Differential scanning calorimetry of rat liver mitochondria[1]

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Summary. Differential scanning calorimetry was employed for studying rat liver mitochondria and extracted mitochondrial lipids. Endothermic transition in the range 15-40 °C was detected for the whole mitochondria and between 10-20 °C for the extracted lipids.

It was reported by different laboratories that Arrhenius plots of the enzymic activities of membrane-bound mitochondrial enzymes show breaks in the curves in a wide range of temperatures[2,3]. In some case there is a correspondence between the temperature of the break as obtained from the Arrhenius plots of the enzymic activity and the temperature of the break detected with spin labels[2], EPR[4] and DSC[5]. The thermal behaviour of mitochondria was studied by three groups[6-8]. They found a phase transition at low temperatures[6,8] or no transition at all[7]. We decided to reinvestigate the thermotrophic behaviour of whole mitochondria and extracted mitochondrial lipids above the 0 °C in the absence of an antifreeze, by employing a very sensitive differential scanning calorimeter.

CR-male rats were starved overnight. The mitochondria were separated by a procedure of Sordahl et al.[9] with small modifications: the livers were removed, homogenized in 0.25 M sucrose + 0.001 M EDTA, the homogenate was centrifugated twice at 750×g for 15 min, the supernatant was separated and centrifugated for 15 min at 4500×g, finally the mitochondrial pellet was resuspended in 0.15 M NaCl and recovered by centrifugation at 18,500×g for 15 min. The mitochondria were stored at 4 °C for not more than 2 days, but usually the experiments were performed on the day of preparation. The pellet contained about 70% water as found after freeze-drying for 3 h. The total lipids of the mitochondria were extracted by the method of Folch et al.[10]. To remove traces of organic solvents the lipids were kept under high vacuum for 3 h. The calorimetric measurements were performed on a DuPont 990 differential scanning calorimeter with a cell base II. The calibrated mode was used, sensitivity of 0.01 mcal/sec·inch and heating rate of 5 °C/min. Hermetically sealed aluminum pans were used. The wet mitochondria or dry lipids were transferred directly into the pans, to the lipids appropriate amounts of water (in large excess) were added and the pans were sealed. They were left for equilibration at room temperature for at least 12 h. As no antifreeze was used, the scans were started above 0 °C, so the transition in the lower temperatures as found by others[6-8] could not be detected.

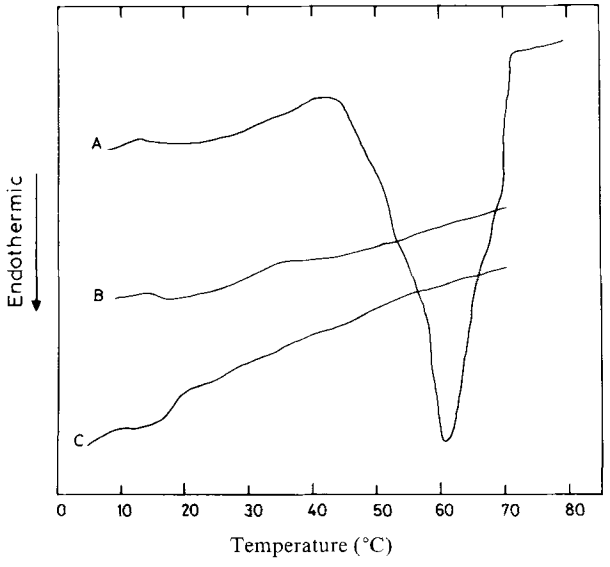
The figure presents differential scanning calorimetry thermograms of rat liver mitochondria and of the extracted lipids. As shown in the figure, the mitochondria undergo melting in the range of ~15-40 °C, with an enthalpy of melting of ~0.13 mcal/mg dry mitochondria. Figure 1A represents the thermogram of the first scan of the mitochondria (heating from 2-80 °C) during which the proteins are denaturated. The denaturation of the proteins appears as a peak centered at ~60 °C. The protein peak disappears on subsequent cycles of cooling and heating (figure 1B, second scan), while the peak in the range 15-40 °C and its enthalpy of melting are almost unchanged. Figure 1C presents the thermograms of the extracted mitochondrial lipids. As seen from the figure, the transition region of the lipid moved to lower temperatures (~10-20 °C). Also, the enthalpy of melting decreased as compared to that of the whole mitochondria. The DSC data are summarized in the table.

As shown in this report, mitochondria undergo phase transition in a region which coincides with the discontinuities in the activation energies as found previously[2,3].

The table shows the difference in the thermotrophic behaviour of the whole mitochondria and the mitochondrial lipids. It was shown by Chapman (private communication) that membrane proteins tend to accumulate in the molten lipid regions and they actually may spearate from the lipid moiety the lower melting components. This may cause an overall increase in the temperature and in the enthalpy of melting of these lipids which are not associated with the protein moiety. On the other hand, Curatolo et al.[11] reported that in the case of interaction of dimyristoyl lecithin with myelin apoprotein the 'boundary' lipid melts at higher temperature than the bulk lipids. The thermotrophic behaviour of rat liver mitochondria is similar to those of rat liver microsomes, as shown previously by us[5].

Enthalpy of melting and the melting range of the whole mitochondria and the extracted mitochondrial lipids

	ΔH mcal/mg (± SE)	Melting range
Whole mitochondria	0.139 ± 0.011	15-40 °C
Extracted lipids	0.076 ± 0.007	10-20 °C



The differential scanning calorimetry thermograms of mitochondria and extracted lipids. *A* 15 mg wet mitochondria (3.2 mg dry), first scan; *B* Second scan of mitochondria presented in A; *C* 2.7 mg lipids + 3.1 mg H_2O; heating rate 5 °C/min, sensitivity 0.01 mcal/sec·inch. The experiments were performed at least in triplicate, employing different mitochondrial preparations.

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Gas chromatographic evidence of the antheridiogen of *Lygodium japonicum* (A_{LY}) (Schizaeaceae)

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Summary. The antheridiogen in culture medium of *Lygodium japonicum* was separated by GC. It shows different polarity compared with *Anemia* antheridiogens.

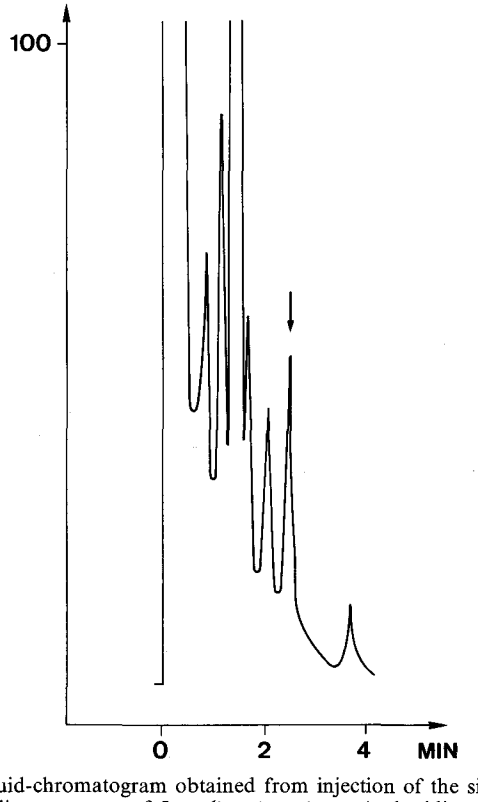
Antheridiogens are phytohormones which are able to induce the male gametangium in ferns. They are synthesized during distinct phases of prothallia development and excreted into the medium. Antheridiogens are described in 3 species of the Polypodiaceae (*Pteridium aquilinum*[3], *Onoclea sensibilis*[4], and *Ceratopteris thalictroides*[5]) and in 2 species of the Schizaeaceae (*Anemia phyllitidis*[6,7], *Lygodium japonicum*[8,9]).

Up till now, qualitative as well as quantitative determinations of fern antheridiogens have been mainly performed by a combination of TLC and biotest. These procedures need at least 10–25 days, depending on the concentration present in the preparation. To improve the analysis, a method for separation and determination of the 2 antheridiogens of *Anemia phyllitidis*[7] by GLC has been worked out in our laboratory[10]. The application of this method to antheridiogens of other species of the Schizaeaceae was obvious, since a combination of GC with mass spectrometric methods should enable the analysis of the as yet unknown chemical structure of these compounds.

Conditions for the culture of the prothallia, the extraction, the TLC and GC methods, as well as the procedure for the biotest of the hydrolyzed TMS-derivatives separated by GC, have already been described[10,11].

The figure shows the GC profile of the TMS-derivatives of the antheridiogen preparation from the culture medium of *Lygodium japonicum*. The arrow indicates the peak which showed antheridiogen activity in the subsequent biotest. The retention time of the TMS-derivative of the *Lygodium* hormone is 0.22 relative to that of TMS-GA_3, whereas the relative retention time of the main hormone of *Anemia* is 1.2.

The result of the present study permits a comparison of the 3 antheridiogens isolated so far from Schizaeaceae (*Anemia* antheridiogen 1 and 2[6,7], A_{Ly}[8,9]) with gibberellic acid. In TLC system these compounds have different R_f values indicating 4 different molecules. The chromatographic properties in TLC, as well as in GC systems, show that the *Lygodium* hormone is less polar than GA_3 or the *Anemia* antheridiogen. The biological and chromatographic properties of A_{Ly} suggest that it also belongs to these biologically active diterpens with a structure comparable to gibberellins. Further studies on structure analysis are in progress.



Gas-liquid-chromatogram obtained from injection of the silylated antheridiogen extract of *Lygodium japonicum*. Antheridiogen fraction (A_{LY}) is marked ↓. Attenuation was 10×256. Equipment: FVT 2400 (Carlo Erba, Mailand); glass column: 2 m, ∅6/2 mm filled with 3% silicone GE SE - 30 on gaschrom Q 100/200 mesh. Temperature: Injector/column/detector = 275/220/260 °C. Carrier: nitrogen, 75 ml/min.

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