Differential scanning calorimetry of chromaffin granule membranes[1]

Diana Bach, K. Rosenheck and A.S. Schneider

Department of Membrane Research, The Weizmann Institute of Science, Rehovot (Israel), and Sloan-Kettering Institute for Cancer Research and Cornell University Graduate School of Medical Sciences New York (New York 10021, USA), 4 September 1978

Summary. Differential scanning calorimetry thermograms of native chromaffin granule membranes exhibit several peaks in the 15–35 °C region. Extraction of cholesterol increases the size of the melting peaks. Addition of Ca^{2+} ions does not seem to influence the lipid transitions.

The chromaffin granule of the adrenal medulla has in recent years attracted major research efforts as a readily accessible system from which information can be gained on the molecular mechanisms of catecholamine secretion[2]. Since secretion is believed to occur by exocytosis[3], the properties of the granule membrane and its component lipids and proteins are of direct relevance to the elucidation of these mechanisms. Thus, fluorescence[4] and spin-label[5] studies of chromaffin granule membranes have indicated a structural transition around 30 °C that was ascribed to the melting of membrane lipids.

By using differential scanning calorimetry (DSC), lipid transitions occurring close to physiological temperatures have previously been observed in sarcoplasmic reticulum membranes[6] and rat liver microsomes[7] and mitochondria[8]. This paper reports an application of the DSC technique to the study of the melting behaviour of chromaffin granule membranes, before and after extraction of cholesterol. The effect of added Ca^{2+} ions, which are required for catecholamine secretion in the intact chromaffin cell[9,10], were also examined.

Chromaffin granules from the bovine adrenal medulla were prepared according to Trifaro and Dworkind[11]. The membranes were obtained by osmotic lysis of the granules with 1 mM Tris buffer, pH 7.4. The membrane pellet, after being washed twice in the same buffer, was obtained by centrifugation at 40,000 rpm for 2 h and contained about 85% water as determined by freeze drying of the membranes. To decrease the water content of the membranes, the pellet was kept in a desiccator over silica gel for about 48 h at 4 °C. The final water content was 60–70%. Cholesterol was extracted from the membranes by ether, employing a procedure developed by Roelofsen and van Deenen[12] for removal of cholesterol from erythrocyte membranes. The total lipids of the chromaffin granule membranes were extracted by the procedure of Folch et al.[13].

The measurements were performed on a DuPont 990 differential scanning calorimeter with a cell base II. The calibrated mode was used with a sensitivity of 0.02 mcal/sec/inch/-($3.3 \cdot 10^{-5}$ J/sec/cm) and heating rate of 5 °C/min. Wet membranes were transferred directly into hermetically sealed aluminium pans. Extracted dry membranes or dry lipids were transferred into the pans, appropriate amounts of water or $CaCl_2$ solutions were added and the pans were sealed. They were left for equilibration at room temperature for at least 12 h. All experiments were done in triplicate at least, using different membrane preparations.

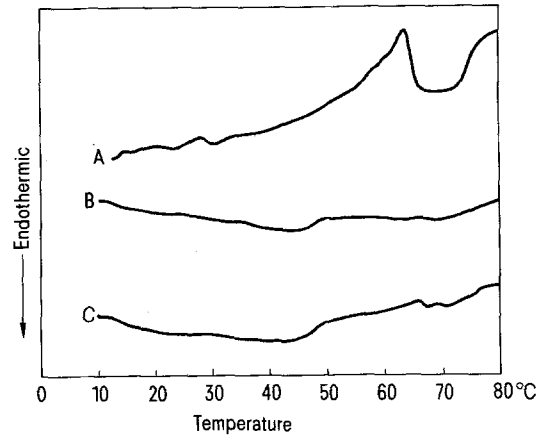
The figure shows the differential scanning calorimetry thermograms of native chromaffin granule membranes and of membranes after extraction of cholesterol. Scan A is that of the native membranes. A number of small peaks appear between 15 and 35 °C, with a somewhat larger peak centered at ~32 °C. The very large peak at ~70 °C is attributed to the denaturation of the membrane proteins, since it disappears in subsequent scans of the same sample. The peaks in the 15 to 35 °C region appear also in consecutive scans, so it may be concluded that they reflect the melting of lipids. The thermograms of the total extracted lipids (not shown in the figure) are very similar to those of the native membranes, except for the absence of the protein peak at 70 °C. The enthalpy of melting of the ~32 °C peak is very low, $\sim 8.4 \cdot 10^{-5}$ J/mg dry membrane.

Extraction of cholesterol from the membranes causes a shift and broadening of the melting range of the lipids, and a large increase in the enthalpy of melting to $\sim 2.5 \cdot 10^{-3}$ J/mg dry membrane (Scan B). Incubation of the cholesterol depleted membrane with 1 mM Ca^{2+} seems not to influence the thermotropic behaviour (Scan C). The DSC data are summarized in the table.

The small size of the melting peaks of the intact membrane may be explained by its relatively high cholesterol content[14]. Because cholesterol intermixes with other membrane lipids, it will interfere with the crystallization of lipids having relatively high temperature melting ranges. The fact that a small part of the lipids of intact chromaffin granule membranes do melt in a physiological temperature range,

Enthalpy of melting and melting temperature of the main peak of native and cholesterol extracted chromaffin granule membranes and of the extracted lipids

	$\Delta H \pm SE$ ($J/mg \times 10^3$)	T_m (°C)
Native membranes	0.075 ± 0.008	~32
Extracted lipids	0.084 ± 0.025	~34
Cholesterol extracted membranes incubated with water	2.5 ± 0.16	~43
Cholesterol extracted membranes incubated with 10^{-3} M $CaCl_2$	2.7 ± 0.25	~44



A 18 mg wet chromaffin granule membranes (5.4 mg dry weight) 1 scan. *B* 1.0 mg dry chromaffin granule membranes after extraction of cholesterol, incubated with 5.2 mg water; 1 scan. *C* 1.1 mg dry chromaffin granule membranes after extraction of cholesterol incubated with 4.9 mg of 1 mM $CaCl_2$; 1 scan.

is probably an indication that these lipids are located in cholesterol-free regions.

Removal of cholesterol leads to an increase in the fraction of lipids undergoing melting, as well as a shift to higher melting temperatures. This observation lends support to the suggestion, by Marsh et al.[5], of a preferential segregation of cholesterol at these temperatures. Similar effects with cholesterol-depleted membranes were observed for myelin[15] and erythrocyte ghosts[16] that show no melting at all in the physiological range of temperatures, as long as cholesterol is present. Our experiments indicate that Ca^{2+} at physiological concentrations does not induce any further segregation of membrane lipids, in addition to that already present, in cholesterol-depleted membranes. This does not mean that at the lower degrees of segregation of the lipids in native granule membranes Ca^{++} effects are also absent. However, due to the smallness of the melting peaks in the thermograms of native membranes the measurement of any such effects will be difficult.

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Desiccation in the black dragon, *Hagenius brevistylus* Selys[1]

Barbara Jean Tracy, C.R. Tracy and D.S. Dobkin[2]

Department of Zoology and Entomology, Colorado State University, Fort Collins (Colorado 80523, USA), 23 August 1978

Summary. *Hagenius brevistylus* lost mass by evaporation in a moderately desiccating environment at the rate of 20.4 mg h^{-1}, and died of desiccation in less than 1 day at a body mass of 79.8% of their normally hydrated mass. It was estimated that *Hagenius* minimally would have to consume the equivalent of 60% of its body mass each day to meet its daily water requirements. This amount of food is equivalent to that necessary to power flight of a dragonfly for 4.6 h.

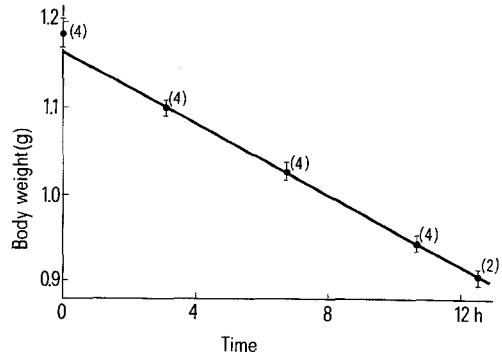
During a study of the role of posturing in the thermoregulatory repertoire of the black dragon[3] *(Hagenius brevistylus)* Selys (Odonata), we noticed that this large dragonfly, held without water in a large flight cage, would position itself in the environment so that its body temperature would remain low. This seemed to differ from the behavior of free-ranging dragonflies which periodically basked in the sun, presumably to raise their body temperatures. We hypothesized that the 'unusual' behavior exhibited by black dragons deprived of water in the flight cage, was due to behavioral hydroregulation to reduce evaporative water loss.

To see if evaporative loss rates in *Hagenius* could be great enough to become 'stressful', we captured 4 dragonflies, weighed each to the nearest tenth mg and hung them up to desiccate under laboratory conditions in bags made of a single thickness of cheesecloth. The laboratory air temperature was 25 °C, relative humidity was 65%, and wind speed was 0–0.5 mph.

Periodic weighings during desiccation (figure) showed that the dragonflies lost mass at a rate of 20.4 mg h^{-1}. This rate led the 4 dragonflies to die in about 12 h at a mean mass of 79.8% (±3.0) of their initial mass.

The conditions under which this experiment was run can be regarded as minimally stressful relative to natural conditions for black dragons which are vigorous fliers and bask often in the sun. However, even under laboratory conditions, these dragonflies lost water at rates equivalent to 48% of their body mass per day. Indeed, the dragonflies lost water at rates faster than reported for larger insects even during flight[4]. Thus, if these insects receive all of their water from food, they would have to consume the equivalent of 60% of their body mass per day (assuming 80% water in food) minimally.

It should be made perfectly clear that these dragonflies could conceivably consume the equivalent of 60% of their body mass in food each day. Tucker[5] reports the minimum cost of locomotion for flying animals such that the power



Body mass of *Hagenius* during desiccation as a function of time.