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CALORIMETRIC AND DSC STUDIES OF MITOCHONDRIA ISOLATED FROM CYTOPLASMIC MALE STERILE LINE OF RICE

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Abstract

In this paper, the thermogenesis and differential scanning calorimetry (DSC) curves of energy release of the mitochondria isolated from variant strains of cytoplasmic male sterile lines of rices have been determined by using an LKB2277 Bioactivity Monitor and a DuPont 910 differential scanning calorimeter. The regularity and characteristics of the energy release of the mitochondria at constant and changing temperature were investigated, the differences in shape of the curves and the thermodynamic and kinetic characteristics of the thermogenesis of the mitochondria were compared, the thermodynamic and kinetic parameters of energy release of the mitochondria in the thermogenesis increasing stage were calculated, and the experimental thermokinetic equations describing the different thermogenesis processes were established.

Keywords: calorimetry, cytoplasmic male sterile line of rice, DSC, mitochondria

Introduction

Mitochondria are important organelles involved in ATP production, they are semi-autonomous organelles, contain the components necessary for the synthesis of some of their own proteins, and are the power plants of all eukaryotic cells. Study of mitochondria is not only of theoretical significance but also of applied value. Many aspects of the relations between mitochondria and hardiness of plants, cytoplasmic male sterility of plants, disease and aging, etc. have been studied in recent years [1, 2].

Mitochondria contain large amounts of enzymes, mainly the enzymes which catalyses cellular oxidation reactions are the specific loci of the oxidation. These enzymes still are active after the isolation of mitochondria and there remain large amounts of nutrients in the mitochondria, hence enzymatic reactions can occur and release energy. The heat effects of the process can be monitored using sufficiently

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sensitive calorimeters, and the thermogenesis curves were obtained [3, 4]. However, few calorimetric studies on the thermogenesis of energy release of mitochondria isolated from higher plants have been reported before to our knowledge [5–8].

As a cellular center for energy metabolism, the mitochondria play essential functions in the development of eukaryotic organisms. In higher plants, one of the developmental transitions that appears to be particularly influenced by mitochondrial function is male reproductive (pollen) development. Mutations in the mitochondrial genome most commonly result in the inability of the plant to shed viable pollen. This phenomenon, known as cytoplasmic male sterility (CMS), is observed in more than 150 plant species [9]. The hybrid seeds can be produced with the help of male sterile lines, and the fine varieties of crops can be supplied by the hybrid seeds to raise yield of crops. Rice breeding has been very successful during the last two decades and yields have been raised in many parts of the world. The identification and development of cytoplasmic male sterility, maintainer and restorer lines were a major step in the success of this technology [10]. The studies of the male sterility of rice and hybrid rice began with Long-Ping Yuan in China, who discovered a male sterile individual-plant of rice first in 1964. After that the CMS individual-plant and its restorer and maintainer lines of YieBai type rice were discovered by Bi-Hu Li in 1970, and the CMS and its restorer and maintainer lines of HongLian type rice (Guangchong 41 etc.) were bred by Wuhan University in 1975 in China, and also Ying-Guo Zhu *et al.* discovered a male sterile individual-plant and its restorer and maintainer lines of MaXie type rice in 1984 in the Hainan province of China [11, 12]. At present the hybrid rice have been widely used in farming in China, and the grain yield has raised greatly for many years.

Many results achieved have shown that the CMS mechanism is closely related to the mitochondria, and the genes of the pollen abortion may be situated at the mitochondria [2, 9]. The studies on the difference of the mitochondrial energy release of different CMS lines are very important and significant for investigations of the CMS mechanism. In this paper, the thermogenesis and differential scanning calorimetry (DSC) curves of energy release of the mitochondria isolated from YieBai, HongLian and MaXie type cytoplasmic male sterile lines of rices have been determined by using an LKB 2277 Bioactivity Monitor and a DuPont 910 differential scanning calorimeter. The regularity and characteristics of the energy release of the mitochondria at constant and changing temperature were investigated. The differences in the shape of curves were compared, and the thermodynamic and kinetic parameters and the experimental thermokinetic equations of the energy release of the mitochondria at the thermogenesis increasing stage have been calculated and established.

Materials and methods

Materials

The YieBai (ZhenShan 97 cytoplasmic male sterile line, ZA in short), HongLian (GuangChong 41 cytoplasmic male sterile line, GA in short) and MaXie (MaXie cy-

toplasmic male sterile line, MA in short) type rice were provided by Research Institute of Genetics, College of Life Sciences, Wuhan University, Wuhan, China.

Isolation of rice mitochondria

According to Pring's method the mitochondria were isolated from the three rice types described above [13], and some changes of the experimental procedure have been made in the isolation process.

The seeds of the rice (*Oryza sativa L*) were sown in vermiculite in flats, which were placed in growth chambers at 25°C in the absence of light. Seven to ten days after planting, etiolated mesocotyl and coleoptile tissue were weighed and immediately homogenized for 30 s in a high speed Waring blender. The cold homogenization buffer was 0.5 mol L^{-1} sucrose, 0.005 mol L^{-1} Na_2EDTA, 0.1% bovine serum albumin (BSA), and 0.05 mol L^{-1} Tris-HCl, pH 7.5, 10 mL of the buffer were added to each gram of the sample. The preparation was filtered through eight layers of cheesecloth prior to centrifugation for 13 min at 1000× g and 4°C. The supernatant was then centrifuged for 20 min at 16000× g and 4°C. The pellets (about 0.5 g) were resuspended in 10 mL reaction buffer (0.3 mol L^{-1} sucrose, 0.4 mol L^{-1} $MgCl_2$, 0.1% BAS, 0.01 mol L^{-1} Tris-HCl, pH 7.5 and deoxyribonuclease I to 0.05 mg L^{-1}) for 60 min at ordinary temperature. Then the solution (about 10 mL) was added the buffer (0.6 mol L^{-1}, 0.02 mol L^{-1} Na_2EDTA, 0.1% BSA, 0.01 mol L^{-1} Tris-HCl, pH 8.0) in same volume, and then centrifuged for 20 min twice at 16000× g and 4°C. About 0.3 g of the pellets (mitochondria) were resuspended in 0.25 mL buffer (0.001 mol L^{-1} Na_2EDTA, 0.01 mol L^{-1} Tris-HCl, pH 8.0) and stored in refrigerator at 0–3°C for measurement. All the above experiments were carried out under aseptic condition.

Instrument and experimental procedures

An LKB 2277 Bioactivity Monitor, a new type of heat-flow microcalorimeter was used in this experiment. It is designed to monitor continuously a wide variety of processes and complex system over the temperature range 20–80°C. In the system, each measuring cylinder normally contains a sample and a reference in separate measuring cups (twin system). The heat output from the sample flows from the thermoelectric detector to the large heat sink (in close contact with the water bath). In response the detector produces a voltage which is proportional to the power output from the sample. In order to minimize the systematic error and disturbance effect, a differential or twin detector system is used. This system is very sensitive, the detection limit is 0.1 μW and the baseline stability (over a period of 24 h) is 0.2 μW. The performance of this instrument and the details of its construction have been previously described [14, 15].

The thermogenesis curves of rice mitochondria were recorded by ampoule method, using two sterile sealed ampoules, one ampoule containing a reference solution such as sterile buffer (0.001 mol L^{-1} Na_2EDTA, 0.01 mol L^{-1} Tris-HCl, pH 8.0), and the other containing the sample (suspension of the mitochondria). The sample normally occupied position A in the monitor, and the reference occupied position B.

Each ampoule contained 1 mL sample (which contained about 0.3 g of the mitochondria and were kept at 0–3°C for 15 h before the determination) or reference and 2 mL of air. The experimental temperature was at 30°C and the amplifiers of the monitor were set at 10 μW.

The differential scanning calorimetric experiments were performed on a DuPont 910 differential scanning calorimeter and 9900 Computer/Thermal analyzer system. The measurements covered the temperature range from 0 to 80°C at a heating rate of 3 K min^{-1}. The mitochondria were sealed in an aluminum pan for measurement. The atmosphere was a pure nitrogen stream at flow rate of 40 mL min^{-1}. Two or three sets of the test of each sample were made. Prior to the measurement, the temperature and enthalpy calibrations were conducted using pure indium as the standard specimen.

All the above experiments were carried out under aseptic condition.

Results and discussion

Thermogenesis curves and thermokinetics of the CMS rice mitochondria

The thermogenesis curves of energy release of the CMS rice mitochondria (which was kept at 0–3°C for 15 h before the measurement) at 30°C are shown in Figs 1 to 3.

Analysis of the thermogenesis curves showed that the energy release of the rice mitochondria reveals four regions: the lag stage, increasing stage, stationary stage and decline stage. Sometimes the stationary stage is not very obvious, without any clear boundary at the stage. During isolation of the mitochondria, the enzymatic system of the mitochondria is damaged (and is in a resting condition at low temperature) so it takes some time for activity to be recovered and for adaptation to the new conditions. During this period, the lag stage, the thermogenesis curve is a horizontal straight line. These results indicate that the lag stage is for about 5 h at 30°C. Once

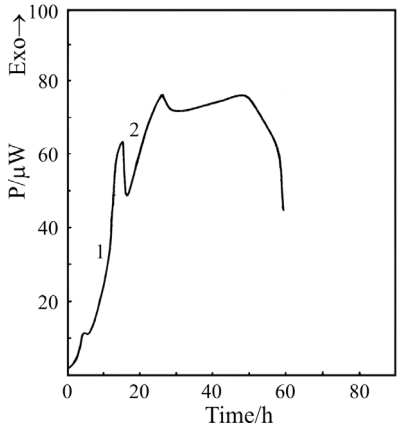

Fig. 1 Thermogenesis curve of energy release of ZA rice mitochondria at 30°C, where 1 and 2 represent two various limit phases on the thermogenesis curves of energy release of ZA rice mitochondria at 30°C, respectively

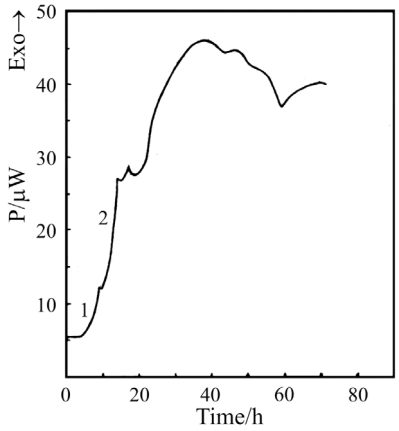

Fig. 2 Thermogenesis curve of energy release of GA rice mitochondria at 30°C, where 1 and 2 represent two various limitless phases on the thermogenesis curves of energy release of GA rice mitochondria at 30°C, respectively

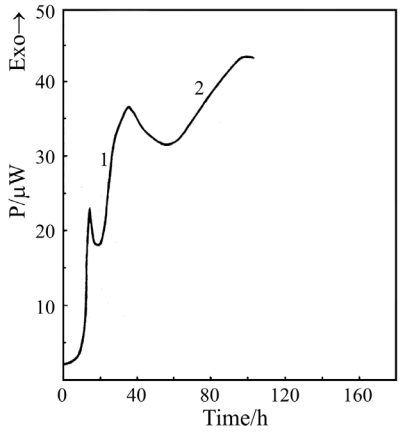

Fig. 3 Thermogenesis curve of energy release of MA rice mitochondria at 30°C, where 1 and 2 represent two various limit phases on the thermogenesis curves of energy release of MA rice mitochondria at 30°C, respectively

the adequate adaptation has taken place, enzymatic activity would gradually recover. Both the substance metabolism and energy metabolism would start, and the energy release of rice mitochondria would increase. At this time, the thermogenesis curve would produce a logarithmic curve or sigmoid curve, i.e. the increasing stage. When all enzymes have become active, the thermogenesis reached a stationary stage without much change in the curves. When nutrients of the mitochondria and oxygen in the ampoule are exhausted, the curve entered the decline stage, and feedback inhibition of enzymes by metabolites also contributes to this decline. The increasing time (t), maximal heat power (P_{max}) and heat effect of energy release of the mitochondria at the increasing stage (ΔH_1) were determined, and are shown in Table 1.

Table 1 Thermodynamic values of the CMS rive mitochondria at the thermogeneses increasing stage (30°C)

Sample	Increasing time t/h	Max. power P_{max}/μW	Heat effect ΔH_1/J
ZA	26	76.5	–3.43
GA	35	46.2	–2.99
MA	96	43.5	–9.36

The increasing stage is a comparatively important stage among the four stages, the thermogenesis of the mitochondrial energy release reveals two phases of the energy release at the stage. One is the limitless phase of the energy release, the thermogenesis curve assume an exponential curve. The other is the limit phase of the energy release, the thermogenesis curve assume a sigmoid curve. The limitless increasing rate constants (k_1) have been calculated from the equation: $P_t=P_0\exp(k_1t)$ or $\ln P_t=\ln P_0+k_1t$, where P_0 is the initial thermal power of the thermogenesis at time t=0, and P_t is the thermal power of the thermogenesis at time t. The values of the thermokinetic parameters are shown in Table 2.

The limit increasing process of the thermogenesis of the mitochondrial energy release present sigmoid curve, the curve can best be expressed as the logistic equation [16]. In the limit increasing process, thermogenesis quantity in the process are time related according to

Table 2 Thermokinetic parameters of the CMS rice mitochondria at the limitless thermogenesis increasing phase (30°C)

Sample	t/h	P_N/μW	k_1/h^{-1}	r
ZA	1–4	2.566	0.4449	0.9968
GA(1)	5.5–9.5	6.020	0.1959	0.9923
GA(2)	10.5–13	12.151	0.2278	0.9962
MA	7–14	2.104	0.3437	0.9948

$$dN_t/dt = k_2(1-sN_t)N_t = k_2N_t - sk_2N_t^2 = k_2N_t - \beta N_t^2 \quad (1)$$

where k_2 is rate constant in the limit increasing process, s is the increase inhibitory factor and N_t represents a number of the mitochondria producing heat at time t in the limit increasing process. The deceleration rate constant is given by $\beta=sk_2$.

The thermal power of the thermogenesis at time t(P_t) is proportion to N_t, then

$$P_t = P_N N_t \quad (2)$$

whence

$$\frac{dN_t}{dt} = \frac{1}{P_N}\frac{dP_t}{dt} \quad (3)$$

from Eq. (1) with Eq. (3), we have

$$\frac{dP_t}{dt} = k_2 \frac{P_N P_t - sP_t^2}{P_N} \tag{4}$$

after integrating Eq. (4) yields

$$\ln \frac{P_t (1-s)}{P_N \left(1 - \frac{sP_t}{P_N}\right)} = k_2 t \tag{5}$$

Equation (5) can be rewritten as

$$\ln\left(\frac{P_N / s}{P_t} - 1\right) = \ln\left(\frac{1-s}{s}\right) - k_2 t \tag{6}$$

or

$$\ln\left(\frac{a}{P_t} - 1\right) = \ln\left(\frac{1-s}{s}\right) - k_2 t \tag{7}$$

where $a=P_N/s$. Using the data P_t and t obtained from the thermogenesis curves of the mitochondrial energy release in the limit increasing stage, suitable values of a have been chosen in the calculations to give the best linearity of Eq. (7). Hence the limit increasing rate constant (k_2), the increase inhibitory factor (s), the deceleration rate constant (β) and the thermal power produced by a mitochondria energy release in the limit increasing process (P_N) can be obtained. The thermokinetic parameters of the thermogenesis in the limit increase process at 30°C are shown in Table 3.

These results reported above indicate that the various kinds of the rice mitochondria have different thermodynamic and thermokinetic parameters and their corresponding experimental thermokinetic equations at the thermogenesis increasing stage. These differences may be related to the composition, structure and energy release mechanism of the mitochondria. These problems are worth to be investigated further.

Table 3 Thermokinetic parameters of the CMS rice mitochondria at the thermogenesis limit activity increasing phase (30°C)

Sample	t/h	P_N/μW	k_1/h^{-1}	s	β/h^{-1}	r
ZA(1)	6–16	10.85	0.2460	0.06253	0.01539	–0.9968
ZA(2)	17–26	50.51	0.1575	0.5550	0.08740	–0.9986
GA	20–38.5	26.28	0.1823	0.5498	0.1002	–0.9976
MA(1)	19–36	13.90	0.2444	0.3686	0.09008	–0.9981
MA(2)	56–96	30.03	0.02041	0.4455	0.009093	–0.9988

DSC curves and thermokinetics of CMS rice mitochondria

The DSC curves of the mitochondria isolated from the CMS rice are shown in Fig. 4. The curves are complete and representative, and also are similar. There is an exothermic peak below 70°C, a turn point exists over the range of 70~80°C in each curve. The initial temperature (T_i), extrapolated onset temperature (T_e), peak temperature (T_p) and total heat effect (ΔH) are listed in Table 4.

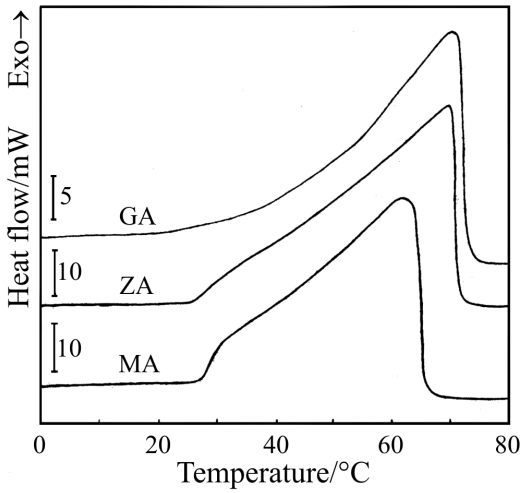

Fig. 4 DSC curves of energy release of the CMS rice mitochondria

Table 4 Thermodynamic parameters of the DSC curves of the CMS rice mitochondria

Sample	m/mg(wet)	T_i/°C	T_e/°C	T_p/°C	ΔH/J g^{-1}(wet)
ZA	7.17	22.70	26.07	69.13	–2607
GA	8.20	20.39	31.43	70.16	–2376
MA	7.51	26.73	27.05	61.70	–2291

Mitochondria are bounded by a double membrane, i.e. outer membrane and inner membrane. The inner membrane is folded to form little shelves, called cristae, which project into the matrix. The membrane is chiefly composed of lipids and proteins. Therefore, water, lipids, proteins and nucleic acids (DNA and RNA) are major chemical components of mitochondria. The DSC results show that the mitochondria started to release energy at about 20°C, and the rate of energy release increases continuously as temperature rises. When the temperature reaches 60–70°C, the curves rapidly fall to the baseline. Due to the energy release of the rice mitochondria over the range of 20–60°C the exothermicity results mainly from the energy release of mitochondria [5–8].

In general, when a change takes place during heating process, the change rate equation in the process can be expressed as follows

$$d\alpha/dt = kf(\alpha) \tag{8}$$

where k is the rate constant of the change, and $f(\alpha)$ is the conversion function reflecting the reaction mechanism. Many change processes can be described by a simple n-th order function [4, 15, 16], it is shown as

$$f(\alpha) = (1-\alpha)^n \tag{9}$$

where α is the degree of conversion or conversion in short in the change process and the values of n represent how complicated in the change mechanism. When Arrhenius equation ($k=A\exp(-E_a/RT)$) is introduced to Eq. (8), the equation rewrites as follows

$$d\alpha/dt = A(1-\alpha)^n\exp(-E_a/RT) \tag{10}$$

Considering the average heating rate ($\beta = dT/dt$), the conversion (α) is temperature (T) related according to

$$d\alpha/dT=(A/\beta)(1-\alpha)^n\exp(-E_a/RT) \tag{11}$$

Integrating Eq. (11), and the Doyle approximation of the temperature integral is used [6, 17, 18], then yields

$$\ln[-\ln(1-\alpha)]\approx\ln\left(\frac{AE_a}{\beta R}\right)5.332-1.052\left(\frac{E_a}{RT}\right)(n=1) \tag{12}$$

or

$$\ln\frac{(1-\alpha)^{1-n}-1}{n-1} \approx \ln\left(\frac{AE_a}{\beta R}\right)5.332-1.052\left(\frac{E_a}{RT}\right)\ (n\neq 1) \tag{13}$$

In general, in the kinetic study of matter change by means of DSC, the change fractional extent (α) is given by

$$\alpha = \frac{\Delta H_T}{\Delta H} = \frac{S'}{S} \tag{14}$$

where ΔH_T is the partial heat effect of the change at a given temperature; ΔH is total heat effect of the change; S' is a peak area from T_0 to T and S is a total peak [17].

Substituting the data of α with temperature change in the energy release of rice mitochondria into Eq. (12) or (13), and evaluating apparent kinetic parameters of the energy release. The suitable values of n have been chosen in the calculations to give the best linearity of Eq. (12) or (13). Hence the apparent activation energy of energy release of the rice mitochondria in the change process (E_a), the pre-exponential factor (A) and the apparent rate constants at 25°C (k_{298}) can be calculated. The results of the experiments and calculations are shown in Tables 5 and 6, respectively.

It follows from Tables 4 and 6 that the values of the total heat effect (ΔH), the apparent activation energy (E_a) and the apparent rate constants (k_{298}) of the rice mitochondrial energy release in the change process are essentially uniform with each other among the different varieties of the CMS rice mitochondria. These results are

Table 5 Date for α and *T* of the DSC curves of the CMS rice mitochondria

α	*T*/K						
	308.12	313.12	318.12	323.12	333.12	338.12	413.12
ZA	0.0498	0.1073	0.1876	0.2919	0.5805	0.7683	–
GA	0.0460	0.0861	0.1471	0.2341	0.4966	0.6980	0.9222
MA	0.0719	0.1565	0.2803	0.4205	0.8198	–	–

Table 6 Apparent kinetic parameters of the energy release of the CMS rice mitochondria as temperature rise

Sample	*n*	*E*/kJ mol^{-1}	ln*A*	$k_{298.12}$/min^{-1}	*r*
ZA	2.8	124.7	43.48	$1.0 \cdot 10^{-3}$	–0.9996
GA	2.7	101.4	33.98	$1.1 \cdot 10^{-3}$	–0.9999
MA	2.3	123.7	43.43	$1.6 \cdot 10^{-3}$	–0.9992

different from that of rice fertile lines [6], and the deeper knowledge for the problem is worth to be investigated further.

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