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ON THE THERMODYNAMICS AND KINETICS OF THE SPIN TRANSITION IN AQUOMETHEMOGLOBIN

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SUMMARY
The temperature-induced transition in aquo-methemoglobin, which is attributed to the spin transition on the basis of the kinetic difference spectrum, is investigated from -20 to +20°C by temperature-jump experiments. Reaction and activation enthalpies of the transition are determined. The equilibrium is almost fully on one side, suggesting that two kinds of spin-equilibria exist in this temperature range. The presence of inorganic ions (Cl^-) should be avoided since these ions exhibit additional effects.

Spin transitions play a central role in the function of hemoglobin and have therefore been subject to detailed magnetic and optical investigations (1-6). The aquo-complex of methemoglobin exhibits special characteristics: A temperature-independent mixed spin state is found above 0°C (1) but at low temperatures several anomalous effects were observed and attributed to the specific influence of the freezing of the solution and further phase transitions in ice at lower temperatures (2,3).

A clear analysis of the temperature-induced optical changes is only possible if the various processes can be separated on the time axis. Schwartz & Schimmel (7) have found a rather slow relaxation process in temperature-jump experiments and suggested a correlation with the spin equilibrium. These experiments were performed at a single temperature (13°C) but no detailed difference spectrum and no thermodynamic analysis were given. Here we report on temperature- and pressure-jump experiments of the transition

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of human MetHb·H_2O [1] in water and water/ethyleneglycol mixtures in the temperature range from -20 to +20°C leading to a thermodynamic and kinetic characterization of the transition in liquid solution. We extended the temperature range to subzero values in order to study the system under conditions where anomalous magnetic effects occurred in the frozen state (2). Moreover, it turned out that low temperatures are required to obtain reliable values of the thermodynamic parameters.

METHODS

Human hemoglobin was prepared from blood-bank samples as described in ref.(8). Oxidation was performed with about fivefold excess of potassium ferricyanide. Organic phosphates and oxidation agents were removed by passing the solution through a Sephadex G25 column, equilibrated with maleate buffer.
For adjustment of a definite pH at subzero temperatures in 50Vol% water/ethyleneglycol mixtures calibration curves were used; these were obtained by extrapolating the pH vs. 1/T curves of maleate buffers, determined above 0°C with a glass-electrode, to lower temperatures. The validity of this procedure has been proved (9). The temperature-jump was brought about by electrical condenser discharge; For pressure-jump experiments a cell with a bursting brass membrane was used. Kinetic difference spectra were determined with an optical bandwidth of 14 nm. Photomultiplier signals were recorded on a Data-Lab transient recorder and punched on a paper tape for further computer analysis.

RESULTS

Effect of chloride: In T-jump experiments of MetHb·H_2O in maleate/0.1M NaCl buffers a fast optical density jump is followed by 2 relaxation processes with time constants of about 1 ms and 20 ms (19°C, pH 5.9). Using different buffers and different salt concentrations it turned out, that the slower relaxation process is entirely due to interactions with chloride ions. At room temperature the chloride-relaxation is even dominant. Both relaxation effects have different wavelength dependences (Fig.1). Other ions, such as nitrate, have a similar effect.

(1) MetHb·H_2O aquo-complex of methemoglobin; IHP inositol-hexaphosphate; DPG 2.3-diphosphoglycerate

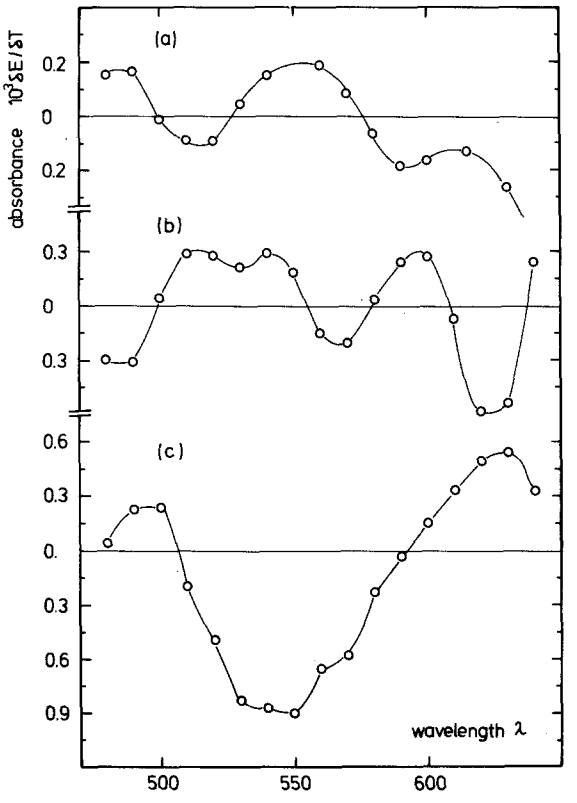

Fig. 1 Kinetic temperature difference spectra of aquo-MetHb (a) Cl^- dependent relaxation (b) fast jump (c) slow relaxation. Conditions (a) 0.17mM heme, 20mM maleate, 0.1M Cl^-, pH 5.9, 19°C (b,c) 0.17mM heme, 20mM maleate, pH 6.1, 5°C

Kinetic difference spectra: Fig. 1b shows the kinetic difference spectrum of the fast not time resolvable optical extinction change after the temperature-jump (time constant < 10 μsec), and Fig. 1c presents the difference spectrum of the slow relaxation process in aqueous chloride-free solution. The slow transition can be observed in both T- and P-jump experiments and will be attributed, on the basis of the difference spectrum, to the spin transition of the ferric iron. Increase in temperature results in decreasing optical absorbance at 550nm (more high-spin) while increase in pressure shifts the equilibrium to the opposite direction (more low-spin).

Effect of ethyleneglycol: The properties of the transition are ba-

sically the same in aqueous solution and in water/ethyleneglycol mixtures. The relaxation amplitude remains unchanged, the time constants are about a factor of 2 faster in the presence of 50Vol% ethyleneglycol.

T-jump experiments. Temperature-dependence of the relaxation process in 50Vol% water/ethyleneglycol: The relaxation data can be described by a simple monomolecular transition. For the reaction $h \rightleftarrows l$ (h,l, denote the high temperature/high-spin and low temperature /low-spin forms respectively) one obtains:

(1) $1/\tau = k_{hl} + k_{lh}$; (2) $\delta E = \Delta\varepsilon C_o \frac{K}{(1+K)^2} \delta \ln K$; $K = l/h$

Notation: τ relaxation time; k rate constants; δE optical absorbance change for 1cm lightpath; $\Delta\varepsilon$ difference of extinction coefficients; C_oMetHb concentration; K equilibrium constant.

Eqn.(2) predicts a maximum of the relaxation amplitude when K = 1. We hoped to observe the maximum in the accessible temperature range since the susceptibility data (2) indicated that the high-spin/low-spin transition which occurs in the range of the freezing temperature is completed at about -25°C. However, this is not found. We have therefore made the approximation that $K \ll 1$, leading to

(3) $\delta E\, T^2 = A \exp(-\Delta H^o/RT)$; $A = \Delta\varepsilon\, C_o \frac{\Delta H^o}{R} \delta T \exp(\Delta S^o/R)$

It is obvious from eqn.(3) that in this case the reaction enthalpy can be obtained independent of the knowledge of the actual value of the equilibrium constant. The experimentally determined quantity $\ln(\delta E T^2)$ is indeed linearly dependent on 1/T. From measurements at 9 different temperatures in the range from -20 to +20°C we obtain under the conditions: 0.27mM heme, 20mM maleate, 50Vol% ethyleneglycol, pH 5.9, 545 nm, δT 4.8°C

$$\ln\left(\delta E \left(\frac{T}{273}\right)^2\right) = -26.0\ (\pm 0.9) + 5.81(\pm 0.23)\cdot 10^3 \cdot \frac{1}{T}$$

(correlation coefficient 0.99; T in Kelvin)

The reaction enthalpy for the transition yields ΔH = -11.5 ($\pm$4%) kcal/mole. Equilibrium constants can be calculated from these

amplitude data and eqn.(3) if the extinction coefficients are known (see discussion).

The temperature-dependence of the time constants yields the activation parameters for l to h transition, since $k_{hl} << k_{lh}$. From the experimentally determined dependence

$$\ln\left(\frac{1}{\tau}\left(\frac{273}{T}\right)\right) = 70.3(\pm 1.8) - 1.77(\pm 0.05)\cdot 10^4 \cdot \frac{1}{T}$$

the activation enthalpy and entropy result

$\Delta H^{\ddagger}_{lh} = 35(\pm 3\%)$ kcal/mole, $\Delta S^{\ddagger}_{lh} = 81(\pm 3\%)$ e.u.

P-jump experiments: Pressure-jump experiments have been performed at 2°C in the aqueous solution; The time constant of the relaxation process is the same as in the T-jump experiments. The optical extinction change is $\delta E = 5\cdot 10^{-4}$ for $\Delta P = 77$ bar, under the conditions stated above. Reaction volumes can be calculated by incorporating the equilibrium constant (see discussion).

Influence of pH and allosteric effectors: The appearance of the relaxation process is strongly dependent on pH and can only be observed in the pH range around pH = 6. The properties of the transition are also dependent on the allosteric effectors. In the presence of a 10fold per heme excess of IHP[1] the amplitude of the effect is increased by a factor of 4-5 at 3°C while the relaxation time becomes slower by the same factor. A less pronounced change is observed in the presence of DPG[1]. The kinetic difference spectrum is identical in the absence and presence of IHP.

DISCUSSION:

The temperature difference spectrum of the relaxation process (Fig.1c) agrees with the difference spectrum of the high-spin/low-spin transition given in ref.(4), which was calculated by correlating the spectral properties of MetHb-hydroxide with magnetic susceptibility data. The temperature difference spectrum of the fast jump (Fig.1b) shows no correlation with the spin state. It

was pointed out that similar difference spectra as in Fig.1c can also be observed without a change in spin state if a quaternary R to T transition occurs, which can be induced with IHP (5). This holds especially for the thiocyanate and imidazole complex, but there is no direct correspondence for the aquo-complex. Very slow kinetic effects are reported on magnetic studies (2). This is to be expected from the high activation enthalpies of 35 (23.5) kcal/mole for the 1 to h (h to 1) transition, and is a further indication that the observed transition correlates with the spin state. Since the equilibrium constant cannot be calculated from the relaxation data alone (the maximum according to eqn.2 is not observed) we used the extinction coefficients given in (4), $\Delta\varepsilon(545) = 4 \cdot 10^{3} M^{-1} cm^{-1}$. Applying this value together with eqn.(3) yields a reaction entropy of $\Delta S^{o} = -50$ e.u. The use of this $\Delta\varepsilon$ value is an approximation, but yields certainly close estimates of the equilibrium constant. One obtains K = 0.005, 0.02, 0.12 at temperatures of 20,0,-20° respectively. The relative amount of low-spin forms turns out to be very small above 0°C, it should, however, become strongly populated at lower temperatures. From the above thermodynamic values one computes a compensation temperature (equal population of h and 1 states) of $T_o = -43°C$. Further kinetic and magnetic data are needed to test that prediction. The above estimate of K shows that at room temperature the equilibrium is almost fully on the high temperature, i.e. high-spin side. Since the magnetic data indicate - although not generally agreed upon - that a substantial amount of low-spin form is present at room temperature (high-spin state only 90% (6) or 78% (1)), one would have to conclude that only a certain fraction of MetHb$\cdot H_2O$ is involved in the observed transition, i.e. that two kinds of spin equilibria exist. The system is then characterized by a temperature-independent trans-

ition ($\Delta H = 0$) of the aquo-complex (1) and another temperature-dependent equilibrium ($\Delta H = -11.5$kcal/mole). The latter equilibrium then reflects a process to which a spin transition is coupled and probably involves the binding of the distal imidazole, as has been proposed on the basis of ESR data (3). The large ΔH value is in fact reasonable for the latter reaction and agrees almost quantitatively with the reaction enthalpies characteristic for low-spin ligand binding to MetHb (10). The reaction volume of $\Delta V = -7$ml/mole calculated from the amplitude in the P-jump experiments and the estimated equilibrium constants is also of the same order as for low-spin ligand binding (11). The coupling of the transition to a protonated group in the protein at acid pH is a further property common to ligand binding to myoglobin and hemoglobin (12). The effect of IHP indicates that the transition occurs in the R- as well as in the T quaternary structure forms.

REFERENCES

1. Messana, C., Cerdonio, M., Shenkin, P., Noble, R., Fermi, G., Perutz, R., Perutz, M. Biochemistry 17, 3652-3662 (1978)
2. Iizuka, T., Kotani, M. Biochim.Biophys.Acta 194, 351-363 (1969)
3. Yonetani, T., Iizuka, T., Waterman, M. J.Biol.Chem. 246, 7683-7689 (1971)
4. George, P., Beetlestone, J.,Griffith, J. in Haematin Enzymes, J.E.Falk, R.Lemberg, R.Morton Eds. pp.105-141, Pergamon Press, New York (1961)
5. Perutz, M., Sanders, J., Chenery, D.,Noble, R., Pennelly, R., Fung, L., Ho,Ch., Giannini, I., Poerschke, D., Winkler, H. Biochemistry 17, 3640-3652 (1978)
6. Perutz, M., Heidner, E., Ladner, J., Beetlestone, J., Ho, Ch., Slade, E. Biochemistry 13, 2187-2200 (1974)
7. Schwartz, A., Schimmel, P., J.Mol.Biol. 89, 505-510 (1974)
8. Rossi-Fanelli, R., Antonini, E., Caputo, A. J.Biol.Chem. 236, 391-396 (1961)
9. Hui Bon Hoa, G., Douzou, P. J.Biol.Chem. 248, 4649-4654 (1973)
10. Beetlestone, J., Epega, A., Irvine, D. J.Chem.Soc.(A) 1346-1351 (1968)
11. Ogunmola, G., Kauzmann, W., Zipp, A. Proc.Natl.Acad.Sci.U.S. 73, 4271-4273 (1976)
12. Goldsack, D., Eberlein, W., Alberty, R. J.Biol.Chem. 241, 2653-2660 (1966)