THE SYNTHESIS AND COMPARISON OF MODIFIED BETAINES AS CRYOPROTECTIVE AGENTS

Andrew W.Lloyd, Ken J.Rutt, Cedric J.Olliff, Pharmaceutical Sciences Research Group, Brighton Polytechnic. Brighton BN2 4GJ UK

N-modified betaines have been shown to be more effective than glycinebetaine as cryoprotective additives for the prevention of freeze-thaw damage to liposomes and erythrocytes (Lloyd et al. 1989). Although various carboxylate anions have been shown to behave similarly in preventing leakage from frozen liposomes (Lloyd et al. 1988), the importance of the betaine carboxylate moeity for cryoprotective activity has yet to be established. This communication describes the effects of chemically modifying the carboxylate moeity of glycinebetaine on the cryoprotection afforded to liposomes and erythrocytes on freezing.

A series of (carboxymethyl)trimethylammonium chloride esters (glycinebetaine esters) were prepared from the appropiate alkylchloroacetates and trimethylamine. Taurine betaine was prepared by refluxing 2-bromoethyltrimethylammonium bromide with sodium sulphite solution. The 2-bromoethyltrimethylammonium bromide was obtained by treating excess dibromoethane with trimethylamine.

Figure 1

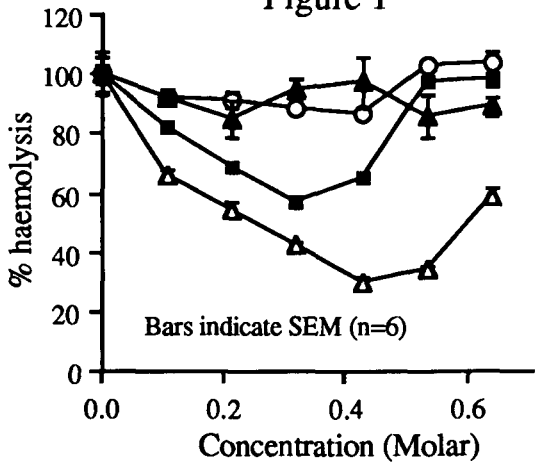
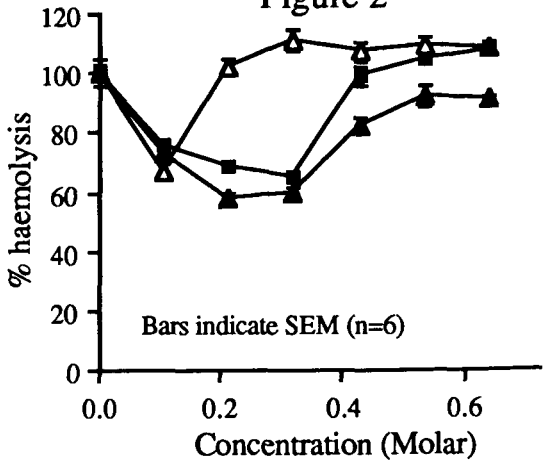


Figure 2



Both liposomes and erythrocytes were used to compare the cryoprotective activities of these compounds with those of glycinebetaine, choline chloride and tetramethylammonium chloride. The methods of determination have been described previously (Lloyd et al., 1988, Brearley et al., 1987). Figure 1 shows the degree of haemolysis which occurs on freezing erythrocytes in the presence of glycinebetaine (Δ), (carboxymethyl)trimethylammonium chloride methyl ester (■), (carboxymethyl)trimethylammonium chloride ethyl ester (○) and (carboxymethyl)trimethylammonium chloride propyl ester (▲). Figure 2 shows the degree of haemolysis which occured on freezing in the presence of taurine betaine (Δ), choline chloride (■) and tetramethylammonium chloride (▲). Choline chloride, tetramethylammonium chloride and the methyl, ethyl and propyl esters were also found to be less effective than glycinebetaine as additives for the cryoprotection of multilamellar liposomes at all concentrations (0.1 - 0.6M).The (carboxymethyl)trimethylammonium chloride octyl ester caused complete haemolysis of erythrocytes and solubilisation of liposomes above its critical micelle concentration (0.035M) and afforded cryoprotection to neither liposomes nor erythrocytes below this concentration. Taurine betaine was shown to afford no cryoprotective activity to the same liposomes. The effects observed for taurine betaine were shown to be typical of other sulphobetaines. It was also observed that sulphobetaines are much less soluble than carboxybetaines.

These results establish the importance of the free betaine carboxylate moeity for cryoprotective activity and show that substitution of this group for a sulphonate group to be detrimental. Clearly this has important implications for the mechanisms of action of these compounds and the future development of novel cryoprotective additives.

Brearley C.A. et al (1987) J. Pharm Pharmacol. 39: 30P
Lloyd A.W. et al (1988) J. Pharm Pharmacol. 40: 27P
Lloyd A.W. et al (1989) J. Pharm Pharmacol. 41: 99P