On the Thermodynamics and Kinetics of the Spin Transition in Human Aquo-Methemoglobin

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Acid human aquo-methemoglobin is known to exhibit a rather slow temperature-dependent spin state change of the ferric iron (1,2). We have investigated this reaction, its temperature and pH dependence and the influence of inositol-hexaphosphate (IHP) by temperature-jump relaxation measurements (3). Using water/ethyleneglycol mixtures we have extended the temperature range to -45°C. No anomalous effects, as found in magnetic measurements at the freezing point, are present in liquid solution.

Two strongly temperature-dependent relaxation phases can be observed (time constants at -26°C 13 and 4000 seconds resp.). Both phases exhibit the same form of the kinetic difference spectrum, characteristic of a spin state change (4). The fast relaxation process can be described by a simple monomolecular reaction h⇄l (h, l: high and low spin forms resp., K = l/h). The table shows the thermodynamic and kinetic parameters of this transition as determined by the temperature dependence of the relaxation amplitudes and relaxation times:

	ΔH (kcal/mol)[a]	ΔS (e.u.)[b]	$\Delta H^{\#}(l \rightarrow h)$ (kcal/mol)[a]	$\Delta S^{\#}(l \rightarrow h)$ (e.u.)[a]	$T_c = \Delta H/\Delta S$ (°C)
stripped	-11.5	-48.5	+35.0	+81.0	-35.9
+ IHP	-11.5	-45.6	+27.0	+49.0	-20.8

(a) Obtained independently of the exact value of the equilibrium constant from the relaxation amplitudes and times in the range between +20°C and -15°C; in this range the condition K<<1 holds.

(b) Obtained with an extinction change $\Delta\varepsilon = 2 \cdot 10^3$ $M^{-1}cm^{-1}$ at 545 nm which gives the best fit of the relaxation amplitudes in the range of the compensation temperature T_c, i. e. under the condition K≈1. This value is by a factor of 2 smaller than that given in ref.(4) which can be unterstood in view of the additional very slow relaxation process.

The values show that the spin equilibrium is largely on the high spin side at room temperature (K = 0.005 for stripped methemoglobin and 0.02 with IHP at 20°C) in agreement with most of the magnetic measurements. The high reaction and activation parameters suggest that the spin transition is coupled to a rather slow structure change of the protein, probably the internal binding of the distal imidazol and the corresponding movement of the E helix. The transition is also observed in the presence of the allosteric effector IHP, which shifts the structure from the R to the T quaternary form.

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