

## Cholesterol creates domains in the lipid bilayer matrix of red blood cell membranes

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### Introduction

The currently accepted model of the structure of biological membranes is that they are comprised of a lipid bilayer matrix in which the various intrinsic membrane proteins are distributed. Domains of eucaryotic plasma membrane which are insoluble in Triton X-100 at 4°C and enriched in cholesterol and saturated sphingomyelin can be separated from other soluble fractions which contain predominantly unsaturated phospholipids [1,2]. Our initial experiments were designed to determine whether domains could be detected in lipid extracts of biological membranes using diffraction methods.

### Materials and methods

Suspensions of red blood cells were incubated in the presence or absence of cyclodextrin. Total polar lipid extracts of the treated and control cells were prepared and dispersed in an equal weight of buffer. The dispersions were examined on Beamline 15A and scattering intensity in the small-angle and wide-angle regions were recorded during a heating scan from 5° to 50°C and a subsequent cooling scan to 10°C at 1°/min.

### Results and discussion

The SAXS/WAXS intensities recorded from a lipid dispersion prepared from control cells during an initial heating and subsequent cooling scan is presented in Fig. 1. At 5°C two lamellar reflections are observed characterized by two orders of reflection at the camera length used. The d-spacings correspond to 7.75 and 7.46nm, respectively. The wide-angle region shows a single broad reflection at a spacing corresponding to 0.46nm characteristic of disordered hydrocarbon chains. Upon heating to 50°C the two peaks remain distinct but the repeat spacings decrease by a few Å.

A lipid dispersion prepared from red blood cells treated with cyclodextrin to partially remove cholesterol was subjected to a similar examination and the results are also seen in Fig. 1. This shows a single lamellar phase of 6.85nm present in the dispersion and that the hydrocarbon

chains remain in a disordered state throughout the temperature scanning profile.

Clearly cholesterol induces the phase separation of a cholesterol-rich lamellar phase. It is presumed to represent raft domains consisting predominantly of sphingomyelin and cholesterol. Further studies are in progress to characterize the components of the cholesterol-rich phase.

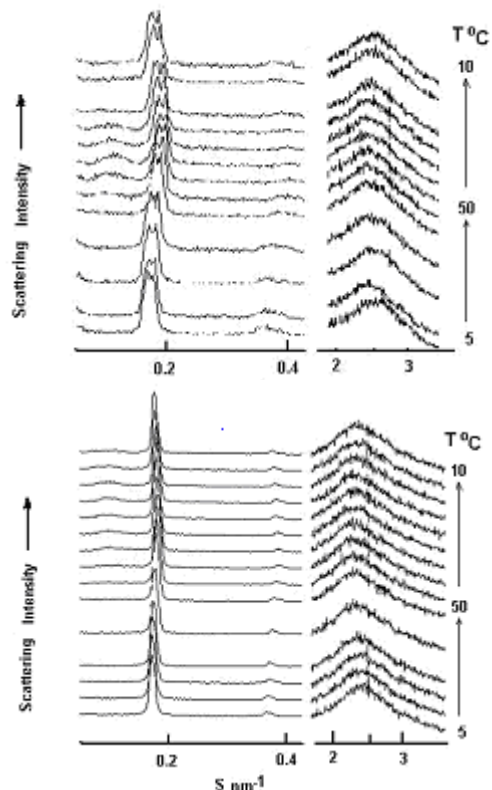


Fig 1. SAXS/WAXS of red blood cell lipids from control (upper) and cyclodextrin treated (lower) cells.

### References

[1] D.A. Brown & E. London *J. Biol. Chem.*, **275**, 17221 (2000)

[2] X. Xu & E. London *Biochemistry*, **39**, 843 (2000).

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