Journal of Thermal Analysis and Calorimetry, Vol. 82 (2005) 225–228

THERMAL STABILITY OF RAT UTERUS DURING DEVELOPMENT DSC approach

Zsuzsanna Vértes[*]

Institute of Physiology University of Pécs, Faculty of Medicine H-7624 Pécs, Szigeti str. 12., Hungary

The age dependence of thermal denaturation was monitored in rats anaesthetized after they're born at 7th, 14th, 21st, 28th, 35th and 42nd days. The samples were stored in rigor or physiological saline solution. The DSC scans in the early age groups show a low temperature exotherm (connective tissues: from gel to liquid crystal transition) and one endotherm (it is very probably the myosin). During further development the endotherms became more and more complex (due to the development of contractile system). At 42 days the scans seem to be similar to the adult ones. In the two buffers the endotherms markedly differ showing that the ATPase activity is present. In adult uterus, treated with nucleotides or estrogen, this activity significantly differs from the skeletal muscle.

On the basis of our results we suppose, that the age dependent changes are decisive processes in the development of rat uterus.

Keywords: *ADP and ADP+V_i, DSC, smooth muscle, thermal stability, uterus*

Introduction

It is well known that one of the main characteristics of smooth muscle is that its contraction and relaxation phases are slower than that of any other type of muscle. The contraction could last for more than 30 s and the muscle does not tire easily. These sustained contractions and ability of the muscle to be stretched beyond its resting states make smooth muscle to be able for the muscular control of uterus.

The energetic background of muscle contraction is widely discussed for different intact muscles [1, 2]. In rabbit skeletal muscle fibers the myosin heads have different dynamic molecular states and thermal stability in the intermediate states of the ATP hydrolysis cycle, as it was shown in previous papers [3–5]. The myosin heavy chain (MHC) isoform content has been shown to be an important determinant of contractile characteristics in striated muscle [6–8]. In smooth muscle the rate of ATP hydrolysis cycle is lower than in skeletal muscle but it works as a more efficient contractile unit. It is well suited for long-term maintenance of tension. This way is a very good object for a long lasting differential scanning calorimetric experiments.

In our experiments we examined the age dependence of thermal denaturation of the rat uterus to see the thermal consequences of the development of its contractile system. It is known that different myosin isoform can be expressed in mammalian smooth muscle in adult and developing stage [9–11]. We used samples of rats anaesthetized after they're born at 7th, 14th, 21st, 28th, 35th and 42nd days. The samples were stored in rigor solution as well as in physiological saline to look for the different function of their ATPase system. These results are compared to observations achieved on adult uteri being in rigor, strong and weak binding states to monitor the changes in the contractile system during development.

As an example for the physiological function of the uterus, we present a thermal unfolding of estrogen treated uterus because it is well known that the main effect of estrogen on the uterus is stimulation of DNA synthesis and cell proliferation [12]. In our previous works we investigated the role of endogenous opioid peptides in the regulation of cell growth and proliferation in the uterus [13–17]. In order to elucidate the possible mechanism by which estrogen treatment increases uterine contractility, the effect of estrogen on thermal unfolding was measured in the isolated rat uterus.

Materials and methods

Animals

CFY strain female rats aged of 7, 14, 21, 28, 35, 42 days and adults were used. The animals were housed in temperature-controlled animal quarters under 12 h light dark cycle and maintained on ad libitum food and water. The rats were killed by decapitation and the uteri were excised and trimmed of adhering fat. The weighed uteri were incubated individually in sealed glass vials containing 4 mL physiological saline or rigor solution (80 mM K-propionate (KPr), 5 mM $MgCl_2$, 2 mM EGTA, 25 mM Tris·HCl,

* zsuzsanna.vertes@aok.pte.hu

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

pH 7.0), rigor solution + 5mM MgADP, rigor solution +5 mM ADP +5 mM V_i (orthovanadate) at pH 7.4 under an atmosphere of O_2–CO_2 (95:5, mass/mass) with continuous shaking. Potassium chloride (KCl), magnesium chloride ($MgCl_2$), ethylene glycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), histidine·HCl, adenosine 5'-diphosphate (ADP) and orthovanadate (Na_3VO_4) were obtained from Sigma (Germany). In some experiments the uteri were incubated with Krebs-Ringer bicarbonate buffer containing 100 nM estrogen (Oe) at the same conditions. The manipulations were done at 0–4°C unless stated otherwise. Our activities were done under the proper law paragraphs and valid permissions.

Calorimetric measurements

The thermal unfolding of muscle proteins in uterus stripes was monitored by a SETARAM Micro DSC-II calorimeter (SETARAM, France). All experiments were done between 0 and 100°C with a scanning rate of 0.3 K min^{-1}. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume in average. Physiological saline and rigor buffer was used as reference sample respectively. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The samples were irreversible denaturated during each cycle.

Evaluation of DSC scans

The repeated scan of denaturated sample was used as baseline reference, which was subtracted from the original DSC scans. Calorimetric enthalpy was calculated from the area under the heat absorption curves using two points setting SETARAM peak integration.

Results and discussion

According to our results the effect of maturation on the thermal stability of the rat uterus can be divided into two main phases. Up to 21 days there is a big low temperature exotherm step, which could be the sign of the gel–liquid crystal transition. It could be originated very probably from the connective tissues, the outer and inner membrane components of whole muscle and muscle fibers respectively. This thermal component appears at all age groups in the same manner in samples kept in both buffers. At very first the myosin melting could be identified in the case of both treatments (Figs 1a, 2a) but in rigor it appears at lower temperature than in physiological saline. After one

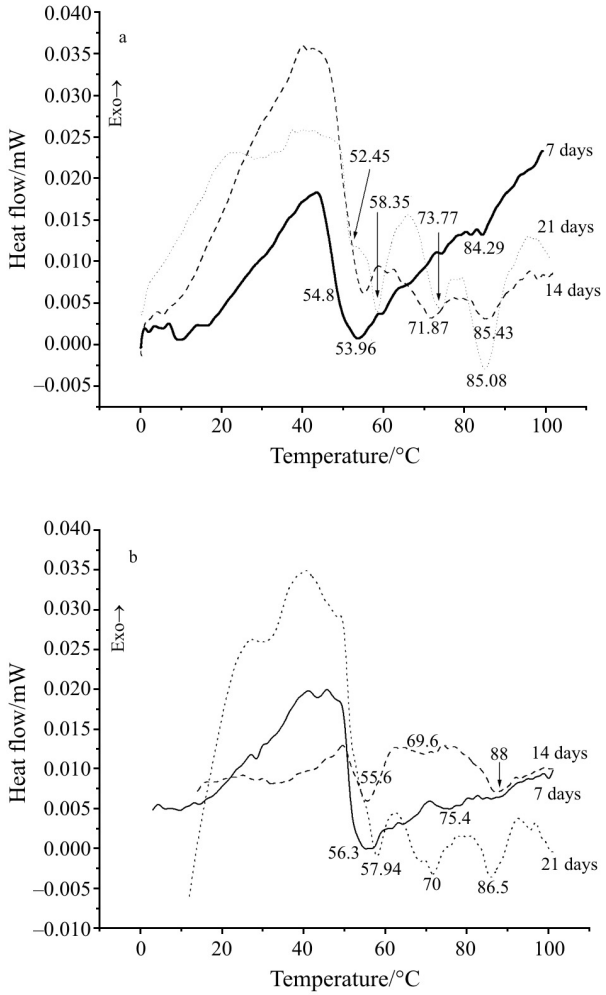

Fig. 1 The thermal denaturation of rat stripes in rigor buffer a – as well as in physiological saline b – up to 21st day of maturation

week appear more and more pronounced manner the endotherms of different muscle proteins and connective tissues (Figs 1, 2).

Up to 42 days the most characteristic change in the endotherms can be observed in the contribution of myosin melting. Its main transition temperature is continuously rising in the function of the age up to ~59°C, with the simultaneous decrease of the difference of this melting temperature in the case of the two treatments (Figs 1, 2). This could be the sign of the function and refinement of the ATPase system of the contractile apparatus [11]. After 42 days of age the thermal denaturation is similar to the adult samples (Fig. 3). The characteristic thermal parameters are shown in Table 1. The unfolding of proteins in muscle fibers by thermal excitation is a complex process and depends on the state of the actomyosin complex as well as the type of muscle [18]. For the sake of comparison the experimental data of cross-striated muscle (prepared from rabbit m. psoas) are used. They suggest [19–22] at least three transitions in the main tran-

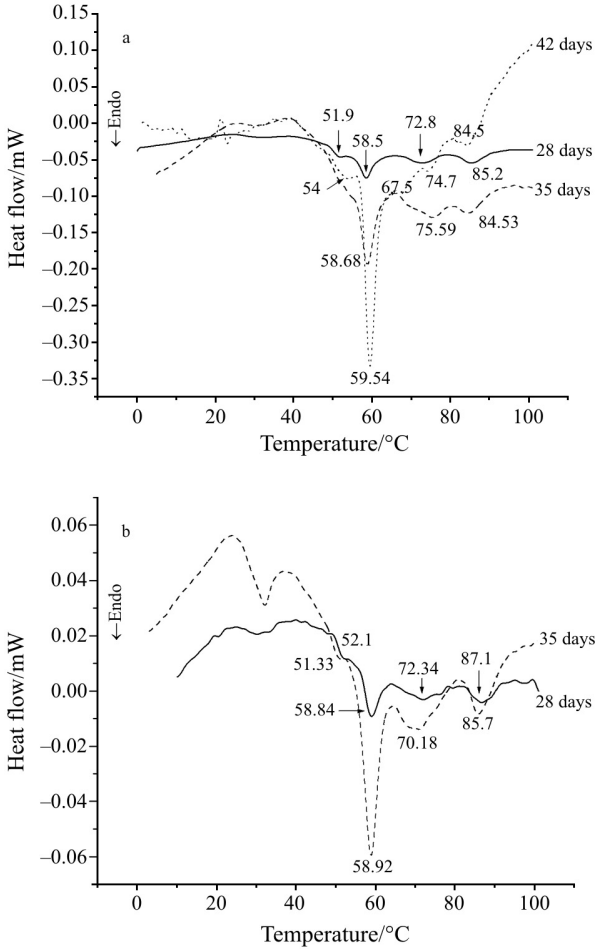

Fig. 2 The thermal denaturation of rat stripes in rigor (a) or saline (b) after the third week of maturation

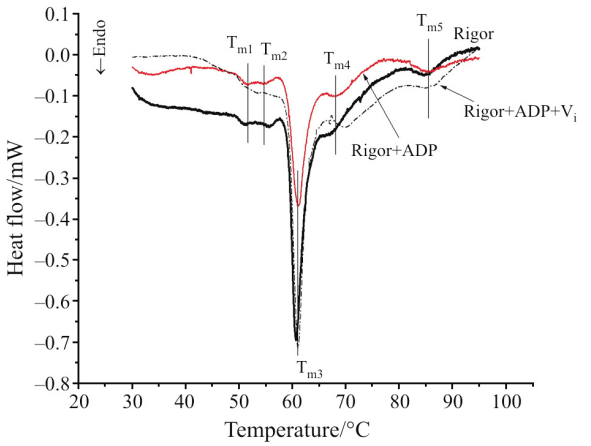

Fig. 3 DSC scans of adult rat stripes in different simulated ATPase cycles

sition temperature range: they can be seen between 45 and 70°C, and the superposition of endotherms is believed to correlate with T_ms of larger domains of myosin and actin filaments. These are in rigor state: T_{m1}=54.05°C, T_{m2}=58.36°C and T_{m3}=62.29°C, in strongly binding state (in the presence of MgADP) T_{m1} decreases to 53.5°C as a consequence of the internal rearrangement of myosin structure [23–25]. In weakly binding state (rigor + 5 mM MgADP + 5 mM V_i) the separation of the first two peaks is less pronounced [26]. It is obvious that addition of nucleotides produces conformational changes in the multisubunit structure of myosin.

Performing the same experiments in adult rat uterus stripes the transition temperature range has been remarkably changed (49–76.7şC, Fig. 3) compared to skeletal fibers. The main thermal transition contains at least five separate denaturations. The main T_{m3} (61.1şC) was the same for rigor and weak binding states, while it moved to 60.7şC in the case of strong binding state as a consequence of structural rearrangement in myosin head (Fig. 3). The total enthalpies of thermal denaturation were 0.45 J g^{-1} for rigor, 0.41 J g^{-1} for strong binding and 0.6 J g^{-1} for weak binding states

From our data we can see that in uterus the transition from rigor into strong binding state is accompanied with a total enthalpy decrease, which is the sign that the system is less stable from global point of view in a good agreement with the skeletal muscle fibers data [3–5]. It is surprising that the total enthalpy change increases and the highest transition temperature goes up in the weak binding state. In the case of smooth muscle the differences in filament stability as a result of phosphorylation – which could appear as a consequence in the thermal stability – are due largely to conformational change occurring in the myosin head, and are not due to differences in filament packing [27]. These molecular changes induced at the active site (myosin head domain) by phosphorylation critically depend upon a stable coiled-coil tail that determines how the regulatory light chains interact at the head/rod junction [28]. It could mean that in smooth muscle the rod part of myosin gives greater contribution to the enthalpy change that can explain our result in weak binding state. It is known that unphosphorylated smooth myosin acts as a load to slow down the rate at which actin is moved by the faster cycling phosphorylated cross-bridges [29]. The rate at which rigor cross-bridges can be recruited to move actin filaments was observed by initiating cross-bridge cycling from rigor in model system by flash photolysis of caged MgATP. A delay at low MgATP concentrations was observed and interpreted as evidence that motion-generating cross-bridges are slowed by a load due to a transiently high percentage of rigor cross-bridges immediately following MgATP release [30]. This experimental data could explain the lower enthalpy change in our strong binding model.

To examine the possible mechanism by which estrogen treatment increases uterine contractility we have checked its effect on thermal stability of uterus.

Table 1 The melting temperatures and transition enthalpy changes of uterus samples treated with different nucleotides as well as with estrogen (average ± standard deviation)

Parameters of samples*	T_M/°C	H/J g^{-1}
Uterus+R (s=4; n=2)	60.7±0.15	0.45±0.04
Uterus+ADP (s=4; n=2)	61.1±0.2	0.41±0.03
Uterus+ADP+V_i (s=4; n=2)	61.1±0.2	0.6±0.05
Uterus+Oe (s=4; n=2)	62.4±0.3	0.56±0.04

*s=number of different rat samples, n=number of measurements from the same sample batch

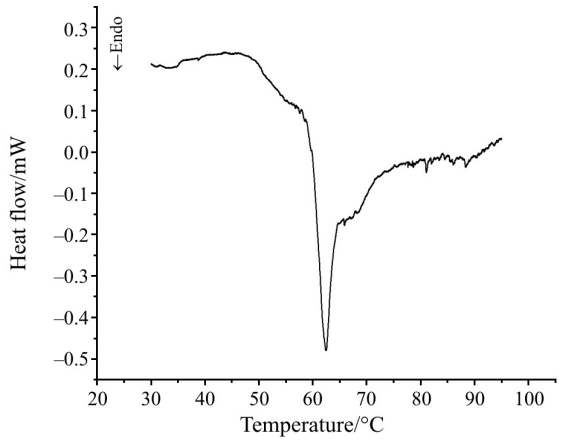

Fig. 4 Denaturation curve of estrogen treated uterus

It was observed (Fig. 4 and Table 1) that estrogen treatment resulted in the increase of melting temperature and transition enthalpy. It means that in estrogen activated state the contractile system of uterus is in an intermediate state between rigor and ADP state.

To summarize we can say that the DSC is a useful tool in monitoring the consequences of age dependent changes in the composition and the state of uterus.

It seems our interpretation is in reasonably agreement with the experimental data obtained in earlier experiments for skeletal fibers [3–5], there is a difference in the global behavior of the intermediate ATPase cycle in rat uterus smooth muscle too. To look into the deeper details of these processes further experiments are required for the molecular dynamic behavior (e.g. EPR and fluorescence spectroscopy) and thermal properties of rat uterus smooth muscle and its protein compounds.

Acknowledgements

This work was supported by grants from the National Research Foundation (OTKA T 029267, CO-272).

References

1 R. C. Woledge, N. A. Curtin and E. Homsher, Energetic Aspects of Muscle Contraction, Academic Press, London, 1985.
2 E. Homsher, Ann. Rev. Physiol., 49 (1987) 673.
3 D. Lőrinczy and J. Belagyi, Biochem. Biophys. Res. Commun., 217 (1995) 592.
4 D. Lőrinczy and J. Belagyi, Thermochim. Acta, 296 (1997) 161.
5 D. Lőrinczy, B. Gaszner, F. Könczöl and J. Belágyi, J. Thermal Anal. Cal., 56 (1999) 1203.
6 M. Bárány, J. Gen. Physiol., 50 (1967) 197.
7 B. Swynghedauw, Physiol. Rev., 66 (1986) 710.
8 P. Wagner, J. Biol. Chem., 256 (1981) 2493.
9 C. Kelley, M. Takahashi, J. Yu and R. Adelstein, J. Biol. Chem., 268 (1993) 12848.
10 S. White, A. Martin and M. Periasamy, Am. J. Physiol., 264 (1993) C1252.
11 S. L. White, M. Y. Zhou, R. B. Low and M. Periasamy, Am. J. Physiol., 275 (1998) C581.
12 T. Ördög, Z. Vértes and M. Vértes, Life Sci., 51 (1992) 1187.
13 Z. Vértes, T. Ördög, M. Vértes and S. Kovács, J. Steroid Biochem., 46 (1993) 819.
14 Z. Vértes, J. L. Környei, S. Kovács and M. Vértes, Eur. J. Pharmacol., 291 (1995) 115.
15 Z. Vértes, J. L. Környei, S. Kovács and M. Vértes, J. Steroid Biochem., 59 (1996) 173.
16 Z. Vértes, A. Sándor, K. A. Kovács, A. Oszter, J. L. Környei, S. Kovács and M. Vértes, J. Endocrinol. Invest., 23 (2000) 502.
17 A. Oszter, Z. Vértes, B. Törőcsik, J. L. Környei, K. A. Kovács and M. Vértes, J. Steroid Biochem., 74 (2000) 25.
18 I. Gazsó, J. Kránicz, Á. Bellyei and D. Lőrinczy, Thermochim. Acta, 402 (2003) 117.
19 D. Lőrinczy and J. Belágyi, Eur. J. Biochem., 268 (2001) 5970.
20 D. Lőrinczy, N. Hartvig and J. Belagyi, J. Biochem. Biophys. Method, 53 (2002) 75.
21 D. Lőrinczy, M. Kiss and J. Belágyi, J. Therm. Anal. Cal., 72 (2003) 573.
22 L. Dergez, F. Könczöl, N. Farkas, J. Belágyi and D. Lőrinczy, J. Therm. Anal. Cal., (2005) (in press)
23 I. Rayment, H. M. Holden, M. Whittaker, C. B. John, M. Lorenz, K. C. Holmes and R. A. Milligan, Science, 261 (1993) 58.
24 D. Raucher, C. P. Sár, K. Hideg and P. G. Fajer, Biochemistry, 33 (1994) 14317.
25 A. J. Fischer, C. A. Smith, J. Thoden, R. Smith, K. Sutoh, H. M. Holden and I. Rayment, Biophys. J., 68 (1995) 19S.
26 M. Kiss, J. Belágyi and D. Lőrinczy, J. Therm. Anal. Cal., 72 (2003) 565.
27 K. M. Trybus and S. Lowey, J. Cell Biol., 105 (1987) 3007.
28 K. M. Trybus, J. Biol. Chem., 269 (1994) 20819.
29 D. M. Warshaw, J. M. Desrosiers, S. S. Work and K. M. Trybus, J. Cell Biol., 11 (1990) 453.
30 D. M. Warshaw, J. M. Desrosiers, S. S. Work and K. M. Trybus, J. Biol. Chem., 266 (1991) 24339.

DOI: 10.1007/s10973-005-7096-7