A CALORIMETRIC STUDY OF CHLOROQUINE INTERACTION WITH PHOSPHATIDYL CHOLINE VESICLES

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Phospholipid vesicles (liposomes) are often employed as model systems in order to study the interaction of drugs with biological membranes or as a possible drug carrier. Depending on the lipophilicity of the drug molecule, it will reside predominantly in the lipid bilayer or in the aqueous core of the vesicle. Drugs partitioning into the bilayer will change the temperature associated with the gel → liquid crystalline phase transition and the calometrically determined heat content (ΔH). Present investigations examine the molecular mechanisms of the interaction of chloroquine base with vesicles of synthetic phospholipids (Dimyristoyl - (DMPC), Dipalmitoyl - (DPPC), and Distearoyl - (DSPC) - phosphatidylcholines) by the application of differential scanning calorimetery (DSC) and complementary evidence is provided by proton nuclear magnetic resonance (pNMR) spectroscopy.

For calorimetric studies, 10% phospholipid dispersions with the appropriate chloroquine concentration were prepared. The samples were examined in sealed calorimetric containers using a heating rate of 5° min^{-1} and a range setting of 1-2 m cals s^{-1}. All experiments were repeated using two separate preparations. ΔH, the transition enthalpy was calculated from the area under the curve which was determined by weighing. Increasing the concentration of chloroquine in DPPC liposomes reduced the height and broadened the main endothermic peak. The pre-transition peak was abolished by 10^{-1} M chloroquine. At drug concentrations $<10^{-3}$ M no changes were observed. Addition of equimolar concentrations of chloroquine to DMPC, DPPC and DSPC resulted in an inverse linear relationship between % reduction in ΔH and chain length. This suggests maximum interaction of chloroquine with a membrane of greatest lipophilic character.

The addition of 10% of the charged lipids stearylamine or dicetylphosphate almost abolished the main endothermic peak. Incorporation of the drug at 10^{-1} M increased ΔH although failed to restore ΔH to the pure DPPC value. Hence the chloroquine would appear to be competing with the ionic lipids for the same sites within the phospholipid bilayer.

Sonication changes the main transition peak of both synthetic and naturally occurring phospholipids. Prolonged sonication results in a reduced transition temperature, a decrease in enthalpy and entropy and an increase in peak width. (Sheetz & Chan 1972). These findings were confirmed for 10% dispersions of DPPC and addition of increasing molar chloroquine resulted in a further reduction in ΔH on sonication.

pNMR of sonicated liposomes gives information relating to the mobility of protons from both the polar head group region and lipid interior of the bilayer. Egg. P.C. liposomes in D_2O were examined after 1h sonication in a Jeol PFT-100 spectrometer operating in a Fourier transform mode. Measurements of % peak ratios due to the $-(CH_2)_n-$ and the $-N^+(CH_3)_3-$ protons showed that with increasing chloroquine concentration the ratio increases rapidly for low chloroquine concentrations and plateaus for ratios >0.1 chloroquine/Egg. P.C. These results confirm the calorimetry studies that chloroquine base is predominantly associated with the hydrocarbon interior of the membrane.

Sheetz, M.P. & Chan, S.I. (1972) Biochemistry 11, 4573-81