Journal of Thermal Analysis and Calorimetry, Vol. 65 (2001) 575–582

THERMAL STABILITY OF HAEMOGLOBIN SOLUTIONS UNDER DC AND AC MAGNETIC FIELD AND UV AND IR RADIATION

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Abstract

The influence of electromagnetic field in wide range of frequency (extra low frequency magnetic field, monochromatic laser light 840 nm, ultraviolet light) and static magnetic field on the stability of aqueous and NaCl 0.9% haemoglobin solutions was studied using differential scanning calorimetry (DSC) method. Three steps of denaturation process can be derived from experimental data. The shifts of transition temperatures are more marked for NaCl 0.9% than for aqueous solutions. The results suggest that static magnetic field is effective in protecting protein, while ultraviolet radiation destabilises haemoglobin. The laser light and alternating magnetic field have a little effect on the thermal stability of haemoglobin.

Keywords: DSC, electromagnetic field, haemoglobin, thermal stability

Introduction

An influence of electromagnetic field on biological systems has been shown in many investigations [1–3]. An interest in this problem is connected with increase of the electromagnetic field application in medical diagnosis and therapy.

Haemoglobin is an important protein responsible for oxygen transport in the body. The iron ions in the hem group can be in various spin states and can modify the tertiary structure of haemoglobin. It is known that the conformational stability of haemoglobin and its derivatives are strongly affected by ligand binding. The conformation stability of proteins can be examined by various methods. However, differential scanning calorimetry (DSC) is the most direct experimental technique to resolve the energetics of conformational transitions of biological macromolecule [4].

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1418–2874/2001/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

Materials and methods

Haemoglobin (Hb) solutions with concentrations: 0.4, 0.7 and 1 mg mL^{-1} were prepared from lyophilised haemoglobin from bovine erythrocytes produced by 'Sigma' (since native haemoglobin is readily oxidised in air, the preparation may be predominantly methaemoglobin).

Two different solvents: H_2O and NaCl 0.9% solution were used in experiments. Then the prepared solutions of haemoglobin were exposed to different electromagnetic fields for 15 min. Electromagnetic field exposures were grouped into four categories: static magnetic field (DC), extremely low frequency magnetic field (AC), infra-red radiation (IR), ultra violet light (UV).

The static magnetic field up to 0.9 T was used for the experiment. AC magnetic field of maximum amplitude about 0.09 mT and complex shape was produced by device MRS2000 applied in medicine for magnetostimulation. A biostimulating laser BL 20 (λ=890 nm, f=30 kHz) was used as IR radiation source and the Hg lamp was applied for UV exposition (λ_{min}=246 nm).

DSC scans were obtained in the temperature range 10–120°C by using the VP-DSC Microcalorimeter. The samples were heated adiabatically at a constant rate 1 K min^{-1}.

DSC measurements were analysed in the framework of different curve-fitting models using the Levenberg–Marquardt non-linear least-square method. The thermodynamic parameters as a thermal midpoint of a transition and calorimetric heat change were determined for each thermal profile.

Statistical analysis of the results was done with STATISTICA using one-way ANOVA.

Results and discussion

The representantive raw DSC profile of haemoglobin aqueous solution with concentration 0.7 mg mL^{-1} is presented in Fig. 1a in order to show heat capacity curves before and after denaturation. The water scan is shown as a dotted line and the haemoglobin scan as a dashed one. The solid line illustrates the data after subtraction of the baseline scan from the sample scan. The courses of DSC scans are consistent with expectations [4]. Figure 1b illustrates raw DSC profiles for haemoglobin aqueous solutions exposed to various kinds of electromagnetic field. All the temperature dependencies of heat capacity obtained for the examined samples are similar. The curves have a marked peak connected with denaturation process. DSC profiles show broad endothermic transition of denaturation ranging from 40 to 80°C followed by an exothermic reaction. Since the aggregation of proteins is largely exothermic, it seems most likely that this exothermic transition corresponds to the aggregation of haemoglobin immediately after or perhaps partly during thermal denaturation. Repeated scans of the denatured samples of haemoglobin show only a flat baseline and no melting profile indicating that haemoglobin denatured irreversibly.

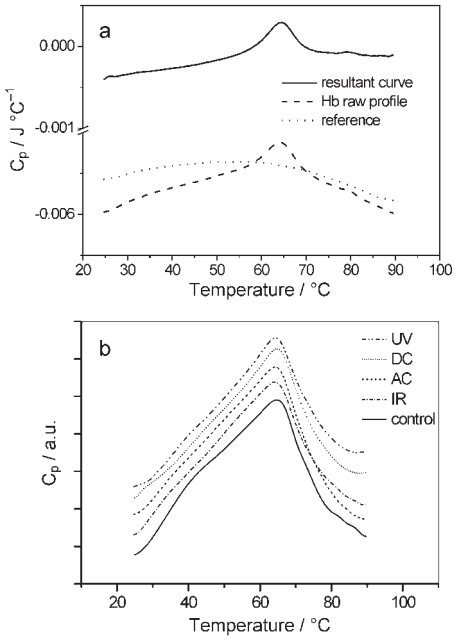

Fig. 1 The raw heat capacity data for haemoglobin aqueous solution (0.7 mg mL^{-1}) (a), the raw DSC profiles for haemoglobin aqueous solution (0.4 mg mL^{-1}) exposed to the various kinds of electromagnetic field (b)

The small shifts of curve maximum (Figs 1–3) for Hb solutions after electromagnetic field exposition in comparison to control sample should be noticed. Similar behaviour was found for H_2O and NaCl 0.9% solutions.

To obtain detailed information about thermodynamic properties a deconvolution of DSC traces were performed in MicroCal Origin DSC. DSC profiles were analysed within the framework of 2-State model and of Non-2-State model. The best curves fitting for NaCl 0.9% and aqueous solution of haemoglobin were obtained in 2-State model and in Non-2-State model, respectively. 2-State model allows the determination of the enthalpy change ΔH_i and the temperature of the maximum, T_{mi}, associated with the unfolding process for each component transition while the Non-2-State model additionally lets to determine the van't Hoff heat change ΔH_{vi}.

Figures 2a–d illustrates the fitting of experimental DSC data (NaCl 0.9%+0.4 mg mL^{-1} haemoglobin) to a 2–State transition model that yields a set of 'best fit' parameters. These results show that the haemoglobin denatures in three steps. Three single transitions seem to constitute the overall denaturation process. The biological meaning of the deconvoluted three components is not quite explained. The observed transitions can be originated with changes of haemoglobin structure. Unfolding of haemoglobin should involve dissociation from tetramer to dimer next to monomer and, finally, unfolding of the individual chains. Kinderlerer and co-workers [5] suggested that β-subunits denatured before α-subunits. The spectrophotometric

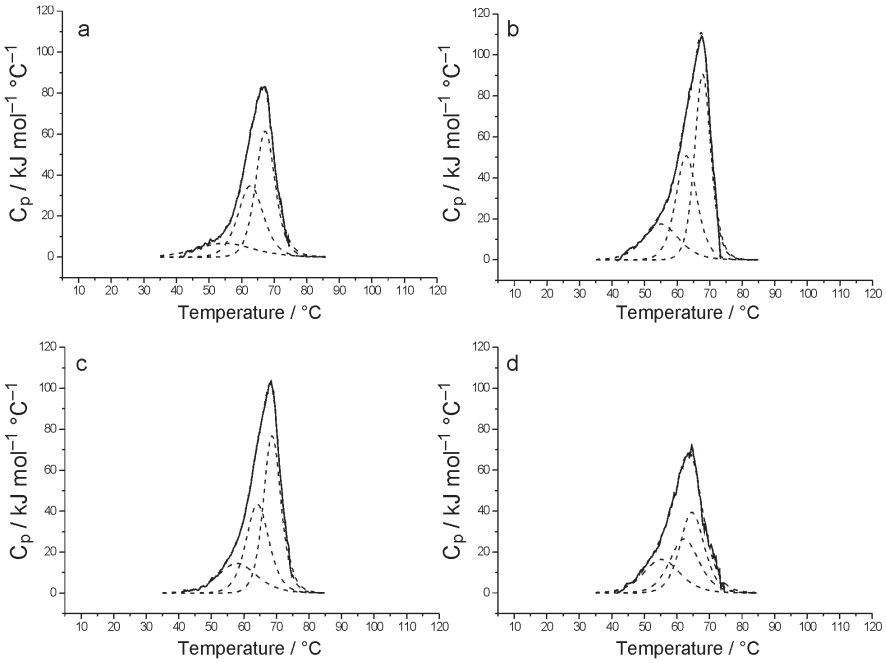

Fig. 2 The curve fitting of DSC profile for NaCl 0.9% solution with Hb concentration 0.4 mg mL^{-1}; a – control; b – after IR radiation; c – after DC magnetic field; d – after UV radiation

observations [6, 7] indicate the greater stability of the core of the globin fold compared to the haeme pockets – the haeme pockets become disordered before further unfolding of the globin chains. On the other hand, overlapping peaks may arise from different Hb derivatives existing in our Hb solutions. It was found [8] that the sequence of decreasing stability of the undissociated tetrameric form is following: deoxyhaemoglobin>carboxyhaemoglobin>oxyhaemoglobin~cyanmethaemoglobin. The later investigations [6, 7] showed carboxyhaemoglobin is more stable than methaemoglobin and that cyanide stabilises methaemoglobin in the same manner that carbon monoxide stabilises normal haemoglobin.

In Table 1 the thermodynamic parameters of the three component transitions for the thermal unfolding process of NaCl 0.9% haemoglobin solution are listed. The main component of denaturation transition is a peak with maximum T_{m1}. The average T_{m1} value of this peak for control samples about 67.4±0.7°C is close to the temperature 67.5°C obtained by Cho and Choy [9] for methaemoglobin at pH=10.5 (T_{m1} value depends on pH). This result is coherent with UV VIS spectroscopy measurements that indicated the high percentage content of various Hb derivatives, mainly methaemoglobin in our studied Hb solutions [10].

Table 1 The thermodynamic parameters of the three component transitions for the thermal unfolding process for NaCl 0.9% solutions Hb

Denaturation peak	Control sample	After AC field	After DC field	After IR radiation	After UV radiation
T_1/°C	67.4±0.7	68.0±0.6	68.3±0.6	67.7±0.2	65.8± 1.5
ΔH_1/kJ mol^{-1}	514.9±3.8	560.8±5.1	539.9±2.5	546.2±4.5	524.5±11.7
T_2/°C	62.6±0.5	63.2±0.4	63.8±0.5	62.8±0.4	62.4± 0.1
ΔH_2/kJ mol^{-1}	368.4±3.7	410.5±2.5	371.9±2.5	382.9±4.8	342.2±10.4
T_2/°C	55.6±1.2	55.3±1.9	58.1±0.9	52.1±4.3	55.6± 0.2
ΔH_3/kJ mol^{-1}	191.6±4.8	259.2±8.2	229.9±3.1	212.5±5.6	243.0± 7.9

It follows from preliminary analysis that UV radiation decreases the main transition temperature by about 2°C while static magnetic field increases the temperature of each component transition (T_1 and T_2 more than 1°C and T_3 by 3°C). The effect of IR radiation and AC magnetic field on T_{mi} values is less significant. The values of enthalpy derivated from 2-State model presented in Table 1 indicate that the first transition step is about 46–48, the next 31–34 and the last 18–22% the over denaturation process. The most significant changes are obtained for enthalpies connected with the third transition that takes place in the lowest temperature. The 22% participation of enthalpy for the solution exposed to UV radiation was obtained.

Figures 3a–d present the fitting for aqueous solution of haemoglobin in Non-2-State model. In this case also three peak components with similar temperatures are obtained but the main components of denaturation transition are peaks T_{m2} and T_{m3} (~34 and 40%, respectively). The thermal midpoints of the transitions are listed in Table 2. T_{mi} values for aqueous solutions are lower than the respective T_{mi} values for NaCl solutions.

Table 2 Comparison of denaturation peak components T_{mi} for examined Hb solutions

Denaturation peak/°C	Control sample	After AC field	After DC field	After IR radiation	After UV radiation
NaCl 0.9% T_1	67.4±0.7	68.0±0.6	68.3±0.6	67.7±0.2	65.8±1.5
H_2O T_1	66.1±0.6	66.5±0.3	66.4±0.5	66.1±0.2	64.3±1.5
NaCl 0.9% T_2	62.6±0.5	63.2±0.4	63.9±0.5	62.9±0.4	62.4±0.1
H_2O T_2	62.8±0.9	62.9±0.9	63.0±0.9	63.2±0.1	60.4±0.1
NaCl 0.9% T_3	55.6±1.3	55.3±1.9	58.1±0.9	52.1±4.3	55.6±0.2
H_2O T_3	57.3±1.2	56.8±1.0	57.0±1.1	56.8±4.2	54.1±0.2

One can see that static magnetic field causes a small shift of the peak temperatures towards higher temperatures. Application of other kinds of electromagnetic field (UV, AC, IR) decreases the denaturation peak temperatures a little. The observed changes seem to be independent of Hb concentration.

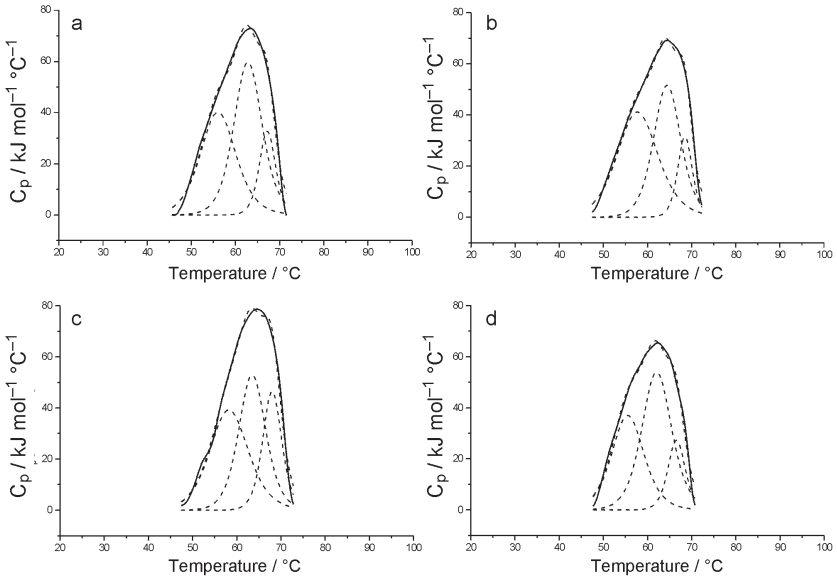

Fig. 3 The curve fitting of DSC profile for aqueous solution with Hb concentration 0.4 mg mL^{-1}; a – control; b – after AC magnetic field; c – after DC magnetic field; d – after UV radiation

It follows from statistical analysis that essential differences (p<0.05) were obtained for the haemoglobin solutions after UV radiation (water and NaCl solutions) and DC magnetic field (only for NaCl solution) exposure in comparison with the control one. The other exposition factors caused no significant differences however the tendency of the observed changes can be taken into consideration.

Higher transition temperatures T_{mi} (Table 2) for NaCl solutions than for aqueous ones indicate a slight stabilisation effect of NaCl on haemoglobin. A slight stabilisation with the addition of NaCl (concentration 0.15 M) was also observed for another protein (aFGF) [11]. Moreover, no effects of ionic strength (up to 0.2 M NaCl) on the thermal stability of various methaemoglobin derivatives were reported [9]. The different contributions of the deconvoluted three components to the total effect under various kinds of exposition may be also due to the specific modifications of the interactions and forces important to the maintenance of the folded, native structure of proteins [12]. Additionally, the effects of various solvents [13, 14] on dissociation and denaturation of haemoglobin point out on their importance for the protein stability.

In Fig. 4 the temperatures of the main denaturation peaks T_{m1} and T_{m2} as a function of electromagnetic field frequency are compared. They decrease a little with the frequency of electromagnetic field used during exposition. This figure provides the same tendency in conformational transitions for aqueous and NaCl 0.9% solutions of haemoglobin. Moreover, the changes of T_{mi} values are less significant for aqueous than for NaCl 0.9% solutions of haemoglobin. It was reported that water plays an essential role in the maintenance of a protein macromolecule in its native state [2]. In

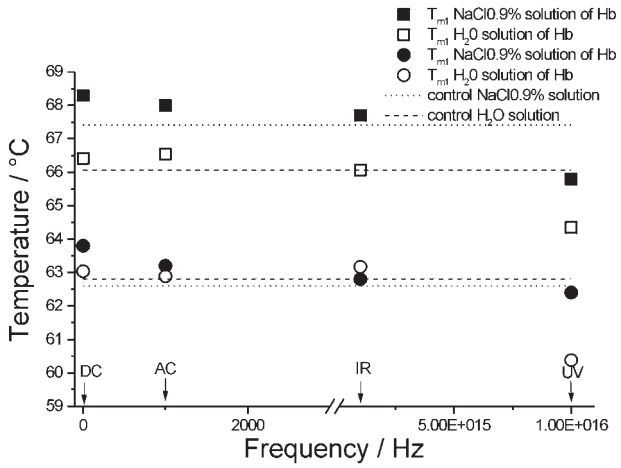

Fig. 4 The denaturation peak temperatures T_{m1}, T_{m2} as a function of frequency of the electromagnetic field used in experiment for examined solutions

the case of the static magnetic field, the length of hydrogen bonds in water decreases and the bonds become stronger.

In general, the interpretation of DSC data of denaturation process is complicated and requires future microcalorimeter studies and deeper analysis.

Conclusions

Differential scanning calorimetry enables to study the thermal stability of haemoglobin solutions. The denaturation process seems to occur in three steps. The shifts of transition temperatures observed under DC and AC magnetic field and UV and IR radiation are more significant for NaCl 0.9% than for aqueous solutions. The results suggest that UV radiation destabilise haemoglobin, while the static magnetic field is effective in protecting the haemoglobin solutions. IR radiation and AC magnetic field have a slight effect on the thermal stability of haemoglobin.

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This study has partly been supported by the State Committee for Scientific Research of Poland (KBN), grant no. 2 P03B 093 19 and apparatus grant no. 3256/IA/160/99.

References

1 J. McCann, F. Dietrich and C. Rafferty, Mutation Research, 411 (1998) 45.
2 M. M. Atef, M. S. Abd El-Baset, Abd El-Kareem, S. Aida and M. A. Fadel, Int. J. Biol. Macromol., 17 (1995) 105.
3 T. Itoh, H. Murakami, K. Orihashi, T. Sueda and Y. Matsuura, Hiroshima J. Med. Sci., 45 (1996) 15.

4 I Jelesarov and H. R. Bosshard, J. Mol. Recognit., 12 (1999) 3.
5 J. Kinderlerer, H. Lehmann and K. F. Tripton, Biochem. J., 135 (1973) 805.
6 F. L. White and K. W. Olsen, Arch. Biochem. Biophys., 258 (1987) 51.
7 T. Yang and K. W. Olsen, Arch. Biochem. Biophys., 261 (1988) 283.
8 G. Guidotti, J. Biol. Chem., 242 (1967) 3685.
9 K. C. Cho and C. L. Choy, Biochim. Biophys. Acta, 622 (1980) 320.
10 A. Michnik, A. Misztal, M. Bartoszek and Z. Drzazga, presented on XXV European Congress on Molecular Spectroscopy, Coimbra 2000, Portugal.
11 D. Adamek, A. Popovic, S. Blaber and M. Blaber, Biocalorimetry, Part VIII: Protein Denaturation Studies, p. 235, Edited by J. E. Ladbury and B. Z. Chowdhry (1998).
12 C. N. Pace, B. A. Shirley, M. Mcnutt and K. Gajiwala, Review, 76 (1996) 75.
13 D. Elbaum and T. T. Herskovits, Biochemistry, 13 (1974) 1268.
14 D. Elbaum, E. R. Pandolfelli and T. T. Herskovits, Biochemistry, 13 (1974) 1278.