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DIFFERENTIAL SCANNING CALORIMETRY STUDY OF HAEMIN THERMAL STABILITY

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Abstract

A differential scanning calorimetry study of the thermal behaviour of haemin in N,N-dimethylformamide solution was carried out. The samples were scanned with different scan rates in the temperature range 25–130°C. The UV VIS spectrophotometry was used as assistant measurement method. The scan rate dependent and irreversible exothermic peaks were found. The activation energies of observed transitions were calculated assuming kinetically controlled process. The nature of thermal behaviour of haemin in DMF solutions is discussed.

Keywords: DSC, exothermic transitions, protoporphyrin-IX chloride, UV VIS spectra

Introduction

Haemin is a highly active molecule participating in many vital processes such as oxygen bonding in haemoglobin and myoglobin. Moreover haemin derivatives, for example HPD (hematoporphyrin derivative), have been used in diagnosis and treatment of malignant diseases in recent years. Efforts have been made to prepare new porphyrin derivatives and to study the interaction of porphyrin with proteins for their application in medicine. We hope to carry out such studies in the future. However, before the photophysics of more complex systems can be understood, it is useful to have results on the simpliest type of protoporphyrin. Haemin is the iron(III) complex of protoporphyrin-IX which formally has a chloride ion attached at one of the axial positions. Metal iron is pulled out of the plane by 0.048 nm towards the chlorine [1]. Haemin has been widely used as model system for haeme proteins. Due to these facts the investigation of thermodynamic stability of haemin solutions is important.

This paper presents differential scanning calorimetry (DSC) studies of thermal behaviour of haemin in N,N-dimethylformamide solution. This solvent is usually chosen because of its good solvating properties for porphyrins and reduction ability. However, the exact nature of the DMF–porphyrin interaction is not well understood [2].

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Materials and methods

Bovine haemin (protoporphyrin-IX chloride) and DMF (N,N-dimethylformamide) were purchased from Sigma Chemical Company (lot 40K1561 and lot 11K1702, respectively) and used without further purification and desiccation. Haemin solutions were prepared by dissolution in DMF at $9.2 \cdot 10^{-5}$ M (0.06 mg mL^{-1}) for all DSC measurements.

DSC was carried out on a VP DSC microcalorimeter (MicroCal Inc., Northampton, MA) with cell volume 0.5 mL at heating rates 0.78–1.56°C min^{-1} in the temperature range 25–110°C. Additional constant pressure of about 1.7 atm over the liquids in the cells was applied. The calorimetric data were corrected for the instrumental baseline (by subtracting DMF-DMF scan) and for the difference in heat capacity between the initial and the final state by using a sigmoidal baseline. DSC curves were analysed with Micro-Cal Origin software.

UV VIS spectra were recorded in the wavelength range of 220–900 nm on JASCO V-530 spectrophotometer with 2 nm band width.

Statistical analysis of the results was done with Statistica 5.1 using one-way ANOVA or Kruskal–Wallis tests.

Results

Scan rate dependence

Figure 1 shows three original DSC haemin scans after subtracting DMF–DMF baseline, obtained at different heating rate. Usually three exothermic transitions were observed but in some cases at the highest (1.56°C min^{-1}) scan rate only two exothermic peaks appeared. The mean values of transition temperatures for three (I, II, III from lower to higher temperature) exothermic processes are listed in Table 1. The transition temperatures of all observed exothermic processes increase with the rise of the scan rate.

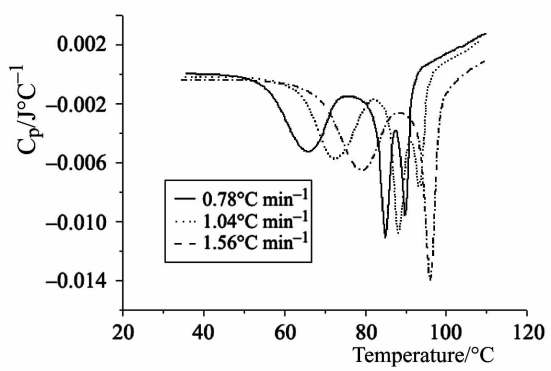

Fig. 1 DSC curves of the relative heat capacity *vs*. temperature for haemin DMF solution (0.06 mg mL^{-1}) at different scan rates

Table 1 The scan rate effect on the mean transition temperature values T_1, T_2, T_3 for observed in haemin DMF solutions three exothermic transitions I, II, III, respectively

β/K min^{-1}	T_1	T_2 (°C±ΔT^*)	T_3
0.78	65±2	85±1	89±3
1.04	72±1	89±1	92±3
1.56	79±2	97±1	101±4

*ΔT – standard error of the mean (SEM)

Irreversibility

It should be noted that the thermal transitions are not reproducible on reheating a sample, i.e. the whole process is irreversible. The reversibility of the transitions was tested by the preliminary heating of haemin solution up to 85, 95, 110°C with subsequent cooling to 25°C after each given temperature. Figure 2 confirms the calorimetric irreversibility of the thermally induced transitions in haemin solution.

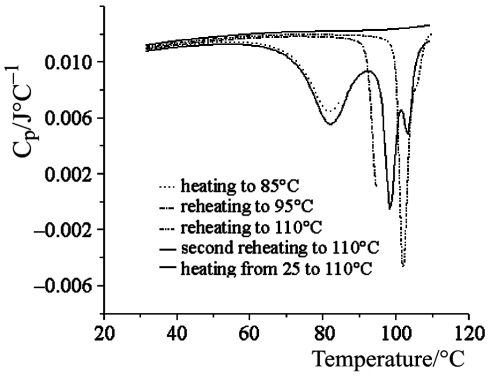

Fig. 2 The raw DSC profiles for haemin DMF solution heated to a given temperature and reheated to higher temperature after the cooling from the previous run (scan rate 1.56 K min^{-1})

Time instability

The thermostability of haemin solutions in DMF seems to be time dependent. Figure 3 presents DSC scans of haemin solution performed in subsequent days of experiment at constant scan rate. All exothermic peaks shift to lower temperatures with time. The first and second peak gradually disappear while the third peak grows. The intensity of all peaks becomes smaller, the line width greater and additional fourth peak appears in DSC curves if haemin solution samples are old (after a few weeks of storage in the refrigerator).

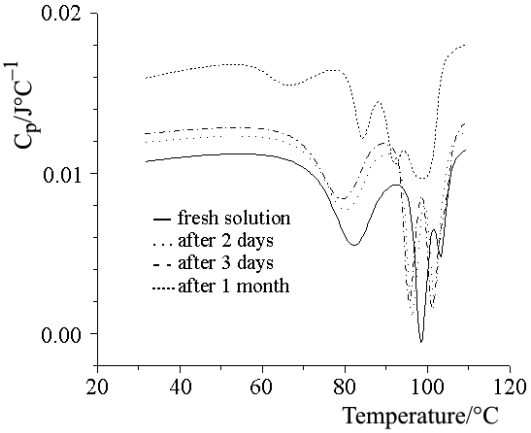

Fig. 3 DSC curves of haemin stored for different times (scan rate 1.56 K min^{-1})

Calorimetric analysis

Detailed analysis of calorimetric results was carried out only for samples not older than two days. The fact that calorimetric traces were found to be irreversible and highly scan-rate dependent, precludes their interpretation in terms of equilibrium thermodynamics. Thus some kind of kinetic analysis should be used.

It was assumed that observed irreversible exothermic processes can be represented as

$$A \xrightarrow{k} B \tag{1}$$

where A is the initial, B is the final state and k is a first-order kinetic constant, which changes with temperature according to the Arrhenius equation.

A mathematical transformation (see Appendix in [3]) allows us to calculate the activation energy of the kinetic process in the following ways:

A) The energy of activation, E, can be obtained from the values of k at several temperatures by using the Arrhenius equation

$$k = A\exp(-E/RT) \tag{2}$$

The rate constant of the reaction at a given temperature, T, can be obtained by using

$$k = \nu c_p / (Q_t - Q) \tag{3}$$

where v (K min^{-1}) stands for the scan rate, c_p for the excess heat capacity, Q_t for the total heat of the process, and Q for the heat evolved at a given temperature, T. The energy of activation can be calculated from the slope of the Arrhenius plot, ln$k$ *vs*. *1/T*.

B) The dependence of the heat evolved with temperature can be expressed as

$$\ln\left(\ln\frac{Q_t}{Q_t - Q}\right) = \frac{E}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right) \tag{4}$$

Thus, a plot of $\ln[\ln Q_t/(Q_t-Q)]$ *vs.* $1/T$ should give rise to straight lines, the slope of each one being $-E/R$.

C) The activation energy can be also calculated from the heat capacity at the maximum of the trace, c_p^m, according to

$$E = eRc_p^m T_m^2 / Q_t \tag{5}$$

On the basis of this model, the values of the rate constant as a function of temperature and the activation energy have been calculated. Figure 4 illustrates plots obtained by method (B) for the first (I) transition. The corresponding enthalpy value for this exothermic transition is the order of 10^2 kJ mol^{-1}. The value of the rate constant extrapolated to room temperature gives the value of the order 10^{-11} min^{-1}.

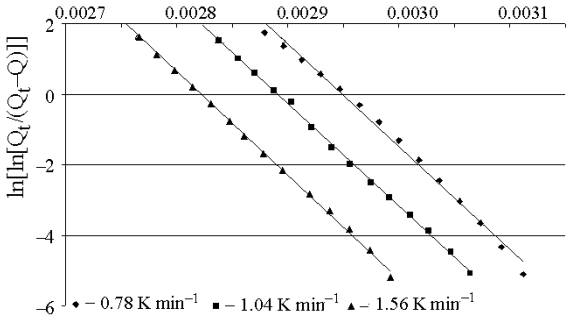

Fig. 4 Method (B) of the activation energy determination

The second and third peak were separated by deconvolution (this deconvolution was done only technically (visually) because the use of equilibrium thermodynamic is incorrect in this case) before kinetic elaboration.

The calculated activation energies were statistically analysed in detail. Selected results of analysis are presented in Fig. 5 for the second (II) transition.

No essential differences were observed between the activation energies of transitions for particular scan rates and methods (p>0.05). Table 2 contains the average values for the activation energies including all the results obtained by the three methods. The activation energy for low temperature transition I is much lower than for transitions II and III.

Table 2 The average activation energies for observed three exothermic transitions in haemin DMF solutions

Activation energy/kJ mol^{-1}	Exothermic transition		
	I	II	III
Mean value	242	853	1016
Confidence interval*	(234; 250)	(805; 901)	(964; 1069)

*95% probability

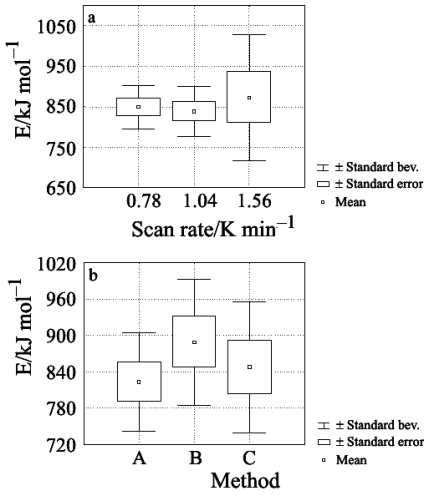

Fig. 5 Activation energies of the second exothermic transition a – at different scan rate and b – calculated by three methods: (A), (B) and (C)

UV VIS spectrophotometric analysis

In the absorption spectrum of the haemin dissolved in DMF, similarly as for all metallated protoporphyrin-IX the following bands are observed: the Soret band near 400 nm, *Q* bands (α known also as Q_o and β known as Q_v) between 500–600 nm and charge transfer (CT) band near 625–630 nm. The spectroscopic properties of haemin are dependent on the concentration of haemin in the solution. The maximum of Soret band does not shift significantly in the concentration range tested (0.03–0.15 mg mL^{-1}). The marked changes occur in the *Q* and CT bands (Fig. 6). There are four maxima: 499, 504.5, 539 and 630.5 nm and three minima: 481.5, 528.5 and 603.5 nm for the highest tested concentration and only one maximum 571.5 nm and one minimum 531 nm at the lowest concentration.

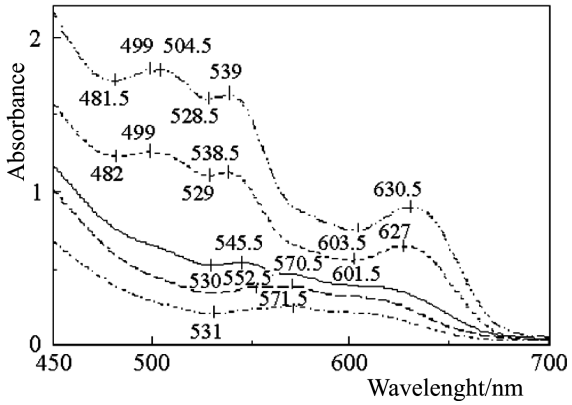

Fig. 6 Absorption spectra of haemin dissolved in DMF: –·–·– – 0.03 mg mL^{-1}, – – – – 0.045 mg mL^{-1}, —— – 0.06 mg mL^{-1}, - - - – 0.1 mg mL^{-1} and –·–·–·– – 0.15 mg mL^{-1}

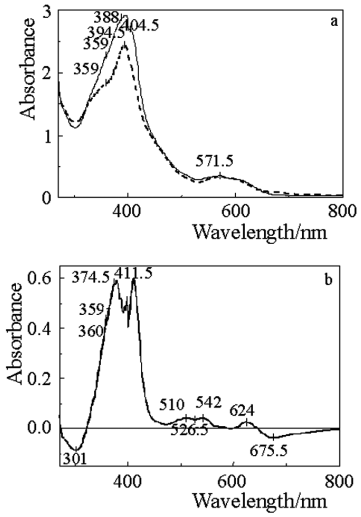

Fig. 7 a – VIS spectra of haemin in DMF solution (0.04 mg mL^{-1}) before (——) and after (– – –) DSC run, b – the differential spectrum between (——) and (– – –)

In order to elucidate the nature of the observed calorimetric exothermic transitions, the absorption spectra of haemin in DMF were recorded before and after DSC experiment. The results are shown in Fig. 7a. Marked differences appear in the Soret band and the Q bands in the visible region become more compact. Figure 7b presents the differential spectrum. The maxima of this spectrum point out the bands which disappear after DSC heating. This mainly refers to the Soret band but also to the α-band (Q_o) at 540 nm and charge transfer band near 625 nm.

Discussion

DSC measurements of bovine haemin as a powder does not exhibit any phase transition in studied temperature range (25–110°C) (data are not shown in this paper). Earlier Wolf *et al*. [4] reported that there was no decomposition, no changes in the crystal structure, no process of phase separation and no melting point in the temperature range between 25 and 275°C of this compound.

A quite different thermal behaviour was observed in our studies of haemin solution in DMF. The calorimetric curves point to some phase transitions that were found to be exothermic and irreversible. The calculated activation energies of these transitions are high (Table 2). The interpretation of the obtained results is not easy due to the complex thermal behaviour of haemin in DMF solution. The observed DSC peaks may be associated with: temperature initiated interactions between solute and solvent molecules, the aggregation of molecules and the polymerisation processing or the degradation of the compound. The spin crossover in the iron complexes may be also monitored in DSC [5, 6].

The stability of haemin solution depends on the kind of solvent. Haemin dissolved in aqueous alkali is unstable [4]. In the coordinating solvent such as DMSO or DMF, the chlorine is displaced and one or two solvent molecules occupy the axial sites [7, 8]. Moreover, owing to the ligand dissociation process the complex $[Fe(III)PPIX(DMSO)_2]^+$ occurs. In DMF the complexes $Fe(III)PPIX(DMF)_2Cl$ and Fe(III)PPIX(DMF)Cl form but the dissociation process in this solvent is less probable [9].

The presence of ligands at axial position tends to modulate the electronic and magnetic properties of the iron centre. It was shown [10] by means magnetostatic methods, AC-susceptibility measurements and EPR spectroscopy that iron ion in the studied haemin exists in two spin states: S=5/2 and 1/2. The spin state changes are also reflected in the spectroscopic characteristics. It is thought that β band associated with high spin complexes shifts from 500 to 540 nm and the α band shifts from 540 to 580 nm with the change of the spin state [7]. This tendency is observed in our spectroscopic measurements for decreasing haemin concentration (Fig. 6). The vanishing band near 500 nm indicates a change from high to low spin state due to haemin – DMF complex forming. It is reported that Fe(III) is associated with two molecules of DMF at high DMF concentrations and no DMF molecules at low concentration. At the same time upon reduction of Fe(III) to Fe(II) the complex with only a single DMF molecule forms [2]. Processing of the iron complexes can be connected with spin state changes.

It is interesting to compare UV VIS spectra of haemin in DMF recorded before and after DSC runs (Fig. 7a). One can see that after DSC heating the Soret band, the α-band at 540 nm and the charge transfer band near 625 nm decrease (Fig. 7b). These changes may be related to the disappearance or the processing of $Fe(III)PPIX(DMF)_2Cl$ and {Fe(III)/Fe(II)}PPIX(DMF)Cl complexes. It is suggested in [2] that at low scan rate and high temperature, TPPFe(DMF) has time to interconvert to $[TPPFeCl]^-$. The temperature initiated interactions between solute and solvent molecules can induce a modulation of the electronic and magnetic properties of the iron ion due to field ligand changes. DSC peaks accompany the spin crossover. The enthalpy change in the solid state connected with the spin crossover in Fe(II) complexes was reported to be near 20 kJ mol^{-1} [6]. However, in our measurements of the haemin in DMF solutions the observed exotherms in DSC curves were markedly stronger.

Another possible explanation of DSC peaks may be the polymerisation or the aggregation process which are thought as strongly exothermic. Such exothermic transitions were observed for acrylonitrile polymerisation and cyclization in the presence of DMF [11].

When intermolecular interactions between solute molecules become energetically more favourable than the interactions between solute and solvent molecules, the dimerization process occurs. Different kinds of porphyrin dimers may be form in the solutions, for example: stereospecific [12], covalently – linked [13] ones. The presence of even small residual amounts of water can enhance dimerization of porphyrin in organic solutions [14]. Specific spectroscopic features accompanying dimer formation are described in review paper by Owens [9]. Changes in the splitting of the Soret band, de-

crease of the charge transfer band (near 624 nm) and forming of one unresolved band in the visible region (above 530 nm) seen in Fig. 7 may be due to dimerization.

On the other hand, the observed decrease of intensity of the Soret band for the haemin solution after DSC heating can be caused by decomposition of the compound in DMF solution with the increase of temperature. The exotherm is often characteristic of a thermal decomposition involving the formation of stable compounds what was discussed in [15].

Detailed interpretation of the nature of the observed exothermic transitions for haemin dissolved in DMF based only on the performed measurements is rather impossible. Further studies with the use of other techniques such as NMR, IR, X-ray are required.

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