BIOLOGIJA. 2008. Vol. 54. No. 3. P. 167–170
DOI: 10.2478/v10054-008-0034-4

Differential scanning calorimetry (DSC) analysis of isolated liver and heart mitochondria

Laima Degutytė-Fomins[1],

Rasa Žūkienė[1],

Žaneta Maijorovaitė[2],

Zita Naučienė[1],

Vida Mildažienė[1, 3]*

[1] *Centre of Environmental Research, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania*

[2] *UAB "Sicor Biotech", V. A. Graičiūno 8, LT-02241, Vilnius, Lithuania*

[3] *Institute of Cardiology, Kaunas University of Medicine, Sukilėlių 17, LT-50009, Kaunas, Lithuania*

Differential scanning calorimetry (DSC) is widely acknowledged for investigation of phase transition of membrane lipids as well as for denaturation of proteins. The aim of the present study was to analyse the thermal behaviour of the components of mitochondria isolated from male rat heart and liver in order to detect the phase transition events that might be responsible for different functional response of heart and liver mitochondria to febrile temperature as well as for the acute loss in membrane barrier function induced at the lower limit of supra-physiological temperature. The complex phase transition in liver and heart mitochondria was determined by DSC measurements in temperature range from 25 °C to 75 °C. The DSC results of heart mitochondria showed phase transition temperatures identical to those of liver mitochondria, except more intensive transition (1.7, 2- and 1.5-fold) at temperatures T_m 46, 54 and 60 °C, respectively, and an additional phase transition peak at T_m 33 °C which was inherent for heart mitochondria only.

Key words: heart mitochondria, liver mitochondria, phase transition, DSC, hyperthermia

Abbreviations: differential scanning calorimetry, DSC; phase transition temperature, T_m; phase transition temperature of peak 1, 2…, T1, T2…; calorimetrically determined enthalpy of the transition, ΔH_{cal}; specific heat, C_p

INTRODUCTION

The elucidation of the molecular mechanism of cellular response to moderate heating is of importance for understanding the events that occur in the cell upon the use of heating for therapeutic purposes or during illnesses associated with fever [1, 2]. The death or survival of different cells upon hyperthermia is determined by a complex response that simultaneously involves different biomolecules and supramolecular structures, complex changes in metabolic activities, signal transduction and gene expression [2]. However, the causal relationships and the sequence of events in the multifactorial chain of stress response induced upon exposure of different cells to heating is still far from being understood [2, 3].

There are indications that mitochondria can be considered as the major targets of hyperthermic stress inside eukaryotic cells [4, 5]. Mitochondria are highly dynamic organelles that frequently move inside cells and exhibit morphological as well as biochemical changes during physiological cell metabolism and stress responses [6]. These organelles may capture a central directory in the cell's fate when affected by physical, biochemical or environmental stress factors. Therefore, we have focused the study on the response of mitochondria from normal tissues to hyperthermia.

We have recently shown that the response of mitochondrial activity to heating in the febrile range is tissue-dependent. Increase of temperature from 37 to 40 °C slightly activated respiration and increased the phosphorylation flux in male rat heart mitochondria [7], but an adverse effect – inhibition of respiration and phosphorylation – was observed in liver mitochondria (unpublished observations). The further increase in temperature by only one degree above the febrile range (up to 42 °C) induced a very sudden increase of the inner membrane permeability and led to the uncoupling of oxidative phosphorylation both in heart [7] and liver mitochondria. In this study, we aimed to analyse the thermal behaviour of the components of mitochondria isolated from male rat heart and liver in order to detect the phase transition events that might be responsible for different functional response of heart and liver mitochondria to febrile temperature as well as for the acute loss in membrane barrier function induced at the lower limit of supra-physiological temperature.

Differential scanning calorimetry (DSC) is a widely acknowledged method for investigating protein denaturation and phase transition of lipid membranes [8]. However, most DSC trials have been only carried on pure lipid bilayers and single proteins. The kinetics of lipid phase transitions in complex systems are not

* Corresponding author. E-mail: v.mildaziene@gmf.vdu.lt

well studied, and calorimetric data on mitochondrial thermal transitions are very scarce. In biological membranes, because of their chemical heterogeneity, the calorimetric transition occurs over a broad range of temperature (10–30 °C) as compared to the restricted, well-defined transition in a pure phospholipid bilayer (2–5 °C) [8, 9]. There is still a big controversy concerning the impact of proteins on thermotropic phase transition in biological membranes. Papahadjopoulos et al. [10] suggested to classify proteins into three types by the protein interaction mode with the membrane lipids and induced characteristic effects on the phase transition; however, it is evident from the results of more recent studies that this classification scheme is not completely appropriate for naturally occurring membrane proteins [9]. An opinion exists that presence of peripheral and integral membrane proteins in biological membranes has only a small effect on temperature, enthalpy and cooperativity of the lipid chain-melting phase transitions *in vivo* [11], although an individual membrane protein can have a fairly marked effect on the thermotropic phase behaviour of a single molecular species of lipid.

A preliminary comparative assessment of hyperthermia-induced changes in heart and liver mitochondria showed that heat (40–45 °C) more strongly increased membrane permeability in heart mitochondria. Hypothetically, the impairment of mitochondrial membrane barrier functions during hyperthermia could be associated with phase transitions of the molecular components, i. e. lipids and proteins.

For the first time we performed a comparative DSC analysis of mitochondria isolated from male rat liver and heart and revealed specific differences in their phase transitions.

MATERIALS AND METHODS

Isolation of mitochondria. Mitochondria were isolated from hearts and livers of male Wistar rats (250–300 g) as described previously [7, 12]. The isolation medium contained 160 mM KCl, 20 mM Tris, 10 mM NaCl, 5 mM EGTA, 1 mg/ml BSA (pH 7.7) (for heart mitochondria), and 10 mM Tris, 3 mM EGTA, 250 mM saccharose (pH 7.7) (for liver mitochondria). After isolation, heart mitochondria were suspended in a suspension buffer containing 180 mM KCl, 20 mM Tris, 3 mM EGTA (pH 7.3) (SB_H), and liver mitochondria were suspended in a buffer containing 5 mM Tris, 250 mM saccharose (pH 7.3) (SB_L) and stored on ice. The concentration of mitochondrial preparation was approximately 55 mg and 111 mg of mitochondrial protein per ml of stored suspension of heart and liver mitochondria, respectively. Protein content was determined by the modified biuret method [13].

Mitochondrial transition measurements by differential scanning calorimetry (DSC). Phase transitions of mitochondria isolated from heart and liver were performed with a MicroCal VP-DSC calorimeter (MicroCal, LLC, Northampton, USA) equipped with 0.5 ml sample and reference cells as described [14]. Prior to measurements, the baseline (medium against medium) was registered. The media used were SB_L or SB_H for liver and heart mitochondria measurements, respectively (see "Isolation of mitochondria"). Mitochondrial suspension was diluted with vacuum degassed SB and scanned from 30 up to 75 °C against SB, scan rate 1 °C/min. Data were registered and analysed with Origin 7.0 specialized software (MicroCal, LLC, Northampton, USA), calculating heat capacity (C_p) and transition temperature (T_m).

Data presentation and statistical analysis. The results are presented as means ± SEM (n = 3).

RESULTS AND DISCUSSION

The complex phase transition in liver and heart mitochondria was determined by DSC measurements in a temperature range from 40 °C to 75 °C. The extended transition peak in liver mitochondria was fitted by five main individual transitions (the grey line in Fig. 1A) of combined enthalpy ΔH_{cal} = 9.07 kcal/mg protein (Table). The transition temperatures T_m and enthalpies ΔH were independent of the variation of mitochondrial concentra-

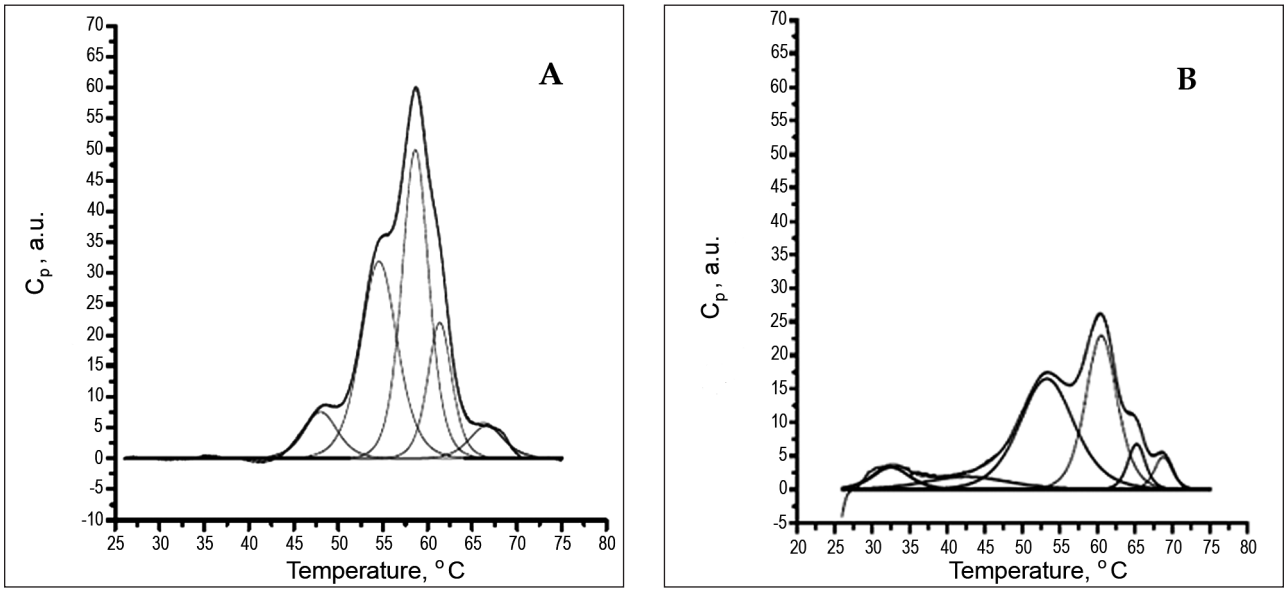

Fig. 1. DSC curves (black lines) of isolated liver (A) and heart (B) mitochondria with simulated putative peaks (grey lines). Base line was approximated by sinusoid. Optimal mitochondrial concentrations: A – 57 mg protein/ml, B – 25 mg protein/ml

tion in a sample, but the concentration affected the intensity of transition peaks and peak resolution (Fig. 2 A, B).

The best peak resolution of a liver mitochondria sample was obtained in a suspension of 57 mg protein/ml SB_L. Increasing the concentration up to 111 mg protein/ml SB_L (using a less diluted liver mitochondria suspension), the peak intensity decreased, with the appearance of an additional peak at T_m 60 °C as a result of the aggregation of thermally denaturated protein. The appearance of an additional high-temperature peak with an increase in protein concentration in the high-density lipoprotein complex was demonstrated earlier [15].

The resolvable transition peaks of the studied liver mitochondria are consistent with Lepock's et al. [14] data ((T_m) 44, 50.8, 57, and 65 °C), with a slight shift to higher temperatures, possibly due to the different animal diet, rat liver mitochondrial isolation procedure, or suspension buffer. Our results showed that the main transitions of liver mitochondria occurred at temperatures T_m 54 °C and 58 °C, whose enthalpies ΔH_{cal} made together 71% of a combined enthalpy. Lepock J. R. et al. [16] presumed that in hepatocytes and in liver mitochondria at 44–57 °C soluble and cytoplasmic components and at 57–70 °C membrane proteins undergo transition.

The DSC results of heart mitochondria indicated identical phase transition temperatures (Fig. 1B and Table) to those of liver mitochondria, except that transitions at temperatures T_m 46, 54 and 60 °C were more intensive (1.7-, 2- and 1.5-fold, respectively). Besides, an additional phase transition peak for heart mitochondria was observed at a temperature (T_m) of 33 °C. It remains to establish whether it is determined by transition or pretransition of specific membrane lipid species.

Historically, the first DSC studies of whole cells and natural membranes were performed on mycoplasma *Acholeplasma laidawii* [17], and it was demonstrated that biological membranes undergo a gel to liquid-crystalline lipid phase transition. Two relatively broad endothermic transitions were reported, and their analysis has revealed that the reversible lower-temperature transition is determined by membrane lipids, whereas the irreversible higher temperature transition is due to protein denaturation [16]. A comparative analysis of available literature data confirms that transition of membrane lipids starts at lower temperatures (42–48 °C) and is followed by protein (50–60 °C) and nucleic acid (58–64 °C) denaturation [3]. Therefore, we conclude that transitions characterised by T1 and T2 (Table) reflect lipid phase transitions of liver and heart mitochondria, and T3–T6 are related to denaturation of mitochondrial proteins.

Phospholipids are the major lipids of biological membranes, and their relative composition is very similar in heart and liver mitochondria, although the amount of lipids per mg protein is different – heart mitochondria contain much more cardiolipin [18], coenzyme Q (4-fold) and neutral lipids (12%), but less cholesterol (by 17%) as compared to liver mitochondria [19]. In DSC measurements, cholesterol strongly abolishes phase transitions in phospholipid bilayers [9, 20]. The higher content of cholesterol in liver mitochondria may result in much lower peak enthalpies both for lipid phase transition and for protein denaturation (Table). It is known that liver and heart

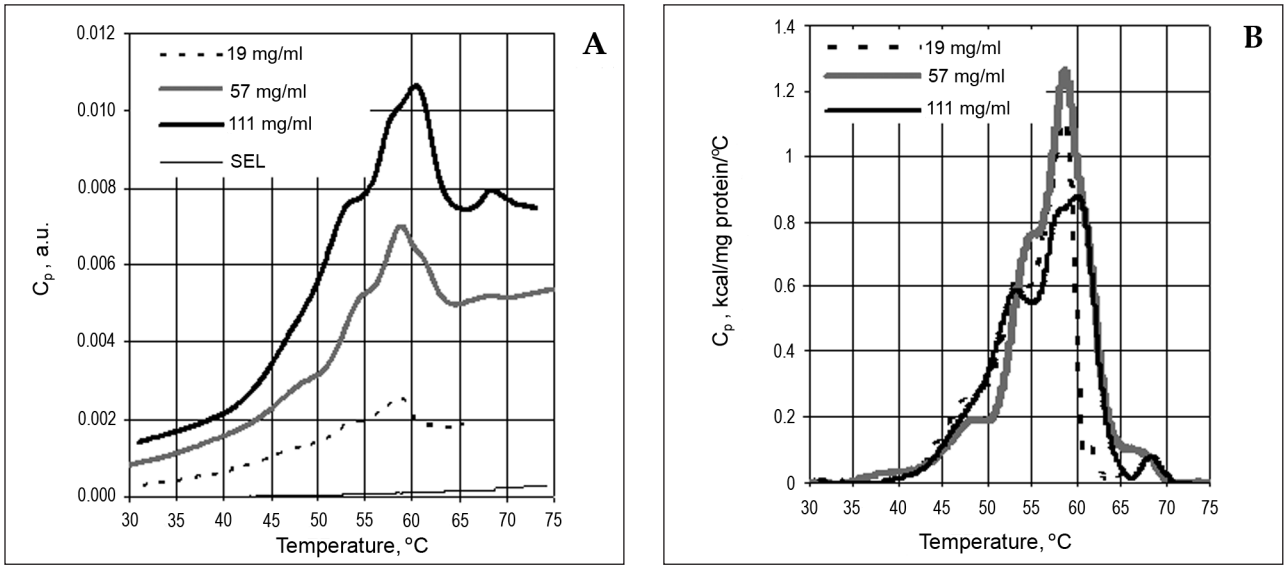

Fig. 2. Dependence of DSC curve profile on liver mitochondria concentration evaluated by protein content: A – raw DSC curves (SB_L – suspension buffer without mitochondria); B – DSC curves with subtracted base line and C_p expressed per mg of protein

Table. **Tansition temperatures T_m and enthalpies ΔH_{cal} for each resolvable peak in DSC profiles of excess C_p vs temperature**

Object	Parameter	T1	T2	T3	T4	T5	T6
Liver mitochondria	T_m (°C)	–	48.0 ± 0.0	53.7 ± 0.7	58.4 ± 0.4	61.0 ± 0.5	67.6 ± 1.3
	ΔH (kcal/mg protein)	–	0.66	3.04	3.51	1.39	0.47
Heart mitochondria	T_m (°C)	33.0 ± 0.7	45.7 ± 4.2	54.6 ± 1.7	60.1 ± 0.7	65.3	68.6 ± 0.3
	ΔH (kcal/mg protein)	0.87	1.13	6.41	5.13	0.85	0.61

mitochondria differ in their overall structure and in protein composition, liver mitochondria having less and shorter cristae of the inner membrane and a denser matrix than heart mitochondria [19]. A recent proteomic comparison has revealed very striking quantitative differences in the abundance of mitochondrial protein between the tissues [21], confirming that the amount of oxidative phosphorylation enzymes is 3- to 10-fold higher; however, the content of enzymes of other metabolic pathways (e. g., carbamoyl-phosphate synthase, glutamate dehydrogenase, glutathione transferase) are 10-fold lower in heart than in liver mitochondria. Although is still difficult to estimate the differences in overall protein amount between these two kinds of mitochondria, it is obvious that different subsets of proteins may be responsible for variations in the quantitative parameters of protein thermal denaturation.

The main difference in the DSC curve of heart mitochondria as compared to liver is an apparent reproducible transition at 33 °C. This transition proceeds at a lower than physiological temperature, and it is difficult to estimate whether it could be related to the activation of respiration in mitochondria at a febrile temperature. Neither in heart nor liver mitochondria had we succeeded to obtain DSC evidence for the phase transition of mitochondrial components at a temperature ranging between febrile and supraphysiological hyperthermia when a very abrupt membrane permeability increase starts [7]. These changes are very likely related to alterations in membrane fluidity rather than to phase transitions, since fluorescent measurements of membrane phospholipid polarization have revealed a transition in the membrane order between 40 and 43 °C, a finding consistent with an increased membrane proton conductance [22].

ACKNOWLEDGEMENT

This work was supported by the Lithuanian State Science and Studies Foundation, project No. P-02/2007-1.

Received 08 April 2008
Accepted 25 August 2008

References

1. Roti Roti JL. J Hyperthermia 2008; 24(1): 3–15.
2. Park HG, Han SI, Oh SY et al. Cell Mol Life Sci 2005; 62(1): 10–23.
3. Despa F, Orgill DP, Neuwalder J et al. Burns 2005; 31(5): 568–77.
4. Qian L, Song X, Ren H et al. Cell Stress & Chaperones 2004; 9(3): 281–93.
5. Ko S, Yuen WF, Fung KP et al. Life Sci 2000; 67(25): 3113–21.
6. Jakobs S. BBA-Mol Cell Res 2006; 1763(5–6): 561–75.
7. Zukiene R, Nauciene Z, Ciapaite J et al. Biologija 2007; 53(4): 34–9.
8. Melchior DL, Steim JM. Ann Rev Bioph Bioeng 1976; 5(1): 205–38.
9. Lewis RNAH, McElhaney RN. In: The Structure of Biological Membranes. Yeagle PP (ed.). Boca Raton–London–New York–Washington, D. C.: CRC Press, 2005: 53–120.
10. Papahadjopoulos D, Moscarello M, Eylar EH et al. BBA-Biomembranes 1975; 401(3): 317–35.
11. McElhaney RN. In: Temperature Adaptation of Biological Membranes. Cossin AR (ed.). London: Portland Press, 1994: 31–4.
12. Mildaziene V, Nauciene Z, Baniene R et al. Toxicol Sci 2002; 65: 220–7.
13. Gornal AG, Bardawill CJ, David MM. J Biol Chem 1949; 177: 751–66.
14. Lepock JR, Frey HE, Rodahl AM et al. J Cell Physiol 1988; 137(1): 14–24.
15. Gursky O, Atkinson D. Proc Natl Acad Sci USA 1996; 93(7): 2991.
16. Lepock JR. J Cell Biol 1993; 122(6): 1267–76.
17. Steim JM, Tourtellotte ME, Reinert JC et al. Proc Natl Acad Sci USA 1969; 63(1): 104–9.
18. Hoch F. J Bioenerg Biomembr 1998; 30(6): 511–32.
19. Fleischer S, Rouser G, Fleischer B et al. J Lipid Res 1967; 8(3): 170–80.
20. Mannock DA, Lewis R, McElhaney RN. Biophys J 2006; 91(9): 3327.
21. Forner F, Foster LJ, Campanaro LJ et al. Mol Cell Proteomics 2006; 5(4): 608–19.
22. Willis WT, Jackman MR, Bizeau ME et al. Am J Physiol – Regul Integr Comp Physiol 2000; 278(5): 1240–46.

Laima Degutytė-Fomins, Rasa Žūkienė, Žaneta Maijorovaitė, Zita Naučienė, Vida Mildažienė

IZOLIUOTŲ KEPENŲ IR ŠIRDIES MITOCHONDRIJŲ DIFERENCINĖ NUSKAITANTI KALORIMETRINĖ (DSK) ANALIZĖ

Santrauka

Diferencinės nuskaitančios kalorimetrijos (DSK) metodas taikomas tiriant baltymų denatūraciją ir fazinį membranų lipidų virsmą. Šio darbo tikslas – palyginamoji terminė izoliuotų žiurkės patino širdies ir kepenų mitochondrijų fazinių virsmų analizė, siekiant įvertinti ir paaiškinti mūsų anksčiau nustatytus hipertermijos sukeltų mitochondrijų funkcijų pokyčių skirtumus. Faziniai virsmai buvo tirti atliekant DSK matavimus 25–75 °C temperatūros intervale. Nustatyta, kad širdies ir kepenų mitochondrijose faziniai virsmai vyksta esant tai pačiai temperatūrai, tačiau širdies mitochondrijų faziniai virsmai T_m 46, 54 ir 60 °C temperatūroje yra intensyvesni (1,7, 2 ir 1,5 karto atitinkamai), lyginant su kepenų mitochondrijomis. Tik širdies mitochondrijų sandams būdingas fazinis virsmas ties T_m 33 °C.