THE TEMPERATURE DEPENDENCE OF THE OPTICAL ABSORPTION SPECTRA OF SOME METHAEMOGLOBIN DERIVATIVES

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SUMMARY

The variation of the optical absorption spectra of the hydrate, fluoride, azide, hydroxide and cyanate derivatives of Methaemoglobin (MetHb) as a function of temperature are reported. The results for the hydroxide and azide are consistent with a two state thermal equilibrium having a low spin ground state. The fluoride is observed to be in a single spin state. Measurements on the hydrate indicate that it is anomalous.

INTRODUCTION

Measurements of the magnetic susceptibilities of haemoglobin derivatives at room temperature show that there exist derivatives having values of magnetic susceptibility intermediate between those of derivatives presumed to have entirely low spin and those of entirely high spin states (1, 2). Group theoretical considerations indicate that the intermediate spin state S = 3/2 is never a stable ground state (3, 4) and the suggestion that thermal mixtures of spin states exist in those derivatives possessing intermediate values of magnetic susceptibility was shown to be sound (5, 6). Variation of the magnetic susceptibility at low temperatures has been used to examine thermal mixing in various haemprotein derivatives but the interpretation of these results and measurements of changes in optical absorption spectra under cryogenic conditions are not entirely compatible (7, 8).

Absorption in the alpha and beta bands and the charge transfer bands of haemprotein derivatives is related to the spin state. A linear relationship has been found between the extinction coefficient in the beta band and the magnetic susceptibility (9). It is similarly known that the magnetic susceptibility is related to the position of the Soret band, for most complexes.

It is thus to be expected that reducing the temperature of the protein will drive the complex towards its ground spin state and this should appropriately change the intensity of absorption in various bands. Further, the absorption spectra of derivatives which do not consist of a thermal mixture of spin states should be unchanged. The absorption ratios in the beta and D bands (at 540nm and 630nm respectively) were measured and the absorption ratios in the E and D bands (at 500nm and 630nm). Since the band at 500nm is in the wing of the Soret band, a change in its absorption relative to that of the other charge transfer band will reflect movement of the Soret band (10). Movements of the Soret band have been shown to indicate a change of spin state.

EXPERIMENTAL TECHNIQUE

The measurements of absorption were made using a Perkin-Elmer Hitachi-EPR3 spectrophotometer for which was constructed a nitrogen, helium gas flow system in order to maintain both sample and reference cuvettes at the same temperature. Freezing of the samples leads to Rayleigh and Mie scattering; Mie scattering from ice crystallites will be a function of wavelength (11) but in difference spectrophotometry the effect should be practically cancelled out since it will occur in both sample and reference. Phase transitions in the ice should not obtrude since the transformation from ice I_h to ice I_c should be effectively prevented by the slow rate of molecular processes at very low temperatures (12). Strains in opposite faces of the frozen sample will deviate the light beam causing its position on the detecting surface to shift implying that absorption values and their ratios at different wavelengths will not be exactly reproducible. However, the manner in which bands and ratios change with temperature will be unaffected.

RESULTS

Haemoglobin hydrate, fluoride, hydroxide, azide and cyanate were examined and in addition a hydrate sample to which had been added inositolhexaphosphate.

In the hydrate, absorption in the beta band at 540nm increases as the temperature falls whilst a small decrease is observed in the D band. The ratio of absorption in the beta and D band (630nm) decreases with rising temperature, and this trend continues down to temperatures of $30^{o}K$. The ratio of absorption at 500nm (the E charge transfer band) to that at 630nm also decreases as the temperature rises, probably indicating a movement of the Soret band to shorter wavelengths. The hydrate with IHP bound shows little spectral variation with temperature and the ratio A_{540}/A_{630} remains constant with temperature within the experimental error. Haemoglobin fluoride was found to exhibit the same characteristics. For the hydroxide (pH 9.5) absorption in the beta band decreased with increasing temperature from 97 to $243^{o}K$ but the ratio A_{540}/A_{630} was not calculated owing to the small value of A_{630}. The azide shows a reduction in absorption in the beta band and a small increase in the D band as the temperature rises. The ratio of absorptions at 540nm and 630nm undergoes a gradual reduction as the temperature is increased.

The absorption spectrum of the MetHb hydrate as a function of temperature is shown in figure 1 and the ratio $^{A}\beta/A_{D}$ as a function of temperature in figure 2.

In the ferrous derivative, oxyhaemoglobin, the intensity of the absorption in the alpha and beta bands was observed to increase as the temperature rose from $30^{o}K$.

DISCUSSION

The behaviour of the hydroxide and azide are consistent with their possessing a low spin ground state, since absorption in the low spin bands decreases with rising temperature. The lack of variation of the

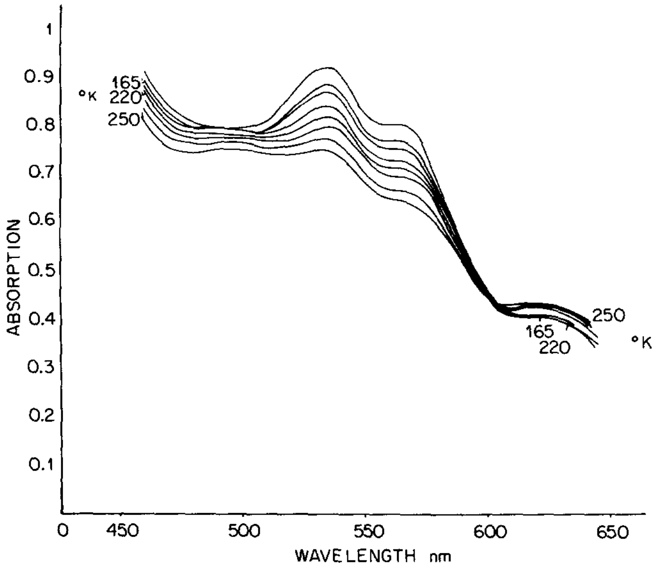

FIG 1. THE OPTICAL ABSORPTION SPECTRUM OF met Hb(H_2O) pH 6.5 WITH TEMPERATURE.

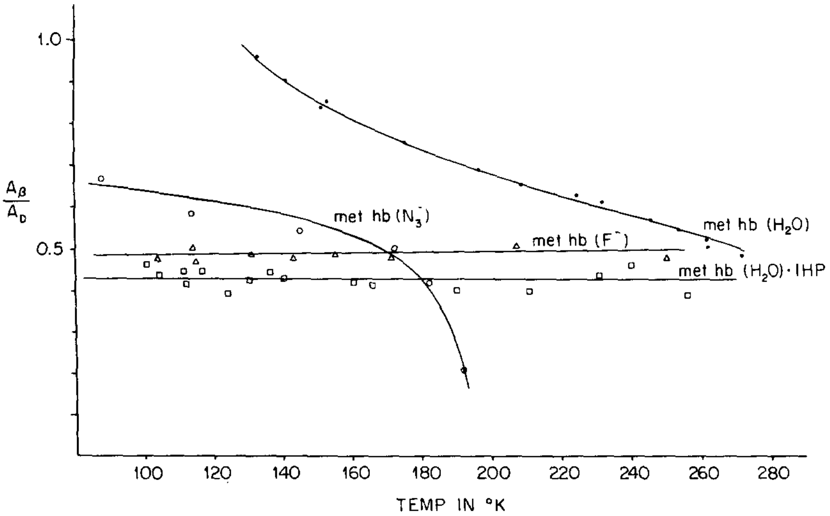

FIG 2. THE RATIO OF THE ABSORPTIONS IN THE β BAND AND D BAND AS A FUNCTION OF ABSOLUTE TEMPERATURE.

absorption bands of the fluoride with temperature indicates that it is almost entirely in one spin state over the entire range of temperature. These results are consistent with magnetic susceptibility measurements.

The ratio of A_{540}/A_{630} for the haemoglobin hydrate + IHP complex was also flat with temperature indicating the presence of only one spin state; this is in accord with EPR and other measurements. A 10% increase in the intensity of the high spin EPR signal at 9GHz has also been observed. It has been shown (13) that these changes are consistent with a change in the protein structure to the allosteric t form.

However, the results for haemoglobin hydrate are radically different from the expected variations. On the basis of a two spin state thermal mixture they suggest that the hydrate possesses a low spin ground state and this is inconsistent with measurements of EPR spectra and magnetic susceptibility where a large high spin component is invariably observed. It has been suggested (8), that the hydrate is a two component chemical mixture, component I having a high spin state over the entire temperature range and component II being a thermal mixture with a low spin ground state. Our results are consistent with Iizuka's suggestion. It is also observed that the fluoride has a more intense high spin EPR signal than the hydrate which would be expected if the hydrate high spin signal arises from component I only. A two component assessment of the Mossbauer results (14) might remove the assumption of considerable covalency needed to explain the large quadrupole splitting. The two component model does not however appear to be consistent with the temperature dependence of magnetic susceptibility (7) or of the EPR spectra. Alternatively, there may be a possibility that minor high spin bands located at the same wavelengths as the alpha and beta bands may grow as the temperature is reduced so that two superposed absorptions are present. Unless the manner of growth is fortuitous one ought to observe a minimum in absorption at some temperature. This was not observed in the temperature range covered.

Full spectral measurements of the cyanate were not made but transmission in the E band was examined as a function of temperature.

It was found that transmission increased with temperature suggesting a decrease in the proportion of high spin state present. This could be caused by a change in absorption of the E band but is likely also to be caused by a Soret shift to shorter wavelengths, indicating a movement to a high spin ground state at lower temperatures.

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