Differential Scanning Calorimetry of Na,K-ATPase Reconstituted in Proteoliposomes: Correlation of DPPC:DPPE:Cholesterol Ratios and Catalytic Activity.

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The Na,K-ATPase consists of protomers containing one α catalytic subunit and one β subunit of regulatory function. The αβ protomers may be organized as dimmers or higher oligomers in the plasmatic membrane. The lipid domain formation is probably very important in the regulation of the Na,K-ATPase activity and we have related it with the lipid transition temperature. The $(\alpha\beta)_2$ form of Na,K-ATPase rabbit kidney outer medulla was purified and prepared as previously standardized in our laboratory. The DPPC:DPPE-proteoliposome was prepared by the co-solubilization method on a weight ratio of 1:1 lipid:lipid and 1:3 lipid:protein and the DPPC:DPPE:Cholesterol-proteoliposome was prepared on 1:1:1 lipid:lipid:cholesterol and 1:6 lipid:protein. The differential scanning calorimetry (DSC) experiments were performed in the range of 10 to 100°C, using a heating rate of 1.0°C/min, sample and reference volume was 0.3mL. The thermogram of DPPC:DPPE-liposome shows two endothermic peaks, centered at 43.4°C(?H=2.55Kcal/mol) and 64.0°C(?H=0.92Kcal/mol). There was an increase of membrane fluidity and only one transition at 34.7°C(?H=0.068 Kcal/mol) was observed to DPPC:DPPE:Cholesterol-liposome. For the proteoliposome system constituted only by DPPC:DPPE the DSC showed just one large peak at about 50°C(?H=4.54Kcal/mol) that could be correlated with some transitions that occur in a cooperative manner. On the other hand, in the DPPC:DPPE:cholesterol-proteoliposomes, three endothermic transitions temperatures, at 34.9°C (?H=0.0087Kcal/mol), 43.0°C(?H=0.0423Kcal/mol) and 69.3°C(?H=0.0189 Kcal/mol) were observed. In conclusion, although the presence of 33% (w/w) that correspond to 50% mol ratio of cholesterol destabilized the proteoliposome system, it caused a two-fold increase on the Na,K-ATPase catalytic activity, proving that the lipid microenvironment has an important role in the modulation of catalytic enzyme activity. Financial support: CAPES, CNPq and Fapesp.