Altered microviscosity of in vivo lipid-manipulated membranes in *Tetrahymena pyriformis*: A fluorescence study

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Summary. By determination using fluorescence polarization measurements with 1,6-diphenyl 1,3,5-hexatriene, ergosterol-replaced pellicle and microsome membranes of *Tetrahymena* cells become less fluid, whereas those membranes from chimyl alcohol-fed cells are more fluid, when compared with the control native membranes.

A variety of evidence has shown that the physical properties of membrane lipids are closely associated with various functions taking place in the biological membranes[2]. It has been also well accepted that the phospholipid as well as the fatty acyl composition and sterol are primary important factors for regulating membrane fluidities.

In order to study such relationships between physical states of lipids and biological processes in the membrane, it is useful approach to observe the effects on functions by manipulation of the membrane lipid composition. Recently, several methods have been employed: genetic, nutritional, enzymatic, thermal manipulations and even catalytic hydrogenation[3].

We have chosen a ciliated protozoan, *Tetrahymena pyriformis,* as a potentially suitable model cell for membrane studies and have conducted several lines of experiments related to membrane formation[4,5], membrane-bound enzymes[6] and environmental adaptation[7,8]. One of several advantages of using this cell system is the ease of manipulation of the lipid composition of different membranes[9]. In our previous papers we have shown that the supplementation of ergosterol or chimyl alcohol causes marked alterations in polar head group, as well as fatty acyl chain composition in *Tetrahymena* membranes[10,11]. In this communication, we describe changes in membrane fluidity as inferred by DPH fluorescence test of the lipid-manipulated membranes by supplementing ergosterol or chimyl alcohol (1-0-hexadecyl glyceryl ether) in *Tetrahymena pyriformis* NT-I.

Materials and methods. Supplementation of cells with ergosterol or chimyl alcohol. The control cells of *Tetrahymena pyriformis* NT-I strain were grown in proteose-peptone basal medium as previously described[12]. For the ergosterol-replacement, cells were grown in the basal medium but supplemented with exogenous ergosterol (2 mg per 200 ml culture)[10]. The chimyl alcohol-fed cells were prepared according to the procedure previously described[11]. Ergosterol and chimyl alcohol were purchased from Sigma Co. Ltd.

Isolation of membrane fractions. When the cells reached the mid-log phase of growth, they were harvested and washed with 0.2 M phosphate buffer, pH 7.2. Membrane fractions were isolated according to Nozawa-Thompson's procedure[12,13].

Fluorescence labelling and measurements. The fluorescent hydrocarbon 1,6-diphenyl 1,3,5-hexatriene (Tokyo Kasei Co. Ltd.) was employed as a probe for monitoring membrane lipids' fluidity. The labelling procedure of membranes was carried out essentially according to Shinitzky and Inbar's method[14,15]. The degree of fluorescence polarization (P) and intensitiy (I) were measured with an Elscint Microviscosimeter MV-Ia.

$$P \approx \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

$I_{\parallel}$, $I_{\perp}$; the fluorescence intensities polarized parallel and perpendicular to the direction of polarization of the excitation beam, respectively.

Results and discussion. Figure 1 demonstrates the temperature dependence of the fluorescence polarization of DPH in cilia, pellicles and microsomes isolated from the control unsupplemented cells of *Tetrahymena pyriformis* NT-I. This shows pronounced differences in the microviscosity between various membrane fractions, as being compatible with data by electron spin resonance[16]. One can expect that differences of membrane fluidity would reflect different lipid compositions of the 3 membranes, which have been shown elsewhere[17]. The ciliary membrane containing the

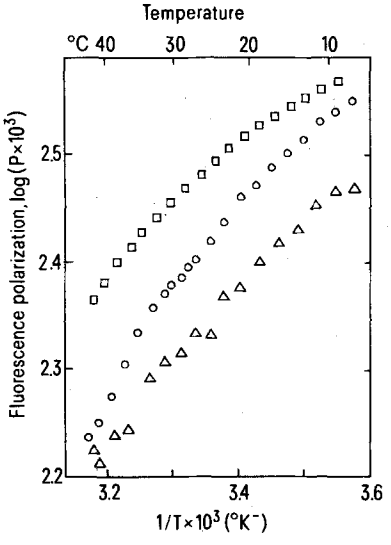

Fig. 1. Temperature dependence of the degree of fluorescence polarization presented as log P vs. 1/T of various membrane fractions from *Tetrahymena pyriformis* NT-I. □, cilia; ○, pellicles; △, microsomes.

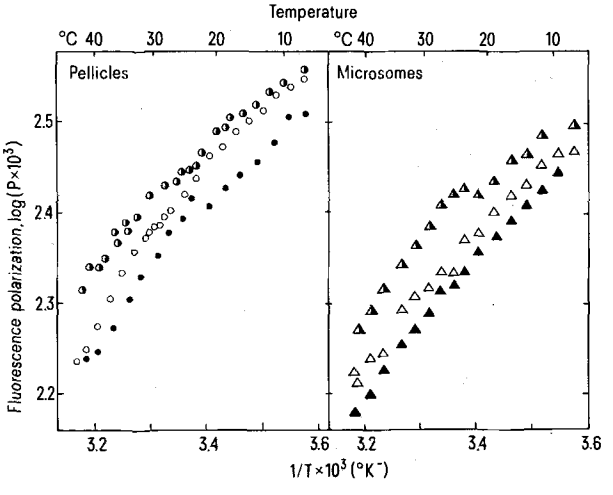

Fig. 2. Alteration of microviscosity in the lipid manipulated pellicle and microsome membranes. ○, native; ◑, ergosterol-replaced; ●, chimyl alcohol-fed; △, native; ▲, ergosterol-replaced; ▲, chimyl alcohol-fed.
The pellicle is the surface membrane comprising the plasma membrane and 2 (outer and inner) alveolar membranes[12].

high level of triterpene, tetrahymanol and 2-amino-ethylphosphonolipid is less fluid than pellicle and microsome membranes. Microsomes are most fluid and have the lowest content of these 2 lipids, together with the highest degree of unsaturation in fatty acyl chains.

Alterations in the microviscosities of pellicle and microsome membranes isolated from supplemented-cells with ergosterol or chimyl alcohol are clearly depicted in figure 2. Ergosterol-replaced pellicle and microsome membranes become more rigid, whereas chimyl alcohol-fed membranes are more fluid, when compared with the control native membranes. It is of interest to note that, in pellicles, rigid-making effect of ergosterol-supplementation and fluidizing effect of chimyl alcohol-feeding are pronounced above and below about at 22–25 °C, respectively. Microsome membranes from ergosterol-replaced cells are markedly less fluid above 22 °C. However, at the present time we have no exact explanation for such phenomena on the basis of the lipid composition alterations of the manipulated membranes. Similar trends were also observed with dispersions of lipids extracted from the manipulated pellicles and microsomes (data not shown).

The in vivo manipulated *Tetrahymena* membranes with altered fluidities would provide a potential clue towards understanding relationships between fluidities and functions of biological membranes. For example, in our recent work on adenylate cyclase in the pellicle membrane, we have obtained data indicating effects of ergosterol-replacement upon the transition of the activation energy of the enzyme, and the detailed results will be reported elsewhere.

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Spirally arranged muscles associated with tracheoles in tsetse fly flight muscles; their possible involvement in sound production

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Summary. A set of muscle fibres have been found associated with certain tracheoles in the flight muscles of the tsetse fly. It is proposed that those muscles may be involved in sound production in the fly.

The production of sounds in insects can be divided into 2 main categories: 1. sounds produced by frictional methods and 2. sounds produced by movement of air[2]. If, as is suggested, within the 2nd category the sounds are produced by air being pulsed out of the spiracles, a mechanism for controlling the air flow would be necessary. Control of spiracular opening is well documented[3] and this operation plays an important part in sound production, however, I propose in addition to the spiracular control that muscle fibres which I have discovered in close association with tracheoles in the indirect flight muscles in the tsetse fly could perform some additional function in sound production.

Ultrathin sections were cut of the indirect flight muscles from the tsetse fly thorax. Fixation, embedding, sectioning and staining for electron microscopical investigation were all carried out according to conventional methods[4].

In the indirect flight muscles of the tsetse fly tracheation is similar to that described in other flight muscles[5], with the tracheoles situated within deep clefts created by invaginations of the sarcolemma. Within these intuckings in certain of the indirect flight muscles an elaborate muscular arrangement has been found closely associated with the tracheoles (figures 1–3). I shall refer to these muscles as tracheal muscles to distinguish them from the flight muscles.

The tracheal muscles clearly arise as extensions of the flight muscle myofibrils (figure 1), and are arranged spirally around the tracheoles (figure 2). The spiral arrangement can be clearly appreciated by the transverse, longitudinal and oblique profiles which are observed in these muscle fibres when either longitudinal or transverse sections of the muscle are examined. Surrounding these tracheal muscles are areas of extracellular space (figure 1), and the membranes of the spiralling muscles make tight junctions with the membrane of the flight muscle fibres (figures 1 and 2).

I propose the following model as to how these tracheal muscles may function. The fibres are spirally arranged around the tracheoles, therefore when the muscle fibres contract they will constrict the tracheoles and when the muscles relax the tracheoles will regain their original dimensions. The extracellular space surrounding the tracheal muscles will allow for any size changes which might take place in the diameter of the tracheoles during such