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A CHARGE-TRANSFER INTERPRETATION OF THE INTERACTIONS OF HEMOGLOBIN WITH OXYGEN AND CARBON MONOXIDE

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SUMMARY

It is shown that the use of the charge-transfer theory to describe the interaction between hemoglobin and O_2 or CO leads to the prediction of a linear relationship between log K and the quantity ($\lambda^{\alpha}_{\mathrm{HbO_2}} - \lambda^{\alpha}_{\mathrm{HbCO}}$), where K is the equilibrium constant of the reaction $HbO_2 + CO \rightleftharpoons HbCO + O_2$ and $\lambda^{\alpha}_{\mathrm{HbO_2}}$ and $\lambda^{\alpha}_{\mathrm{HbCO}}$ are the wavelengths of the so-called α-bands in the absorption spectra of oxyhemoglobin and carboxyhemoglobin. Such a linear relation was long ago observed experimentally for a number of vertebrate hemoglobins, but was never satisfactorily explained. The fact that the charge-transfer theory does explain this seemingly unlikely experimental relationship provides support for the view that the interaction between hemoglobin and O_2 or CO may be regarded and treated as a charge-transfer process.

INTRODUCTION

There have been proposed in recent years at least two significantly different descriptions of the nature of the interaction between hemoglobin and O_2 or CO. It was suggested by WEISS that this is basically an electron-transfer process, with the Fe^{2+} of the hemoglobin acting as the donor and the O_2 or CO molecule being the acceptor[1]; thus the iron in HbO_2 or HbCO could be roughly described as being in the Fe^{3+} state. PAULING, on the other hand, sees the iron as remaining in the Fe^{2+} state but forming a double bond with an O_2 molecule, and a triple bond with CO (ref. 2).

It is the purpose of this paper to point out some experimental evidence which appears to be relevant to this question, and to show that it can be interpreted as support for the charge-transfer model.

RESULTS AND DISCUSSION

It was found some years ago that there exists a direct correlation, for the hemoglobins of thirteen different species of vertebrates, between the equilibrium constant of the reaction $HbO_2 + CO \rightleftharpoons HbCO + O_2$ and the difference between the so-called α-bands in the absorption spectra of HbO_2 and $HbCO$[3]. When log K was plotted against the span, S, defined as $S = \lambda^{\alpha}_{\mathrm{HbO_2}} - \lambda^{\alpha}_{\mathrm{HbCO}}$, the experimental points indicated an approximately linear relationship which could be described by the equation $\log K = 0.05\,S$, for S in Ångstrom units. It was later shown that vertebrate myoglobins seem to follow essentially the same relation[4,5], but that non-vertebrate hemoglobins deviate very markedly[6].

That there should exist a correlation between these particular quantities does not appear obvious, and to the writer's best knowledge, no explanation of this fact has been offered. It will be shown now that this correlation is consistent with a charge-transfer description of the hemoglobin–O_2,CO interaction.

The charge-transfer theory was originally proposed by MULLIKEN as an interpretation of the formation of complexes between electron donors and acceptors[7,8]. In the present work, the theory is used in a somewhat modified form, developed by FLURRY[9]. The basis of this treatment is that the ground state of the complex is described by a wave function Φ_N, given by

$$\Phi_N = a\Phi_D + b\Phi_A \qquad (1)$$

where Φ_D and Φ_A are the wave functions of the molecular orbitals which serve as donor and acceptor, respectively, of the electron which is transferred between the two interacting entities. The complex will also have an excited state, which, in terms of the approximations made in the theory, is

$$\Phi_E = b\Phi_D - a\Phi_A \qquad (2)$$

a and b having the same values as in Φ_N. Since a is usually considerably larger than b, the excited state, in general, simply corresponds to the complex in a much more ionic form; that is, the degree of electron transfer is much greater than in the ground state. The system can undergo transitions between these states, Φ_N and Φ_E, and these can be observed spectroscopically, as so-called "charge-transfer" bands.

In FLURRY's treatment, the energies of the ground and excited states are given by

$$E_N = a^2D + b^2A + 2ab\beta_{DA} - b^2V_{es} \qquad (3)$$

and

$$E_E = b^2D + a^2A - 2ab\beta_{DA} - a^2V_{es} \qquad (4)$$

In these equations, D is interpreted as the negative of the ionization potential of the donor, A as the negative of the electron affinity of the acceptor, β_{DA} is a quantity which reflects the extent of interaction between the donor and the acceptor, and $-V_{es}$ is defined as the potential energy of the electrostatic attraction between the two components of the complex which would result if there were a complete transfer of one electron[9]. The energy of the $\Phi_N \rightarrow \Phi_E$ transition is then

$$\Delta E_{CT} = E_E - E_N = (b^2 - a^2)(D - A + V_{es}) - 4ab\beta_{DA} \qquad (5)$$

The quantity β_{DA} is rather difficult to evaluate, but fortunately it will not be necessary to do so. Since the function Φ_N, as well as the basis orbitals Φ_A and Φ_D, can be taken to be normalized, it follows that $\int \Phi_N{}^*\Phi_N d\tau = 1$, so that $a^2 + b^2 + 2ab\int \Phi_A\Phi_D d\tau = 1$. The approximation is made that the overlap integral, $\int \Phi_A\Phi_D d\tau$, can be set equal to zero, which leaves $a^2 + b^2 = 1$. Substituting this into equations (3) and (5) leads to

$$E_N = (1 - b^2D) + b^2A - b^2V_{es} + 2b(1 - b^2)^{1/2}\beta_{DA}$$
$$\Delta E_{CT} = (2b^2 - 1)(D - A + V_{es}) - 4b(1 - b^2)^{1/2}\beta_{DA}$$

These two equations can be combined to eliminate β_{DA}, yielding finally

$$E_N = 0.5(D + A - V_{es} - \Delta E_{CT}) \tag{6}$$

The energy of formation of the complex in its ground state is $\Delta E_N = E_N - D$, which is given by

$$\Delta E_N = 0.5(A - D - V_{es} - \Delta E_{CT}) \tag{7}$$

It will now be suggested that the interaction between hemoglobin and O_2 or CO is of the charge-transfer type, and that the α-bands in the observed spectra correspond to the $\Phi_N \rightarrow \Phi_E$ transitions*. It is not immediately obvious whether the direction of the charge transfer should be taken as Hb → O_2,CO or O_2,CO → Hb. A recent study of the infrared spectrum of HbCO provides some relevant information, however[11]. The stretching frequency of the C–O bond was observed to be about 1951 cm^{-1}, which represents a decrease from the value for free gaseous CO, 2143 cm^{-1} (ref. 12). Now, it is known that the loss of an electron by CO is associated with an increase in its vibration frequency**, while electrons which are gained are generally assumed to enter an anti-bonding π-orbital, which would presumably have the effect of weakening the bond and lowering the vibration frequency. Hence, the observed decrease, from 2143 to 1951 cm^{-1}, suggests that the charge transfer is from the hemoglobin to the CO. It seems reasonable to expect that the same holds true when O_2 is involved instead of CO.

The energy of formation of the Hb–CO complex will therefore be

$$\Delta E_{N, HbCO} = 0.5(A_{CO} - D_{Fe^{2+}} - V_{es, Hb-CO} - \Delta E_{CT, HbCO}) \tag{8}$$

corresponding to the process Hb + CO → HbCO, while for the Hb–O_2 complex,

$$\Delta E_{N, HbO_2} = 0.5(A_{O_2} - D_{Fe^{2+}} = V_{es, Hb-O_2} - \Delta E_{CT, HbO_2}) \tag{9}$$

corresponding to Hb + O_2 → HbO_2. (It is presumed that the Fe^{2+} is the reactive part of the hemoglobin in these interactions.)

The energy change in a process can be related to the free energy change, ΔF, by $\Delta F = \Delta E + \Delta(PV) - \Delta(TS)$, where P, V, T, and S are, respectively, the pressure, volume, temperature, and entropy of the system. When the process occurs at a constant temperature, then $\Delta F = \Delta E + \Delta(PV) - T\Delta S$; when, in addition, it involves gases, then—if they can be assumed to be behaving essentially ideally, as, for instance, when they are at low pressures—the term $\Delta(PV)$ can be written $(\Delta n)RT$, where Δn is the change in the number of moles of gases in the course of the process. The free energies of formation of HbCO and HbO_2 will therefore be $\Delta F_{HbCO} = \Delta E_{N, HbCO} + (\Delta n)_{HbCO}RT - T\Delta S_{HbCO}$ and $\Delta F_{HbO_2} = \Delta E_{N, HbO_2} - (\Delta n)_{HbO_2}RT - T\Delta S_{HbO_2}$. The $(\Delta n)RT$ term will be the same in both cases, and the $T\Delta S$ terms should also be very nearly the same. Thus, $\Delta F_{HbCO} - \Delta F_{HbO_2} = \Delta E_{N, HbCO} - \Delta E_{N, HbO_2}$, and substituting from Eqns. 8 and 9,

$$\Delta F_{HbCO} - \Delta F_{HbO_2} = 0.5\,[(A_{CO} - A_{O_2}) - (V_{es, Hb-CO} - V_{es, Hb-O_2}) - (\Delta E_{CT, HbCO} - \Delta E_{CT, HbO_2})] \tag{10}$$

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* The suggestion that the α-bands represent charge-transfer transitions has been made previously; see, for example, MAHLER AND CORDES[10].

** The vibration frequency of CO^+ is 2184 cm^{-1} (ref. 12). The effect upon the C–O bond of the molecule's losing an electron has been discussed by POLITZER[13].

The electrostatic attraction between the two parts of the complex should be quite similar in HbCO and HbO_2, so the corresponding terms in Eqn. 10 will be assumed to cancel.

The above free energies of formation do not refer to standard conditions, which would require that the gaseous CO and O_2 be at pressures of 1 atm. It can easily be shown, however, that the difference between the free energies of formation of these complexes will be the same when the gases are at one pressure as when they are at another (see APPENDIX). Thus, $\Delta F_{\mathrm{HbCO}} - \Delta F_{\mathrm{HbO_2}} = \Delta F^0_{\mathrm{HbCO}} - \Delta F^0_{\mathrm{HbO_2}}$, where the superscript denotes standard conditions. So Eqn. 10 becomes

$$\Delta F^0_{\mathrm{HbCO}} - \Delta F^0_{\mathrm{HbO_2}} = 0.5\ [(A_{\mathrm{CO}} - A_{\mathrm{O_2}}) - (\Delta E_{\mathrm{CT,\,HbCO}} - \Delta E_{\mathrm{CT,\,HbO_2}})] \qquad (11)$$

Since the equilibrium constant of a reaction is related to the standard free energy change by $\Delta F^0 = -2.303\ RT \log K$, then

$$\begin{aligned}\Delta F^0_{\mathrm{HbCO}} - \Delta F^0_{\mathrm{HbO_2}} &= -2.303\ RT\ (\log K_{\mathrm{HbCO}} - \log K_{\mathrm{HbO_2}}) \\ &= -2.303\ RT \left(\log \frac{a_{\mathrm{HbCO}}}{a_{\mathrm{Hb}} a_{\mathrm{CO}}} - \log \frac{a_{\mathrm{HbO_2}}}{a_{\mathrm{Hb}} a_{\mathrm{O_2}}}\right) \\ &= -2.303\ RT \left(\log \frac{a_{\mathrm{HbCO}} a_{\mathrm{O_2}}}{a_{\mathrm{HbO_2}} a_{\mathrm{CO}}}\right) \\ &= -2.303\ RT \log K \end{aligned} \qquad (12)$$

the final K being the equilibrium constant for the reaction $HbO_2 + CO \rightleftharpoons HbCO + O_2$, which is the reaction mentioned at the beginning of this paper; this is the equilibrium constant which was observed experimentally to follow the relation $\log K = 0.05\ S$. Combining Eqns. 11 and 12 gives

$$\log K = \frac{1}{4.606\ RT} [(\Delta E_{\mathrm{CT,\,HbCO}} - \Delta E_{\mathrm{CT,\,HbO_2}}) - (A_{\mathrm{CO}} - A_{\mathrm{O_2}})] \qquad (13)$$

The energies of the $\Phi_{\mathrm{N}} \rightarrow \Phi_{\mathrm{E}}$ transitions can be related to the wavelengths of the radiations absorbed by $\Delta E_{\mathrm{CT}} = \boldsymbol{h}\nu_{\mathrm{CT}} = \boldsymbol{h}c/\lambda_{\mathrm{CT}}$, $\boldsymbol{h}$ and c being PLANCK's constant and the speed of light, respectively; hence,

$$\log K = \frac{\boldsymbol{h}c(4.606\ RT)^{-1}}{\lambda_{\mathrm{CT,\,HbO_2}} \lambda_{\mathrm{CT,\,HbCO}}} (\lambda_{\mathrm{CT,\,HbO_2}} - \lambda_{\mathrm{CT,\,HbCO}}) - \frac{A_{\mathrm{CO}} - A_{\mathrm{O_2}}}{4.606\ RT} \qquad (14)$$

The observed wavelengths, $\lambda_{\mathrm{CT,\,HbO_2}}$ and $\lambda_{\mathrm{CT,\,HbCO}}$, each vary by less than 0.4 % in the thirteen species of vertebrates studied, so that the product $\lambda_{\mathrm{CT,\,HbO_2}} \cdot \lambda_{\mathrm{CT,\,HbCO}}$ is essentially constant.

Eqn. 14 therefore represents a linear relationship between the logarithm of the equilibrium constant for the reaction $HbO_2 + CO \rightleftharpoons HbCO + O_2$ and the difference between the absorption wavelengths of HbO_2 and HbCO—exactly the sort of relation found experimentally by ANSON *et al.*[3]. There are differences in detail: the experimental line was drawn as passing through the origin and having a slope of 0.05, while Eqn. 14 would have a non-zero intercept on the log K axis (the constant term $(A_{\mathrm{CO}} - A_{\mathrm{O_2}})/4.606\ RT$), and a slope which comes out to be about 0.0033. Such discrepancies, however, are to be expected. First of all, the degree of uncertainty in the experimental line, as indicated by ANSON *et al.*[3], is such that in reality it may well cross the axes at significant distances from the origin. (The value of the quantity $(A_{\mathrm{CO}} - A_{\mathrm{O_2}})/4.606 RT$ cannot be determined at present; there are not reliable data available for the electron affinities of O_2 or CO.) Furthermore, the derivation of Eqn. 14 involved a number of approximations. These were pointed out at the time, but one important one should be mentioned again; this is the neglect of the overlap between the donor and acceptor orbitals, $\int \Phi_A \Phi_D d\tau$. It is standard procedure in most applications of the charge-transfer theory to set this integral equal to zero; this is a significant approximation, however, and must be expected to have some effect upon the results.

What is important in the present work is not the numerical values of the slope and intercept of the straight line predicted by Eqn. 14, but rather the fact that Eqn. 14 does predict a straight line. It has been shown, then, that by acting upon the assumption of a charge-transfer interaction, it is possible to obtain, in a reasonably straightforward manner, exactly the same type of relationship as has been observed experimentally between two quantities which, on the surface, seem unlikely to have any direct relation at all. The success of the approach which has been used here offers support for the view that the interactions of Hb with O_2 and CO can validly be regarded and treated as charge-transfer processes*.

APPENDIX

From the thermodynamic relation $(\partial F/\partial P)_T = V$, it follows that $(\partial \Delta F/\partial P)_T = \Delta V$, and therefore $\Delta F(P_2) = \Delta F(P_1) + \int_{P_1}^{P_2} \Delta V \, dP$. For the reaction of hemoglobin with CO or O_2, $\Delta V \approx -RT/P$, where P is the pressure of the reacting gas. Thus, $\Delta F(P_2) = \Delta F(P_1) - RT \ln (P_2/P_1)$, and $\Delta F_{\text{HbCO}}(P_2) - \Delta F_{\text{HbO}_2}(P_2) = \Delta F_{\text{HbCO}}(P_1) - \Delta F_{\text{HbO}_2}(P_1)$.

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* This conclusion, and the general treatment presented in this paper, would be just as valid if the direction of the charge transfer were opposite to what has been assumed; the quantity $(A_{CO} - A_{O_2})$ in Eqn. 14 would then be replaced by $(D_{O_2} - D_{CO})$.