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# THERMOTROPIC 'TWO-STAGE' LIQUID CRYSTALLINE ⇌ CRYSTALLINE LIPID PHASE SEPARATION IN MICROSOMAL MEMBRANES

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**The effect of temperature on native microsomal membrane vesicles isolated from *Tetrahymena* is investigated by wide angle X-ray diffraction. A 4.2 Å reflection, typical for lipids in the crystalline state, can be recorded in the temperature range between 0°C and 35°C. Quantitative evaluation of this reflection reveals a broad thermotropic 'two-stage' liquid crystalline⇌crystalline lipid phase separation with a 'breakpoint' at approx. 18°C. This 'breakpoint' coincides with the emergence of lipid-protein segregations in endomembranes of intact *Tetrahymena* cells as previously visualized by freeze-etch electron microscopy.**

The molecular organisation of biomembranes critically depends on temperature. In particular, the membrane lipids can undergo reversible liquid crystalline ⇌ crystalline phase transitions and/or separations, respectively [1–3]. Such lipid phase changes in turn have dramatic consequences for the conformation and distribution of proteins in membranes. Altered protein distribution, for example, can be directly visualized in different membranes of a wide variety of cells by freeze-etch electronmicroscopy (e.g. [4]). This method reveals thermotropic lipid-protein segregations, manifesting themselves as the emergence of smooth domains on membrane fracture faces. To date, however, it is not yet clear (at least in eukaryotic membranes) whether such lipid-protein segregations relate to the onset or the completion of a lipid phase transition or even occur during a broad lipid phase separation.

In the unicellular eukaryote *Tetrahymena*, for example, we previously observed, that temperature lowering from the optimal growth temperature of 28 to 5°C induces the appearance of smooth domains on the fracture faces of different endomembranes below 18° C [5–8]. In order to correlate these lipid-protein segregations with the lipid state in membranes we now investigate native microsomal membranes isolated from *Tetrahymena* by wide angle X-ray diffraction. This method allows the direct detection of at least crystalline lipids in membranes.

Axenic 15 l cultures of the ciliate protozon *Tetrahymena pyriformis* (amicronucleate strain GL) were grown at 28°C and harvested during the logarithmic growth phase as described previously [9]. The cells were disrupted at 28°C in a French press (3000 lb/inch$^2$) and then smooth microsomal membrane vesicles (average diameter: ~ 150 nm) were isolated as detailed previously [10]. Wide-angle X-ray diffraction patterns of the vesicles were recorded using a rotating anode generator, running at 45 kV and 200 mA. The spatial distribution of the diffracted X-ray intensity was one-dimensionally registered with a position sensitive proportional counter with a curved counter wire. This special counter, constructed by Kreutz, allows the registration of diffracted intensity free from parallactic distortions even at high angles.

Fig. 1A shows representative wide-angle X-ray diffraction patterns of the microsomal vesicles at

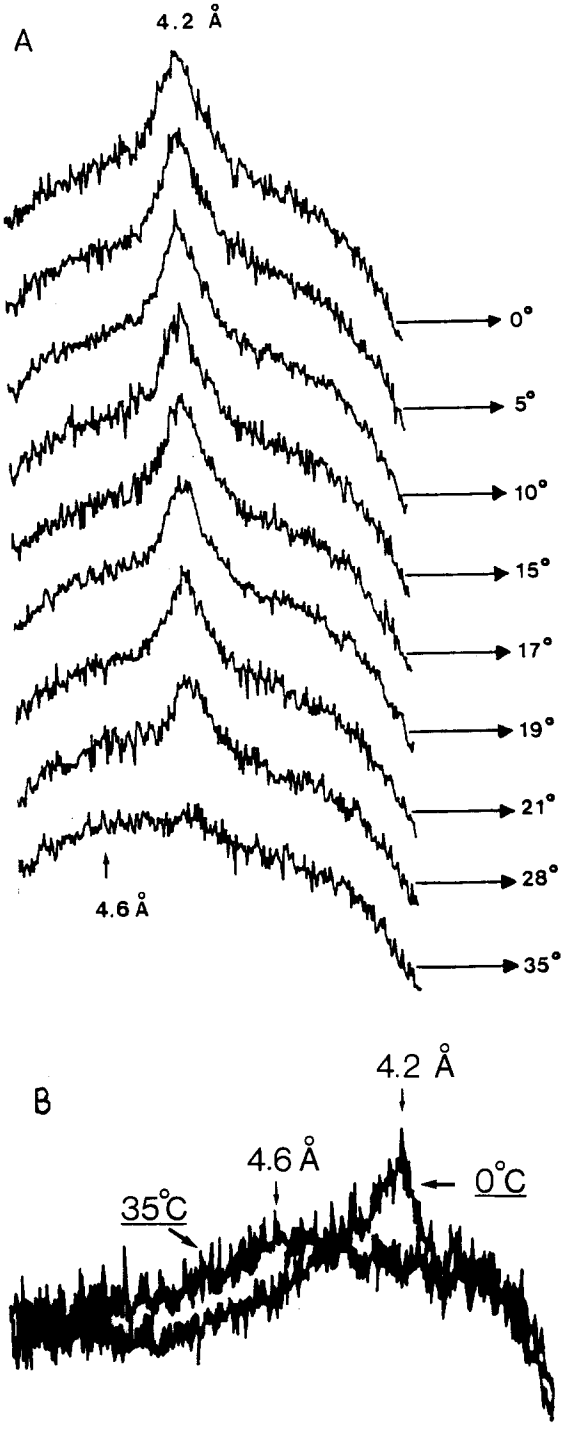


Fig. 1. Wide angle X-ray diffraction patterns of native microsomal membrane vesicles at different temperatures between 0 and 35°C. The spectra were registered by a curved PSP-counter and stored in a multichannel analyser [14]. Exposure time 2000 s.

different temperatures. At 0°C, we can observe a clear 4.2 Å reflection. The integral intensity of this reflection is directly proportional to the number of lipids in the crystalline state, more precisely to the number of fatty acid residues arranged in two-dimensional hexagonal lattice (s). This proportionality is valid regardless whether the scattered intensity is caused by coherent diffraction of one large lattice or by incoherent superposition of the individual diffraction of many smaller lattice domains [11].

Upon raising the temperature, the intensity of this reflection decreases and has almost completely disappeared at 35°C. Concomitantly, we observe an increase of the scattered intensity in the 4.6 Å region (Fig. 1B). This diffuse 4.6 Å reflection is characteristic for disordered lipids in the liquid crystalline state. We therefore conclude that the lipids are majorly transferred from the crystalline into the liquid crystalline state upon raising the temperature from 0 to 35°C. This transformation is fully reversible.

A quantitative evaluation of this lipid transformation can be performed only with the 4.2 Å reflection under our experimental conditions. The reason is that a reliable background subtraction can be done only with the 4.2 Å reflection. In contrast, the statistical error of a quantitative evaluation of the 4.6 Å reflection would be too large since this reflection is very diffuse. Moreover, the signal-to-noise ratio cannot be considerably reduced by longer exposure times since the membrane vesicles are stable only for a few hours.

We determined the integral intensity of this reflection according to the method detailed in Fig. 2. The quantitative evaluation of the 4.2 Å reflection as a function of temperature (Fig. 3) indicates: (i) The total number of crystalline lipids decreases with increasing temperature. Obviously a broad thermotropic crystalline $\rightleftharpoons$ liquid-crystalline lipid phase separation occurs in the membrane over the whole temperature range between 0 and 35°C. Such a broad phase separation rather than a cooperative phase transition is expected due to the very heterogenous lipid composition of the *Tetrahymena* microsomal membranes [10]. (ii) Conspicuously, crystalline lipid domains coexist with liquid crystalline lipids in the membrane even at the optimal growth temperature of the cells at

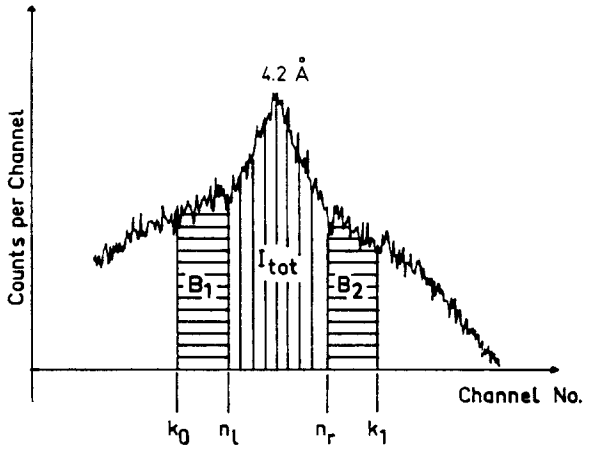


Fig. 2. Method used for the determination of the integral intensity of the 4.2 Å reflection. Integral intensity $I_{4.2}$ of the 4.2 Å reflection:

$$I_{4.2} = I_{tot} - (B_1 + B_2) \Leftrightarrow$$

$$I_{4.2} = \sum_{k=n_1}^{n_r} C(k) - \sum_{k=k_0}^{n_1} C(k) - \sum_{k=n_r}^{k_1} C(k)$$

where $C(k)$ = number of counts registered in channel No. $k$

$$k_0 = n_1 - \frac{(n_1 - n_1)}{2}$$

$$k_1 = n_r + \frac{(n_1 - n_1)}{2}$$

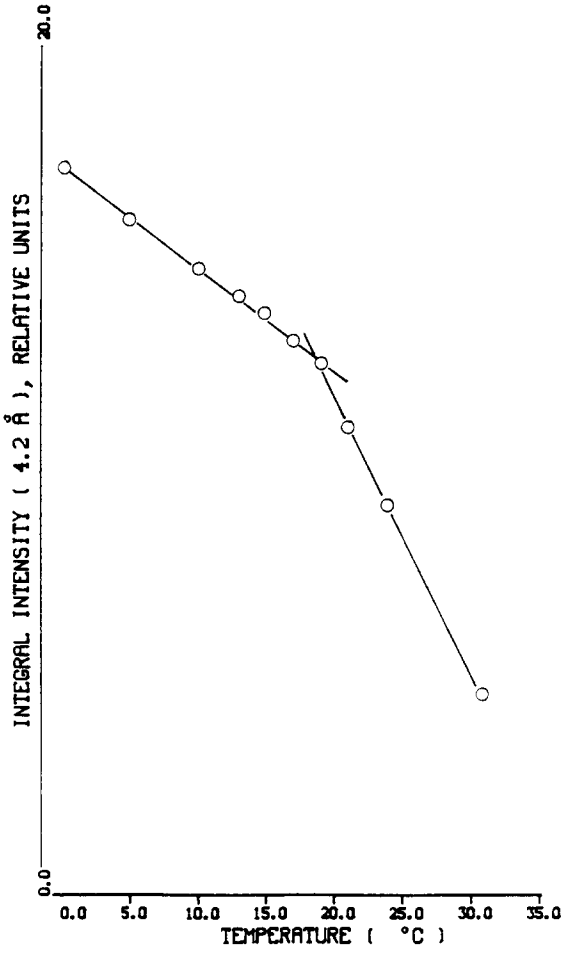


Fig. 3. Temperature dependence of the integral intensity of the 4.2 Å reflection. Values are averages from four different membrane fractions.

28°C. In this context it is noteworthy that crystalline lipids are present at 28°C regardless whether the membranes are stepwise warmed up from 0 to 35°C or are first equilibrated at 35°C and then recooled. (iii) The crystalline ⇌ liquid-crystalline phase separation is not linear but rather 'bisegmental', i.e. it occurs in a two-stage process with a breakpoint at approximately 18°C. Such a breakpoint could be reproduced in each out of eight membrane isolations. Incidentally, such a breakpoint cannot be revealed in membrane preparations which are derived from cells broken at 4°C in a French press or in a Potter-Elvejhem homogenisator or which are prefixed with 2% glutaraldehyde.

The temperature response of the membrane resembles that of extracted lipids. In fact, lipids extracted from *Tetrahymena* microsomal membranes can also undergo a broad thermotropic two-stage phase separation with a breakpoint at approx. 16°C [12]. This breakpoint was attributed to an abrupt lipid redistribution due to a change in the compositional quality of the lipids transformed from the crystalline into the liquid crystalline state below and above the breakpoint (cf. also Ref. 13). It is therefore conceivable that the breakpoint in native membranes reflects a similar lipid redistribution.

In any case, it is conspicuous that the temperature range of the breakpoint in the lipid phase separation in native membranes coincides with the onset of thermotropic lipid-protein segregations in endomembranes of intact *Tetrahymena* cells [5–8]. This indicates that the lipid-protein segregations

do not relate to the onset or completion of a rather cooperative phase transition, but occur during a broad phase separation. We suggest that the lipid-protein segregations are induced by an abrupt change in the interaction between the two coexisting crystalline and liquid crystalline lipid phases.

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