PHOTODISSOCIATION OF CARBON MONOXIDE FROM SOME NEW HEMOGLOBIN DERIVATIVES. A POSSIBLE CASE OF ENERGY TRANSFER.

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Summary : The photodissociation of carbon monoxide bound to the divalent hemes in hemoglobin valency hybrids $\alpha^{+}_{2}(\beta CO)_{2}$ and $\beta^{+}_{2}(\alpha CO)_{2}$ has been studied. A parameter proportional to the quantum yield has been measured for the derivatives having the trivalent heme in the aquo or fluoride form, and compared to the same parameter for an equimolecular mixture of carboxyhemoglobin and ferrihemoglobin having identical overall absorption spectra. It has been shown that the presence of ferrihemes on a given type of chain has a significant effect on the photoreactivity of the carboxyheme situated on the complementary chain.

It is well known that carbon monoxide bound to myoglobin and hemoglobin is dissociated by the action of light. The quantum yield for myoglobin and hemoglobin has been measured by Bücher and Negelein (1) and more recently by Noble et al (2) who extended the measurements to the isolated chains of hemoglobin. Despite much information obtained by Noble et al on the effects of solvent composition (ionic strength) and protein concentration on the quantum yield, some aspects of the reaction are not understood, particularly the reasons why the quantum yield for myoglobin is always higher than that for hemoglobin or the isolated chains including the α chain which is a monomer. In the course of a systematic study of the photodissociation

reaction, we have examined the behaviour of some systems where the dissociation by light takes place in the presence of additional chromophores incorporated into the molecule. In order to be assured that the systems are as close to carboxyhemoglobin in structure as possible, the additional chromophores were provided by a part of the heme groups themselves which, when oxidized to the trivalent state, do not fix carbon monoxide and are thus not directly involved in photolysis. Direct oxidation of hemoglobin by less than equivalent amount of oxidant (c.f. ferricyanide) can in principle provide such material, but was not used in this work; the use of such a procedure leads to a mixture of different molecular species containing from 0 to 4 oxidized groups per molecule with an unknown distribution of oxidized heme groups on the α and β chains and is unfavourable for analysis. We have therefore used the recently described hemoglobin valency hybrids (3), $\alpha_2^+ (\beta CO)_2$ and $\beta_2^+ (\alpha CO)_2$ which, as their compositions show, are pure species carrying the oxidized groups either on the α chains or on the β chains as the case might be. These molecules contain two distinct kinds of chromophores, namely the carboxy hemes (functional) on one hand, where the photochemical event takes place, and, on the other hand the oxidized groups (non functional) which absorb light energy but cannot utilise it <u>in situ</u> for chemical work. Moreover, the absorption spectra of the non-functional groups change as a function of the ligand attached thereon. (Fig. 1).

In this communication, we shall describe the photoreactivity of the valency hybrids when the non-functional group possesses respectively the aquo (H_2O) and fluoride (F^-) spectra.

Materials and methods

Human oxyhemoglobin (Hb O_2) was prepared according to the method of Drabkin (4); methemoglobin (Hb^+) was prepared from Hb O_2 by the action of nitrite. Human hemoglobin valency hybrids, $\beta_2^+ (\alpha CO)_2$ and $\alpha_2^+ (\beta CO)_2$ were prepared as described earlier (3). The experimental solutions (in 0,1 M

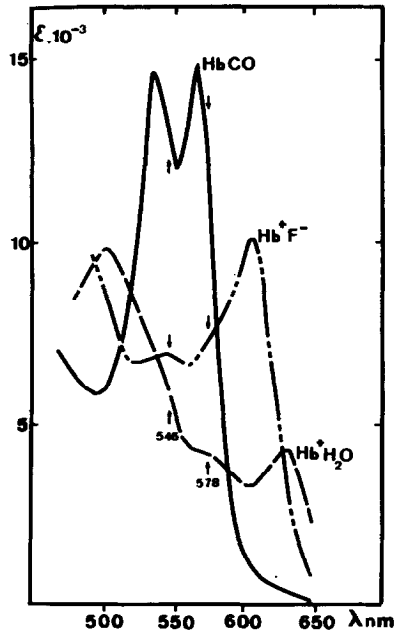

Fig. 1 Absorption spectra of carboxyhemoglobin, aquo and fluoride derivatives of ferrihemoglobin, in 0.1 M phosphate buffer at pH 6.9

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phosphate) contained a total protein concentration adjusted to give 0.96×10^{-6} M in reduced carboxy groups; the fluoride derivatives were obtained by adding potassium fluoride (KF) at a final concentration of 0.15 M; they were compared with the aquo derivatives at the same ionic strength, in the presence of 0.15 M potassium chloride (KCl). The final pH was always 6.9.

The photochemical reactivity of the different compounds was measured at 20° C by the method of Noble et al (2), slightly modified as described elsewhere (5). Briefly, the procedure followed consists in shining light on a mixture of HbCO and HbO_2 initially at equilibrium in the dark and measuring the apparent first order rate constant of approach to a new state of equilibrium in the light (dark $\longrightarrow$ light). If R is this rate constant and R_o is the rate constant of the reverse reaction when light is removed

(light→dark), the quantum yield of photodissociation can be obtained from the relation $R - R_o = 2.3\varepsilon I_o \varphi$, where I_o= light intensity and ε= molar extinction coefficient of the photoreactive species (HbCO) at the given wavelength. Following the suggestion of Noble et al (2), we measured the slope $\frac{\Delta R}{\Delta I}$ for each hemoprotein when the light intensity was successively reduced by neutral calibrated filters. Best straight line alignments of R versus I were obtained in the region of low light intensities. The slope $\frac{\Delta R}{\Delta I}$ multiplied by $\frac{1}{\varepsilon}$ gave a number Φ proportional to the true quantum yield φ; for comparing the different hemoproteins, it was considered sufficient to measure and list the values of Φ.

The change of the proportion of HbCO & HbO_2 in the reaction mixture following illuminations (6) was followed at 4 200 Å with the help of a Cary 14 recording spectrophotometer.

The values of Φ were determined in each case for actinic light of two wavelengths, namely 546 nm and 578 nm.

A summary representation of the results is given in the tables I and II.

Results and discussion

For HbCO, we obtained a value of $\Phi = 25 \times 10^{-7}$ in 0.1 M phosphate and $\Phi = 40 \times 10^{-7}$ in 0.1M phosphate + 0.15 M KCl or KF, at wavelengths 546 nm and 578 nm. The photoreactivity remained unaltered, at a given ionic strength, when the solution contained, in addition to HbCO, an equivalent concentration of Hb^+H_2O or Hb^+F^-. For these equimolecular mixtures also, the value of Φ did not change between 546 and 578 nm.

In contrast, the values of Φ for the valency hybrids were significantly different, both as a function of the ligand bound to the non-functional chromophore as well as the wavelength of dissociating light. The differences were more marked in the case of the compound containing the oxidized heme group on the β chains.

While comparing the quantum yields of the valency hybrids to those of a 1:1 mixture of HbCO and Hb^+F^-, it may be recalled that the overall

Table I

Values of $10^7 \Phi$* for different hemoproteins in 0.1 M phosphate buffer at pH = 6.9. Protein concentration 0.96×10^{-6} M in reduced carboxyheme.

Irradiating wavelength \ Proteins	MbCO	HbCO	$(\alpha^+H_2O)_2\ (\beta CO)_2$	$(\alpha CO)_2\ (\beta^+H_2O)_2$
546 nm	45 ± 3	25 ± 2	28 ± 2	61 ± 3
578 nm	45 ± 3	25 ± 2	23 ± 2	46 ± 2

Table II

$10^7 \Phi$ for different hemoproteins in 0.1 M phosphate buffer plus 0.15 M KCl or KF. The final pH is 6.9. Protein concentration : 0.96×10^{-6} M in reduced carboxyheme.

Irradiating wavelength \ Proteins	HbCO	$(\alpha^+H_2O)_2\ (\beta CO)_2$	$(\alpha^+F^-)_2\ (\beta CO)_2$	$(\alpha CO)_2\ (\beta^+H_2O)_2$	$(\alpha CO)_2\ (\beta^+F^-)_2$
546 nm	40 ± 2	43 ± 2	46 ± 2	50 ± 3	57 ± 3
578 nm	40 ± 2	38 ± 2	39 ± 2	47 ± 2	51 ± 3

* The values of Φ for the different hemoproteins allow comparison of their true quantum yield φ since $\Phi = f(I_o) \cdot \varphi$

spectra are almost identical in the two cases, and the solutions are strictly identical in protein and salt concentration, the proportion of oxidized and reduced heme groups, etc... The fundamental difference lies in the fact that the hybrids carry both types of chromophores on the same molecule and presumably oriented in a given geometry whereas in the case of a 1:1 mixture, the ferri and ferro hemes are carried by different molecules having their own movements. The observed increase in quantum yield of the hybrids implies a sensibilization of the functional ferro groups through their attachment to complementary chains carrying the ferri groups.

The quantum yield of photodissociation for hemoproteins so far studied are not easily understood on the basis of known parameters, and must depend on many factors including the vibrational energy levels of the reactive chromophore. In a general way, after the optical excitation of the heme chromophore (Fe^{II} or Fe^{III}), disactivation occurs which may be accomplished by internal conversion, or, in part, by doing chemical work, for example photolysis. The disactivation of a given chromophore may also take place, under favourable conditions, by transfer to an acceptor. The increased photoreactivity of the hybrid species, particularly $\beta_2^+(\alpha CO)_2$ compared to HbCO , $(\alpha CO)_2$ $(\beta CO)_2$, may thus arise from a change in the vibrational structure of the Fe^{II}-CO chromophore on the α chain in a way that less energy is dissipated by internal conversion, leaving a larger part for chemical work. In this hypothesis, one has to admit that the vibrational structure of the heme group on the α chain suffers a modification when the heme group of the partner β chain changes from the reduced carboxy to the ferri state, involving a mechanism similar to that believed to operate in heme - heme interaction in hemoglobin (7). On the other hand, if the vibrational structure of the photoreactive chromophore is supposed to remain unchanged, the increased photoreactivity of this group may be explained as arising from a coupling between two types of chromophores ($Fe^{II}CO$ and $Fe^{III}H_2O$ or $Fe^{III}F^-$) giving rise to an energy transfer from the non-functional ferri to the functional ferro group.

The present data are not adequate to decide the validity of one or other of the hypothesis; further informations are being obtained by a study of the photochemical action spectra (5).

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