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The effect of nucleotides (ADP and ADP + V_i) on the thermal stability of rat uterus

D. Lorinczy[a,*], Zs. Vértes[b], J. Belagyi[c]

[a]*Biophysical Department, Faculty of Medicine, University of Pécs, Szigeti str. 12, H-7624 Pécs, Hungary*
[b]*Faculty of Medicine, Institute of Physiology, University of Pécs, Szigeti str. 12, H-7624 Pécs, Hungary*
[c]*Central Research Laboratory, Faculty of Medicine, University of Pécs, Szigeti str. 12, H-7624 Pécs, Hungary*

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Abstract

Thermal unfolding of stripes prepared from rat uterus has been studied in the presence of nucleotides by differential scanning calorimetry (DSC). Using ADP, ATP and inorganic phosphate analogue orthovanadate, three intermediate states of the ATP hydrolysis cycle were simulated in the uterus stripes. In the main transition of the DSC pattern at least four overlapping endotherms were detected in rigor (AM), in strongly binding (AM · ADP) and weakly binding state (AM · ADP · V_i) of myosin to actin. It was found that nucleotide binding induced a shift of the main melting temperatures (from 60.7 to 61.1°C) and produced changes in the total calorimetric enthalpies (0.45 J/g for rigor, 0.4 J/g for strong binding, and 0.6 J/g for weak binding state). In the Krebs–Ringer bicarbonate buffer containing 100 nM estrogen (Oe) the main transition temperature shifted to 62.4°C and the total enthalpy change was 0.56 J/g. It seems to be an intermediate phase between the strong and weak binding state. The changes of the parameters of the peak functions suggest global rearrangements of the internal structure in myosin heads in the intermediate states.

Keywords: Smooth muscle; Uterus; Thermal stability; DSC; ADP; ADP + V_i

1. Introduction

The main characteristics of smooth muscle are that their contraction and relaxation phases are slower than that of any other type of muscle and they act rhythmically. Their contraction period could last for more than 30 s and they do not tire easily. These sustained contractions and their ability to be stretched beyond their resting states make smooth muscle to be able for the muscular control of uterus, especially during pregnancy. The cells of smooth muscle tissue are called fibres, the longest ones (about 0.5 mm) are found in the uterus during the latest stages of pregnancy. Despite the fact that troponin is absent in smooth muscle the basic contractile mechanism appears to be much the same as in skeletal muscle. The energetic aspects of muscle contraction are widely discuss for different intact muscles [1,2]. It were shown in our previous papers [3–5] that in rabbit skeletal muscle fibres the myosin heads have different dynamic molecular states and thermal stability in rigor, strong as well as weak binding states of the ATP hydrolysis cycle. In smooth muscle the rate at which ATP is utilised for the contractile process is lower than in skeletal muscle but the smooth muscle is a more efficient contractile unit. It is well suited for

* Corresponding author. Tel.: +36-72-536-260; fax: +36-72-536-261.
E-mail address: denes.lorinczy@aok.pte.hu (D. Lorinczy).

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long-term maintenance of tension. This way it is a very good object for a long lasting differential scanning calorimetric experiments, therefore in this paper we investigate — according to our knowledge at very first in this field — the thermal denaturation of different contraction states in rat uterus fibres. Using ADP, ATP and inorganic phosphate analogue orthovanadate, three intermediate states of the ATP hydrolysis cycle were simulated in rat uterus muscle stripes.

The importance of estrogen in the development of the uterus has been known for almost a century. The main effect of estrogen on the uterus is stimulation of DNA synthesis and cell proliferation [6]. In our previous work we investigated the role of endogenous opioid peptides in the regulation of cell growth and proliferation in the uterus [7–11].

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.

2. Materials and methods

2.1. Animals

CFY strain female rats 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 Krebs–Ringer bicarbonate buffer pH 7.4 under an atmosphere of O_2–CO_2 (95:5, v/v) with continuous shaking.

In some experiments the uteri were incubated with Krebs–Ringer bicarbonate buffer containing 100 nM estrogen (Oe) at the same conditions, in others with rigor solution (80 mM K-propionate (KPr), 5 mM $MgCl_2$, 2 mM EGTA, 25 mM Tris–HCl, pH 7.0), rigor solution + 5 mM MgADP, rigor solution + 5 mM ADP + 5 mM V_i (orthovanadate). 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). The manipulations were done at 0–4°C unless stated otherwise. Our activities were done under the proper law paragraphs and valid permissions.

2.1.1. Calorimetric measurements

The thermal unfolding of myosin in fibres 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. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μl sample volume in average. Rigor buffer was used as reference sample. 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.

2.1.2. Evaluation of DSC scans

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

3. Results and discussion

The unfolding of proteins in muscle fibres by thermal excitation is a complex process and depends on the state of the actomyosin complex. The experimental data for cross-striated muscle (prepared from rabbit m. psoas) suggest at least four transitions in the temperature range examined. The minor transition at $T_{m0} = 18$°C might be attributed to the interaction of LC-2 light chain with the long α-helical part of the myosin head [12]. This transition could be evaluated as the sign of an interdomain communication. The main transition between 45 and 70°C is the superposition of endotherms that are believed to correlate mainly with T_m of the larger domains of myosin and a smaller contribution from thin filaments. In the absence of nucleotides (rigor state) three transitions was decomposed from the DSC scan with $T_{m1} = 54.05$°C, $T_{m2} = 58.36$°C and $T_{m3} = 62.29$°C melting temperatures (Table 1). In the presence of MgADP there was a decrease in $T_{m1} = 53.5$°C and an

Table 1
The melting temperatures and transition enthalpy changes of uterus samples treated with different nucleotides as well as with estrogen (average ± S.D.)[a]

Parameter of samples	T_m (°C)	H (J/g)
Uterus + R ($s = 4$; $n = 2$)	60.71 ± 0.15	0.446 ± 0.04
Uterus + ADP ($s = 4$; $n = 2$)	61.08 ± 0.2	0.404 ± 0.03
Uterus + ADP × V_i ($s = 4$; $n = 2$)	61.08 ± 0.2	0.603 ± 0.05
Uterus + Oe ($s = 4$; $n = 2$)	62.36 ± 0.3	0.559 ± 0.04

[a] s: Number of different rat samples, n: number of measurements from the same sample batch.

increase in the transition enthalpy which could be the energetic consequence of the internal rearrangement of myosin structure in strongly binding state [13–15]. When 5 mM MgADP was added in the presence of 5 mM V_i the separation of the first two peaks is less pronounced. It is obvious that addition of nucleotides produces conformational changes in the multisubunit structure of myosin. The enthalpy of the thermal unfolding depends on the nucleotides, the conversion from a strongly attached state of myosin for actin to a weakly binding state is accompanied with an increase of the transition enthalpy of the third melting peak which might relate to the change of the affinity of nucleotide binding to myosin.

The transition temperature range for rat uterus stripes has been remarkably changed (49–76.7°C) compared to skeletal fibres. The main thermal transition contains at least four separate denaturations. The main T_{m3} was 60.7°C for rigor model (Fig. 1) while it increased significantly to 61.1°C in the case of strong (Fig. 2) and weak binding (Fig. 3) simulation. The end temperature of weak binding transition raised up to 80.4°C which was also more pronounced than in skeletal fibres. The total enthalpies of thermal denaturation were 0.446 J/g for rigor, 0.404 J/g for strong binding and 0.603 J/g for weak binding states. In the case of skeletal muscle fibres the total enthalpy remained approximately constant independently from the type of hydrolysis cycle [5] only the contribution of different structural and functional domains was changed. We did not performed any DSC scan decomposition because in the literature we did not find well accepted data for the thermal denaturation of the different smooth muscle proteins, therefore we have no indication for the identification with the proper structural elements or with the possible internal molecular rearrangements during the different states of ATP hydrolysis cycle. From our data we can see that 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 fibres data [3–5]. It is surprising in the case of weak binding state the increase of total enthalpy change and the shift of the higher temperature of transition temperature range. We have observed for skeletal muscle a greater enthalpy contribution and transition temperature (63.8°C) by the third main transition component but the total enthalpy change remained the same. That finding was explained by the more dense packing of the nucleotide binding domain in 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 [16]. 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 [17]. It could mean that in smooth muscle the rod part of myosin gives greater contribution to the enthalpy change which 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 [18]. 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. Following the flash, which results in a rapid increase in MgATP concentration, actin filaments experienced a MgATP-dependent delay prior to achieving steady state velocity. The delay at low MgATP concentrations was 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 [19]. This experimental data could explain the lower enthalpy change in our strong binding model.

It seems our interpretation is in reasonably agreement with the experimental data obtained in earlier experiments for skeletal fibres [3–5], there should be

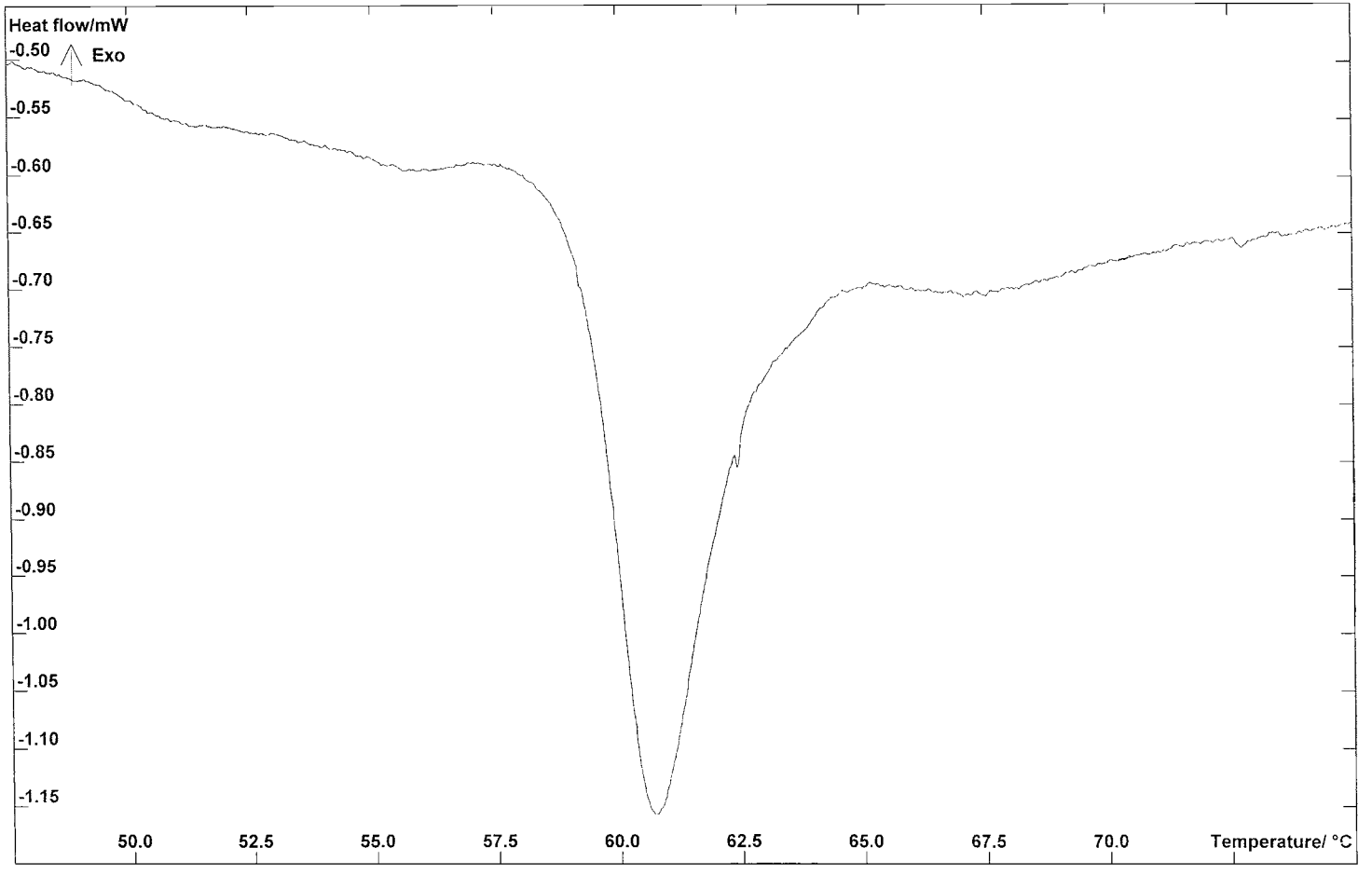

Fig. 1. The thermal denaturation of rat stripes in rigor buffer.

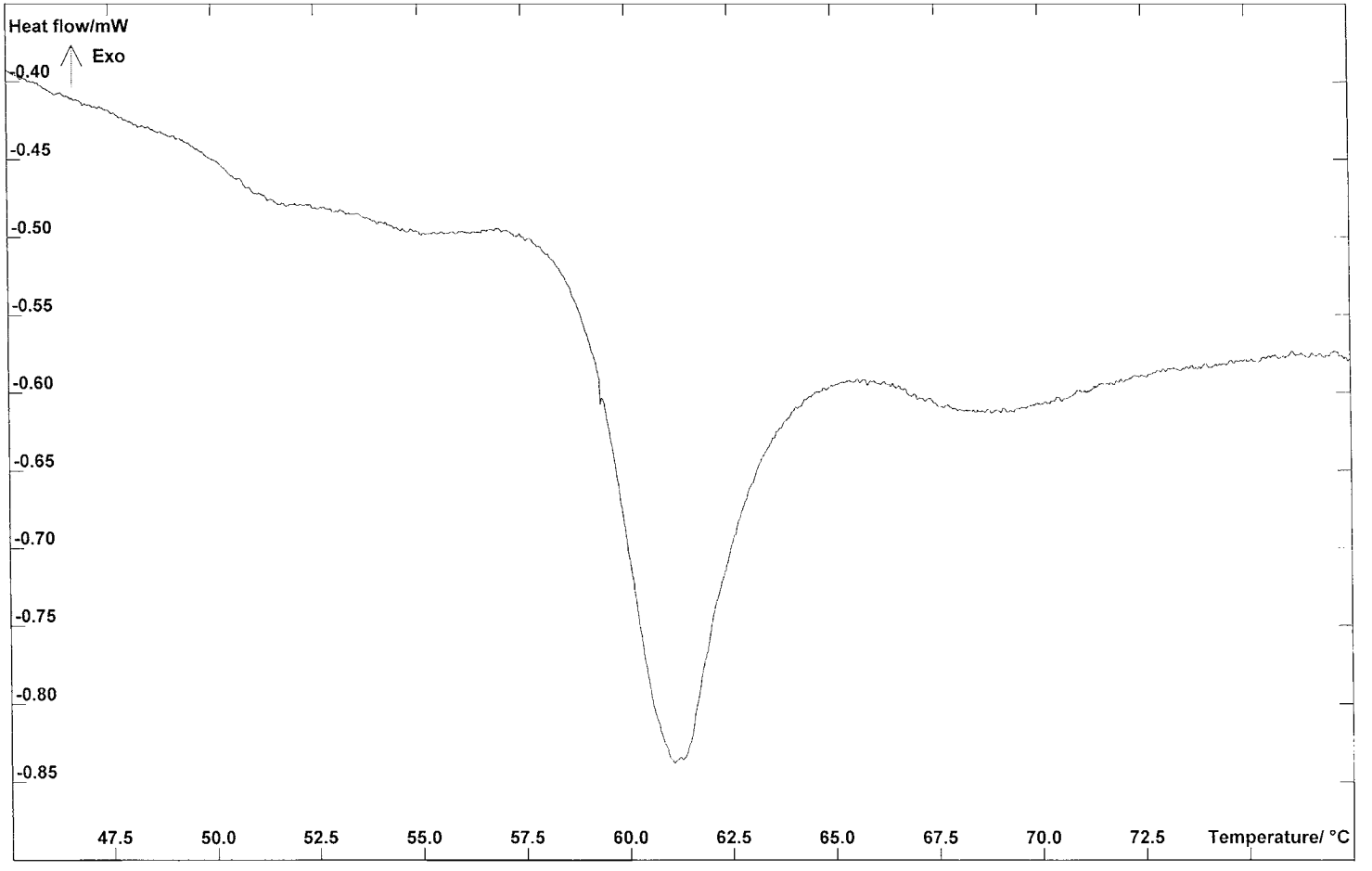

Fig. 2. DSC scan of rat stripes in rigor buffer + 5 mM ADP.

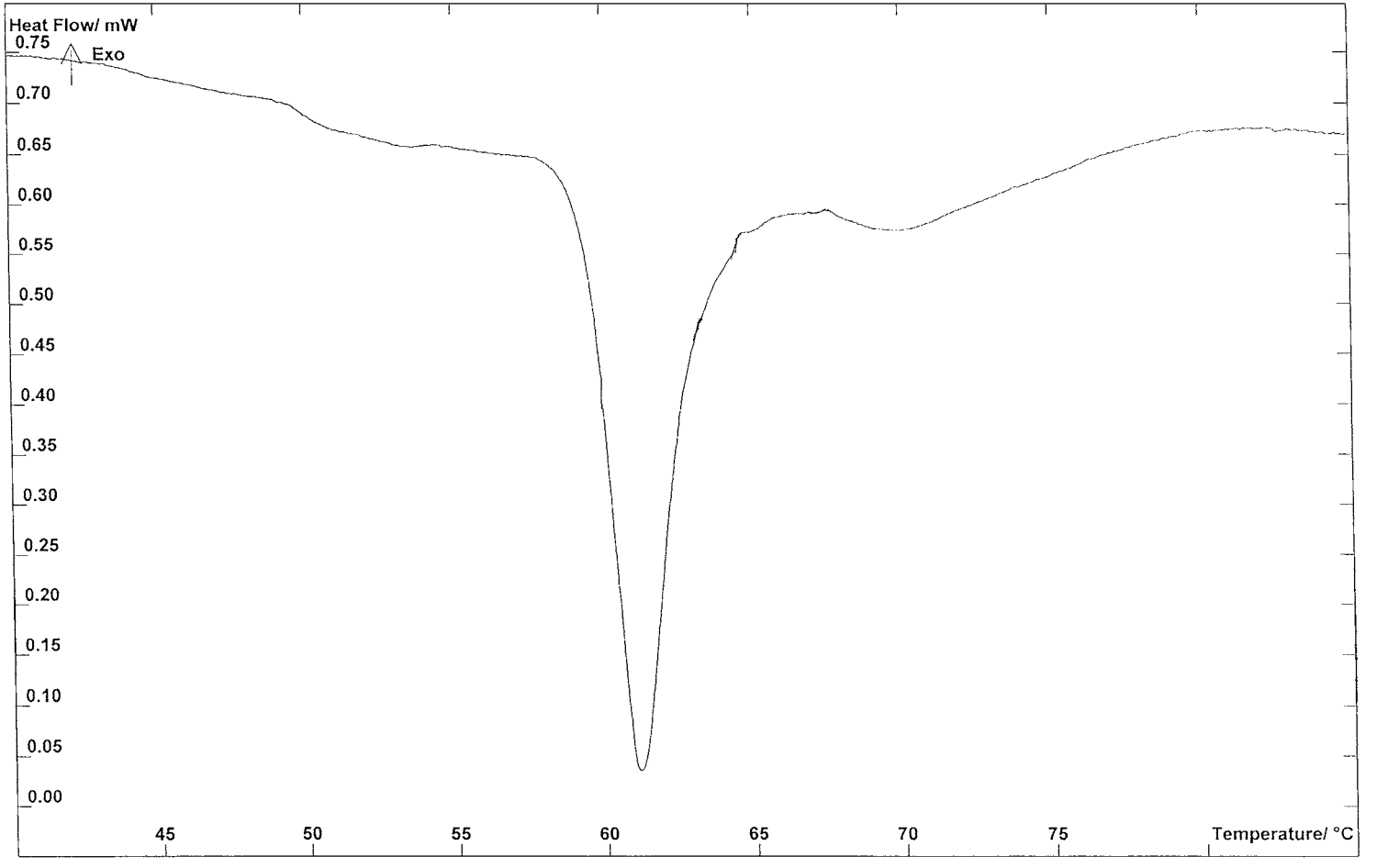

Fig. 3. Heat treatment of rat stripes in rigor buffer + 5 mM ADP and 5 mM V_i.

difference in the global behaviour between rigor and strong binding states in rat uterus smooth muscle. To look into the deeper details of these processes further experiments are required for the molecular dynamic behaviour (e.g. EPR spectroscopy) and thermal properties of rat uterus smooth muscle and its protein compounds.

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