Oleic acid is elevated in cell membranes during rapid cold-hardening and pupal diapause in the flesh fly, *Sarcophaga crassipalpis*.

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Abstract

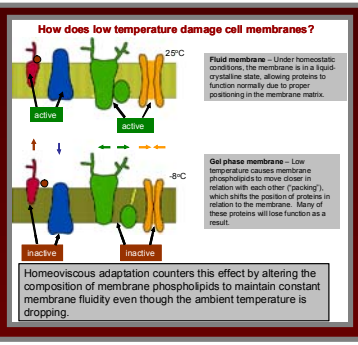
The integrity of cellular membranes is critical to the survival of insects at low temperatures, thus there is tremendous advantage conferred to insects that can adjust their composition of membrane fatty acids (FA's). Such changes, known as homeoviscous adaptation, allow cellular membranes to maintain a liquid-crystalline state at temperatures that are normally low enough to cause the membrane to enter the gel state and lose the ability to maintain homeostasis.

Flesh flies (*Sarcophaga crassipalpis*) were subjected to two experimental conditions that elicit low temperature tolerance: rapid cold-hardening and diapause. FA's were isolated and analyzed using gas chromatography-mass spectrometry. FA's changed in response to both rapid cold-hardening and diapause. In response to rapid cold-hardening, the proportion of oleic acid (18:1n-9) in pharate adults increased from 30% to 47% of the total fatty acid pool. The proportion of almost every other FA was reduced. Diapausing pupae experienced an even greater increase in oleic acid proportion to 58% of the total FA pool. Oleic acid not only increases membrane fluidity at low temperature, but also allows the cell membrane to maintain a liquid crystalline state should the temperature increase. This is the first demonstration of homeoviscous adaptation in a cold-hardy insect with a pupal diapause.

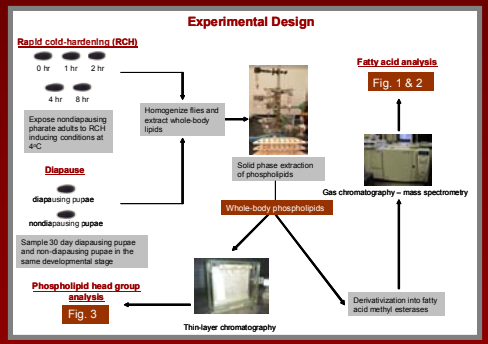
Introduction

Preventing cellular damage due to low temperatures (cold shock) is a major challenge for insects. The flesh fly, *Sarcophaga crassipalpis*, possesses two known mechanisms by which cold shock can be prevented or attenuated: by entering into a cold-hardy pupal diapause and by rapid cold-hardening (RCH). Diapause for *S. crassipalpis* is a photoperiod-induced developmental arrest that features physiological changes that contribute to low temperature survival such as upregulation of heat shock proteins and glycerol (Lee et al. 1987, Hayward et al. 2005). RCH for the flesh fly is induced by short exposures (hrs) to temperatures between 0ºC and 10ºC and is characterized by a three-fold increase in whole body glycerol content. Glycerol alone probably does not fully explain the protection imparted from RCH because glycerol concentrations never reach a level shown to protect proteins and membranes (Macartney et al. 1994), and other insects that possess RCH ability do not have any detectable upregulation of polyols (Kelty and Lee 2001).

Homeoviscous adaptation, the ability to alter the composition of membrane phospholipids to maintain fluidity during temperature changes (Sinensky 1974), has been linked to cold adaptation in a number of organisms, but has not been investigated extensively in insects. In the present study we investigate the hypothesis that homeoviscous adaptation in *S. crassipalpis* is involved in the RCH and diapause responses of this flesh fly by chromatographic analysis of fatty acid constituents of phospholipids as well as by thin-layer chromatographic analysis of phospholipid head groups..



Methods



Results

- Oleic acid (18:1n-9) levels increased by 17% in response to rapid cold-hardening (Fig 1A). All other fatty acids were decreased or remained the same.
- Diapause also increased oleic acid levels by 14% of the fatty acid pool (Fig. 2). All other fatty acids were reduced or remained the same. Unlike rapid cold-hardening, the change in oleic acid increased the unsaturation index of the fatty acid pool.
- Both diapause and rapid cold-hardening cause phopholipid head group changes with an increase in the proportion of phosphatidylethanolamine in relation to phosphatidylcholine (Fig. 3).

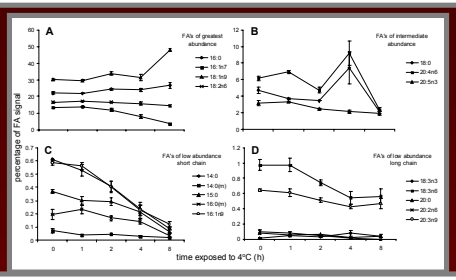


Figure 1. Change in individual fatty acids (FA) as a result of chilling consistent with flesh fly rapid cold-hardening induction. *Sarcophaga crassipalpis* pupae were subjected to 4ºC for a period of 0-8 hrs and their fatty acids were analyzed with GC-MS. Each data point represents the mean ± standard error (n=5). Each point that bears an asterisk is considered statistically significant with respect to the zero-hr treatment group (one-way ANOVA with Tukey's post-test). It is clear from the above data that oleic acid (18:1n9) is dramatically increased (17.6% total increase) between 4 and 8 hrs at the expense of many other fatty acids.

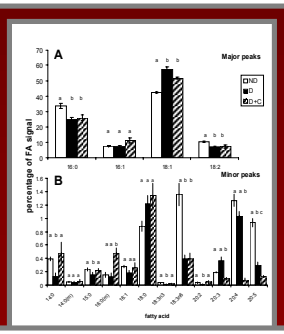


Figure 2. Change in major (A) and minor (B) fatty acid (FA) proportions due to diapause and chilling in the flesh fly, *Sarcophaga crassiplapis*. Fatty acid methyl esterases from non-diapausing pupae (ND) were compared with 30 day diapausing pupae (D) and 30 day diapausing pupae that had been chilled at 4ºC for 8 hrs (D+C). Bars respresent means ± standard error (n=5). Significance for each fatty acid peak was determined through a one-way ANOVA, and significant peaks were assigned statistical groupings (a,b,c) by Tukey's post-ANOVA t-tests.

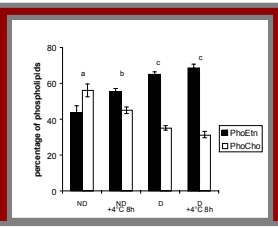
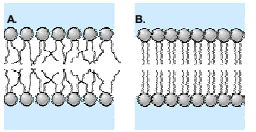


Figure 3. Change in phospholipid class due to rapid cold-hardening and diapause in the flesh fly, *Sarcophaga crassipalpis*. Phosphatidylethanolamines (PhoEtn) are upregulated at the expense of phosphatidycholines (PhoCho) due to rapid cold hardening ($p<0.05$) and diapause ($p<0.01$), but chilling a fly in dipause does not enhance this response beyond that induced by diapause alone. Letters above the bars (mean ± standard error, n=5) represent statistical groupings (Tukey's). D is diapause and ND is nondiapause. Chilling was carried out by exposing the fly to 4oC for 8 hrs

Conclusions

- Our experiments provide strong evidence for homeoviscous adaptation in flesh fly cell membranes during RCH and diapause. This is evidenced by an increase in the overall degree of unsaturation of fatty acids as well as changes in phospholipid head groups. Both promote membrane fluidity at low temperatures.
- Fatty acid change due to RCH and diapause both feature a 14-17% increase in oleic acid, a monoene with a single double bond located in the middle of the acyl chain. This particular fatty acid possesses the unique ability to promote a wide widow of fluidity rather than the shift of a thinner fluidity window to a lower temperature (McIlhaney 1974). In this manner, the flesh fly can maintain membrane fluidity for low temperatures as well as warmer temperatures.
- Increase in the proportion of phosphatidylethanolamine at the expense of phosphatidylcholine aids homeoviscous adaptation by reducing torsional strain on cell membranes as temperatures drop.
- The "kinks" in the acyl chains of fatty acids with double bonds promote membrane fluidity (and homeoviscous adapatation) by increasing overall molecular disorder as lowering temperatures increase molecular order (e.g. packing).



A. Fluid membrane – Contains many unsaturated fatty acids. Maintains fluid state as low temperature "packs" the membrane. "Kinks" in acyl chains from double bonds maintain distance between adjacent phospholipids.

B. Ordered membrane – Contains many saturated fatty acids (shown here) or results from low temperature packing. If low temperature causes this, the membrane transitions to gel state and homeostasis is lost.

References

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