*Py* (0.23mM) *with slow-moving porphyringlobin* (△; 24.0μM), *fast-moving porphyringlobin* (○; 30.0μM) *and a mixture of slow-moving porphyringlobin* (24.0μM) *with fast-moving porphyringlobin* (30.0μM) (▲) *in* 0.05M-*potassium phosphate buffer, pH*7.0, 25°*C*

The reaction was monitored spectrophotometrically at 324nm. □ represents the weighted average of △ and ○.

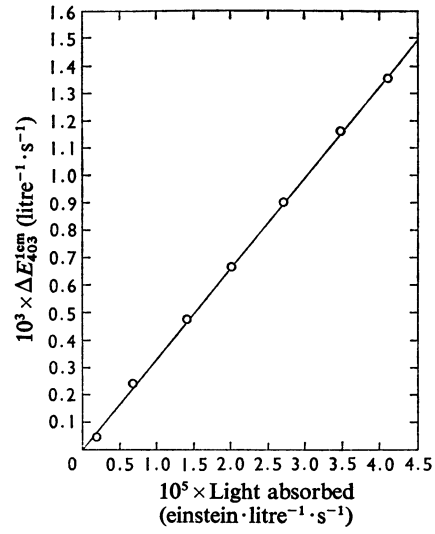

Fig. 4. *Initial rate of absorbance loss at* 403*nm of porphyringlobin* (2.88μM) *in* 0.05M-*potassium phosphate buffer, pH*7.0, 20°*C at equilibrium with atmospheric oxygen, as a function of the light intensity to which it was exposed*

Light absorbed is shown in einstein$\cdot$litre$^{-1}\cdot$s^{-1} (1 einstein = 6.03×10^{23} quanta).

amino acid residues of the globin moiety are destroyed.

Fig. 4 shows the initial rate of absorbance loss at 403nm of an unchromatographed mixture of porphyringlobins, in equilibrium with air, as a function of the intensity of the illuminating light. The linear relationship demonstrates that the photosensitized reaction requires a single photon for each molecule of porphyrin destroyed. The slope of the relationship is 33.2 absorbance units loss per einstein absorbed: a true quantum yield cannot be calculated from this value because it is not known how many of the porphyrin residues are initially oxidizable and because several products with unknown absorbances arise in the reaction: however, assuming that all the porphyrins react to form a single colourless product sets the minimum quantum yield as 2.5×10^{-4} at a pO_2 of 150mm.

Preliminary experiments showed that the rate of photodecomposition of porphyringlobin increased with oxygen partial pressure and that the reaction is therefore a photosensitized oxidation. We shall assume that the initial rate of photodecomposition (as measured spectrophotometrically) is determined by the loss of porphyrin and that the subsequent photodecomposition of the initial product may be ignored. With this assumption, the initial quantum yield (q) is:

$$q = \frac{1}{n}\sum_{j=1}^{j=n} \frac{K_{0j}O_2}{1 + K_{0j}O_2} = \frac{\Delta E}{\Delta I \Delta \epsilon} \qquad (1)$$

where j refers to porphyrin groups which absorb light equally but which are distinguished by different oxidation constants, K_0. The oxidation constant is defined by the ratio $k_o/(k_i + k_f)$, which includes the rate constant for oxidation (k_o), fluorescence, (k_f) and other processes directly competitive with both (k_i). ΔE and ΔI represent the rates of absorbance loss and absorption of light (in einsteins$\cdot$litre^{-1}) and $\Delta\epsilon$ represents the extinction coefficient change in forming the first photoproduct. Fig. 5 shows that $\Delta I/\Delta E$ is linearly related to the reciprocal of oxygen partial pressure, both for fast- and slow-moving porphyringlobin. The intercepts (at infinite oxygen partial pressure) correspond to 40 absorbance units loss per einstein absorbed for fast-moving porphyringlobin as against 27 for slow-moving porphyringlobin. The linear relationship also allows the calculation of an apparent oxidation constant; for fast-moving porphyringlobin, $K_o = 3.58 \times 10^4$M^{-1}, for slow-moving porphyringlobin, $K_o = 1.68 \times 10^4$M^{-1}.

The linear relationship of Fig. 5 suggests either that the photo-oxidized porphyrins are identical or that higher powers in oxygen concentration arising from the expansion of eq. (1) are negligible. Com-

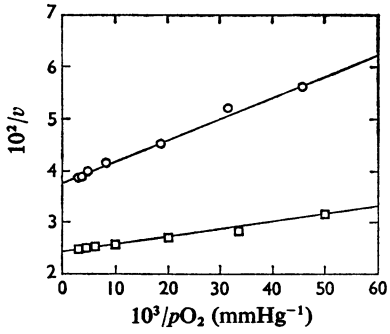

Fig. 5. *Double-reciprocal plot of the initial rate of absorbance loss (v) at 403nm of fast-moving porphyringlobin* (□; 2.26μM) *and of slow-moving porphyringlobin* (○; 2.94μM) *as a function of oxygen partial pressure (mmHg)*

The initial rates were normalized to unit light intensity (expressed in einstein·litre^{-1}·s^{-1}). Potassium phosphate buffer (0.05M; pH7.0, 25°C) was used.

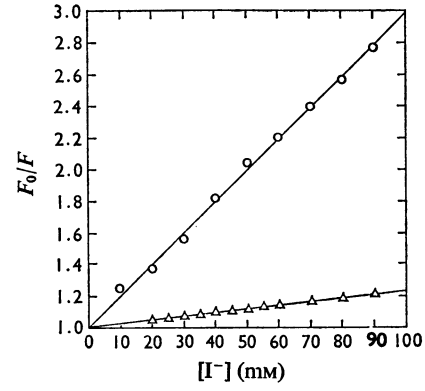

Fig. 6. *Quenching by I$^-$ ions of the fluorescence of porphyrin* (○; 1.09μM) *in* 0.1M-*Tris, excited at* 398*nm, and porphyrin–apomyoglobin* (△; 0.895μM) *in* 0.05M-*potassium phosphate buffer, pH*7.0, *excited at* 403*nm at* 20°C

parison of the values of K_0 with the range of oxygen concentration studied (36.5μM–0.55mM) shows that the latter explanation is untenable. Assuming therefore the identity of photo-oxidized porphyrins, it is tempting to suggest, in keeping with the thiol group reactivity of the pigments, that the relative values of the intercepts and oxidation constants can be explained if the porphyrins of fast-moving porphyringlobin are more accessible to oxygen than those of slow-moving porphyringlobin. This view must be treated with caution, however, for we have found that porphyrin is not photo-oxidized in pyridine or dioxane but is oxidized in glycerol, dimethyl sulphoxide and ethylene glycol. For this reason, it should be remembered that photo-oxidation competes with other processes for the energy of excited porphyrin molecules and that the balance of all these processes depends on the environment.

The model we have employed shows that the vertical intercepts of Fig. 5 equal $n/x\Delta\epsilon$ where n represents the number of porphyrin residues, x of which are photo-oxidized. The large values of $n/x\Delta\epsilon$ obtained in relation to reasonable estimates of $\Delta\epsilon$, make it probable that only a small fraction of the porphyrin residues are initially capable of being oxidized; of course, with time and the disruption of the protein structure (Treffry & Ainsworth, 1974*a*), all the porphyrin residues are ultimately destroyed.

Quenching of porphyrin fluorescence by I$^-$ ions

Fig. 6 shows that I$^-$ ions (Q) quench the fluorescence of porphyrin dissolved in 0.1M-Tris by the Stern–Volmer collisional mechanism, expressed as:

Quantum yield of iodide quenching =

$$\frac{F_0-F}{F_0}=\frac{K_Q Q}{1+K_Q Q}$$

where F_0 and F are fluorescence intensities measured in the absence and presence of quencher respectively. The quenching constant K_Q is defined by the ratio $k_q/(k_1+k_f)$, which includes the rate constant for I$^-$ quenching (k_q), fluorescence (k_f) and other processes directly competitive with both (k_1). The determined value for K_Q is 20.0M^{-1}. Fig. 6 also shows that the quenching of porphyrin bound to apomyoglobin is represented by the same relationship and that K_Q is 2.4M^{-1}.

The quenching of the porphyrin fluorescence of fast- and slow-moving porphyringlobin may be represented by a Stern–Volmer relationship that sums the contributions of the several fluorophors. Assuming each porphyrin absorbs light equally, and neglecting energy transfer between them, we have (Lehrer, 1971):

$$\frac{\Delta F}{F_0}=\sum_j\frac{(q_{0j}-q_j)}{\sum_j q_{0j}}=\frac{1}{\sum_j q_{0j}}\sum_j\frac{q_{0j}K_{Qj}Q}{1+K_{Qj}Q}$$

or

$$\left(\frac{\Delta F}{F_0}\right)^{-1}=\left(\sum_j\frac{f_j K_{Qj}Q}{1+K_{Qj}Q}\right)^{-1} \qquad (2)$$

where q_0 and q are the fluorescence quantum yields corresponding to F_0 and F; f_j is F_{0j}/F_0 and F_0 is $\sum_j F_{0j}$. Fig. 7 shows that $F_0/\Delta F$ is linearly related to the reciprocal I$^-$ concentration, effectively by a single term in the summation. This situation suggests either that the quenched fluorophors of the given pigment are identical or that the higher powers in Q arising from the expansion of eqn. (2) are negligible. The estimated value of K_Q equals 16.3M^{-1} for slow-moving porphyringlobin and 8.8M^{-1} for fast-moving porphyringlobin, indicating that the latter explanation is not tenable. The intercepts (at infinite quencher concentration) show that three times as much fluorescence is accessible to I$^-$ quenching in fast-moving

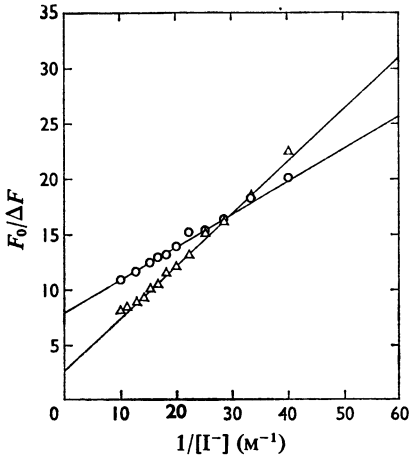

Fig. 7. *Quenching by I^- ions of the fluorescence of fast-moving porphyringlobin* (△; 0.850 μM) *and slow-moving porphyringlobin* (○; 0.676 μM) *in* 0.05 M-*potassium phosphate buffer, pH* 7.0, *excited at* 403 *nm at* 20°C

porphyringlobin as in slow-moving porphyringlobin, an observation that is in keeping with previous data suggesting that fast-moving porphyringlobin has a more open structure than has slow-moving porphyringlobin. The lower quenching constant found for fast-moving porphyringlobin (and the even lower constant found for porphyrin–apomyoglobin) indicates, however, that factors other than the accessibility to I^- determines K_Q. In this connexion it is noteworthy that the quantum yields of fast- and slow-moving porphyringlobin are identical to within a few percent and so therefore are the ratios $k_f/(k_i+k_f)$ for the two pigments. k_f for both pigments are also the same, as shown by their almost identical absorption bands of least frequency: it therefore follows that the corresponding values of k_i are identical and that the differences in K_Q must be ascribed to change in k_q.

It may be noted that the maximum concentration of I^- employed in these experiments (0.1 M) does not affect the spectrum of porphyringlobin and that the fluorescence intensity increased linearly with the concentration of the pigment.

Discussion

The X-ray crystallographic studies of Perutz *et al.* (1968) and Perutz (1970), in conjunction with extensive chemical evidence accumulated by other workers (Benesch & Benesch, 1963) have demonstrated that oxyhaemoglobin differs from deoxyhaemoglobin both in its tertiary and quaternary structure and it is accepted that structural change accompanies the oxygenation of deoxyhaemoglobin (Perutz, 1970; Gibson, 1973).

The results reported in the present paper and by Treffry & Ainsworth (1974*a*) demonstrate that porphyringlobin is also able to take up different structural forms: this fact alone precludes the argument of Noble *et al.* (1972) that structural change requires the introduction of a bulky ligand. Our results, however, go further and show that the two forms of porphyringlobin, fast- and slow-moving, resemble oxy- and deoxy-haemoglobin respectively in some of their properties. The main points in this comparison are as follows.

The method of separating fast- and slow-moving porphyringlobin, both by gel electrophoresis and by chromatography on CM-Sephadex (Treffry & Ainsworth, 1974*a*), indicates that fast-moving porphyringlobin is the stronger acid in the region of neutrality, thus reproducing the behaviour of haemoglobin that is responsible for the alkaline Bohr effect.

The different dissociation constants for the dimer–tetramer equilibria of oxy- and deoxy-haemoglobin reflect directly the differences in the quaternary structures of the two pigments and the stabilizing forces holding the dimers together. Thus the dissociation constant for oxyhaemoglobin of 10^{-6} M decreases to 10^{-12} M when six additional salt bridges form between the subunits of deoxyhaemoglobin. Fast-moving porphyringlobin has a very similar dissociation constant to oxyhaemoglobin, but, although the dissociation constant of slow-moving porphyringlobin is 25 times smaller, it does not approach the very low value now ascribed to deoxyhaemoglobin (Thomas & Edelstein, 1972). On these grounds, it must appear that not all the salt links of deoxyhaemoglobin are formed in slow-moving porphyringlobin. It does appear, however, that slow-moving porphyringlobin is the more-stable porphyringlobin, for it increases gradually at the expense of fast-moving porphyringlobin (Treffry & Ainsworth, 1974*a*).

The differences in the rates of reaction of the β-93 cysteine residues of oxy- and deoxy-haemoglobin with various reagents have been used to identify the respective structures (Antonini & Brunori, 1969). Perutz (1970) has shown that the differences arise from changes in both tertiary and quaternary structure. Thus in the deoxy structure, the salt bridge between histidine $146\beta_2$ and aspartate $94\beta_2$ hinders access to cysteine $93\beta_2$: in the oxy conformation this bridge is broken and cysteine $93\beta_2$ becomes accessible to thiol reagents. Cysteine $93\beta_2$ is also situated in the $\alpha_1\beta_2$ interface, which undergoes the bigger interfacial change as the quaternary structure changes from that of deoxy- to oxy-haemoglobin (Perutz *et al.*, 1968). Similar changes in structure must typify the difference between slow- and fast-moving porphyringlobin, for the thiol group

reactivity of these pigments exactly parallels the corresponding reactivity of deoxy- and oxy-haemoglobin.

Maeda & Ohnishi (1971) and Gibson (1973) have also used the change in thiol group reactivity during oxygenation to demonstrate that structural change may be transferred between subunits and does not exclusively depend on the presence of ligand on the individual chains. We, too, have demonstrated that mixtures of fast- and slow-moving porphyringlobin contain hybrids in which the more-stable slow-moving conformation impresses its structure on the dimer originating in fast-moving porphyringlobin, thereby decreasing the rate of its reaction with a thiol reagent.

Although the photo-oxidation and fluorescence quenching by I^- of porphyringlobin are determined by a number of factors that preclude a single interpretation, the data that were obtained do allow the conclusion that the porphyrin residues of fast-moving porphyringlobin are more accessible than those of slow-moving porphyringlobin: in this there is a further resemblance between these pigments and oxy- and deoxy-haemoglobin respectively.

In summary therefore we have shown that different protein conformations are accessible to porphyringlobin and that two of them have properties typical respectively of oxy- and deoxy-haemoglobin. It appears, in consequence, that the iron atom of haemoglobin, and its ligands, do not so much generate structures as stabilize structures that are already available.

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