The Role of Group Structure in the Action of Some Morpholinium Chloride Derivatives on Model Systems

A. HENDRICH[1], J. SARAPUK[1] and S. WITEK[2]

1 *Department of Physics and Biophysics, Agricultural Academy, Norwida 25, 50-375 Wroclaw, Poland*
2 *Institute of Organic and Polymer Technology, University of Wroclaw, Poland*

Abstract. Effects of morpholinium chlorides, which exhibit fungicidal activity, on model lipid systems is discussed. It was shown by means of DSC and BLM techniques that for different salts possessing alkyl chains of identical length, the interaction with lipids depends strongly on the polar head charge and the structure of the salt. The results obtained and conclusions drawn can be useful in explaining the possible mechanism of the biological effectiveness of the compounds tested; this is suggested by the observation that salts tested showed the same sequence of activity in both biological tests and model experiments.

Key words: Morpholinium chlorides — Lipid membranes — Head group interactions — Biological activity

Introduction

The derivative studied, N-dodecyl-N-[3-(β-methyl-β-nitrovinyl)-4-methoxybenzyl] morpholinium chloride, exhibits a strong activity against some fungi (Witek et al. 1978a, b). We tried to establish which part of this molecule is essential for its biological activity. Two derivatives of the above compound, with modified head group structures and an identical hydrophobic part, were synthesized for this purpose. Earlier experiments with similar substances have shown that the fungicidal activity can be a result of an interaction of the dug molecule with the lipid phase of the membrane (Kuczera et al. 1983; Kleszczyńska et al. 1981; Sarapuk et al. 1984, 1985; Gabrielska et al. 1981). In our experiments, the lipid phase was simulated by dipalmitoyl phosphatidylcholine (DPPC) and egg yolk lecithin. The differential scanning calorimetry (DSC) and black lipid membrane (BLM) techniques were used.

Materials and Methods

The amphiphilic compounds N-dodecyl-N-[3-(β-methyl-β-nitrovinyl)-4-methoxybenzyl]morpholinium chloride (salt IVA), N-benzyl-N-dodecyl morpholinium chloride (salt IVB) and N-

dodecyl-N-methyl morpholinium chloride (salt IVC) were synthesized in the Institute of Organic and Polymer Technology of the Technical University of Wroclaw Their chemical formulae are shown in Fig 1

Samples for DSC measurements were prepared as follows 10 mg of dipalmitoylphosphatidylcholine (DPPC) (Calbiochem) and 40 μl of bidistilled water solution of the chloride derivatives studied (MC) were heated to a temperature above the phase transition of the pure lipid (i e ~ 50 °C), and vortexed over 3—5 min to obtain homogeneous samples The final molar MC to DPPC ratios (MR) in mixtures were 0 005—0 5 After vortexing, the samples were kept in cool for few hours at temperatures slightly above 0 °C Than about 25 mg of the mixture was sealed in an aluminium container and scanned DSC was carried out using a home-made calorimeter at a heating rate of 3 °C/min, the sensitivity of the apparatus was 2 2 mJ/s, the total experimental error of enthalpy determination was less than 20 % the error of temperature estimation was less than $\pm$0 4 °C

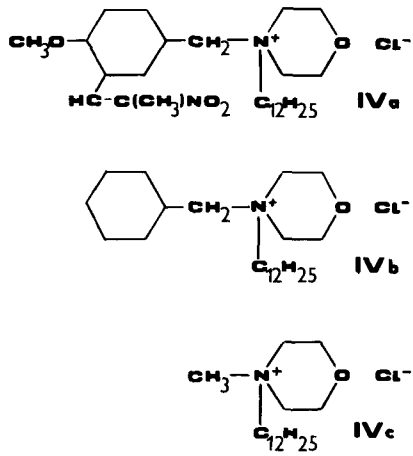

Fig. 1. Chemical formulae of the salts studied

Black lipid membranes were formed from a stock solution containing 1 5 % (w/v) lecithin, extracted from egg yolk according to the method of Singleton et al 1965, in n-decane (Sigma) Physiological saline was used as the bath solution The experiments were performed at room temperature Further data concerning the measurement set-up as well as the measurement procedure were described elsewhere (Sarapuk et al 1981)

Results

The DSC experiments were aimed at detecting possible effects of morpholinium chlorides on the temperature of the phase transition and its enthalpy (ΔH). While there was not change in the first parameter, the enthalpy of transition was influenced by the salts studied The results are summarized in Fig 2 As it can be seen, the action of salts IVA and IVB on DPPC differed from that of salt IVC. All the three salts, when added in molar ratios (mol of salt/mol of DPPC) lower than 0 05 had no effect on the enthalpy of the transition. However, over this MR value changes in enthalpy induced by salt IVC were smaller than those caused

by salts IVA and IVB, respectively. It should however be noted that although the effect of salt IVB on DPPC was similar to that of salt IVA, the latter changed ΔH slightly stronger: salt IVA > salt IVB > salt IVC. Also, it should be stressed that at sufficiently high molar ratios all the morpholinium chlorides tested induced the disappearance of calorimetrically detectable phase transition.

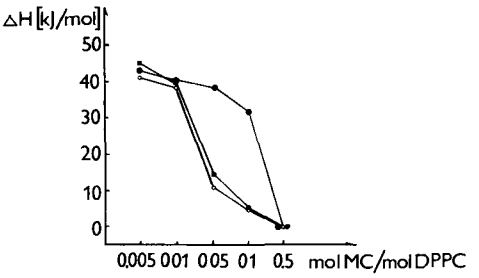

Fig. 2. Dependence of the transition enthalpy ΔH on the molar ratio of the salts studied to DPPC (MR) ○ — salt IVA, ■ — salt IVB, ● — salt IVC

The results of BLM experiments show that all the salts studied, induced instability of planar lecithin membranes, which persisted over considerable periods of time (several hours) in the absence of MC. The measure of this instability was a gradual shortening of the membrane life-time with increasing concentrations of MC. A critical concentration (CC) of MC was introduced to describe this effect. The critical concentration prevented membrane formation. The value of CC for the salts studied were 1.6×10^{-5} mol/l, 2.1×10^{-5} mol/l and 2.1×10^{-4} mol/l for salt IVA, IVB and IVC, respectively. The error of CC determination was smaller than 5 %. The decreased stability was accompanied by changes in membrane resistance. For salt IVC this change reached one order of magnitude (from $1.7 \times 10^{8}\,\Omega$ to $1.8 \times 10^{7}\,\Omega$) for a concentration of the salt of 8.3×10^{-5} mol/l. A similar resistance pattern of BLM was observed for other salts as well. For MC concentrations approaching the critical ones, the time course of the resistance change was typically about 8—10 min., and the process ended in the breaking of the membrane. The effects observed were similar to those reported in previous works dealing with biologically active substances (Kleszczyńska et al. 1981; Sarapuk et al. 1981), and they were consistent with the results of DSC experiments.

Discussion

It is known (Sarapuk et al. 1981, 1984; Gabrielska et al. 1981) that amphiphilic substances including morpholinium chlorides, interact with lipid systems by incorporating their alkyl chains into the hydrophobic interior of the lipid. The depth of this penetration depends, among others, on the length of the alkyl chain of the amphiphilic molecule, and this is one of the factors conditioning the

stability of the lipid systems (Helenius and Simons 1975; Jain and Wu 1977). It has been shown that maximum interaction is reached with alkyl chains of amphiphilic molecules with 10—12 carbon atoms (Gabrielska et al. 1981; Grupe et al. 1978; Frischleder and Gleichmann 1977; Eliasz et al. 1976). On the other hand, the fact that interactions also occur between the polar heads of the lipid and the amphiphilic compounds (Sarapuk et al. 1984, 1985; Kuczera et al. 1983; Boni et al. 1981; Lelkes et al. 1979) must be considered. This type of interactions should be dependent on the size, stereometry and charge distribution of the polar heads. Certainly, interactions occurring in the hydrophilic region also influence processes in the hydrophobic region, as the depth of the penetration into the lipid structure depends on the strength of the interaction of the polar heads. What is observed externally is the global effect of both interactions. Nevertheless, it is possible to estimate the role of the polar head type interaction using a series of compounds differing from each other to a lesser extent as it was the case in our experiments. Comparing the results obtained with salt IVA and salt IVB, it can be seen that these two compounds differed slightly in their action on the systems studied. The slightly weaker action of salt IVB as compared to that of salt IVA, observed in both DSC and BLM measurements, undoubtely was a result of the removal of the nitrovinyl group from the polar head of salt IVA. Since the latter group is electronegative its removal weakens the electrostatic interaction with the polar head group of lecithin. In salt IVC, a possible change in the penetration depth into the lipid structure should be considered since, in addition the polar head of this salt lacks the benzyl group as compared to both salts IVA and IVB. Due to this, the interaction with the lecithin head is further weakened as the benzyl group is also electronegative. However, results obtained by means of both techniques used differ much more from those obtained for salts IVA and IVB. As mentioned earlier, it may be due to the fact that a weaker interaction at the polar region prevents the alkyl chain of salt IVC to incorporate to the same depth as do the alkyl chains of salts IVA and IVB, resulting in additional weakening of the global interaction with the lipid molecules. Another conclusion following from the results obtained with all the salts tested is that the structure of the lipid bilayer gets completely destroyed when the concentrations of these compounds are sufficiently high. This is suggested by the destruction of planar membranes and by abolishing phase transition of DPPC observed in calorimetric measurements. Based on the results obtained the fungicidal action of morpholinium chlorides, especially against *Alternaria tenuis* and *Botrytis cinerea*, seems to be associated with their action on the lipid phase of the membranes of these fungi; this view is strengthened by the fact that the effectiveness of the salts against the above fungi has the same sequence (Witek et al. 1978a, b) as observed in the present work. Our results and conclusions are consistent with those obtained by Kuczera et al. (1983).

References

Boni L T, Steward T P, Alderfer J L, Hui S W (1981) Lipid — polyethylene glycol interactions II Formation of defects in bilayers J Membrane Biol **62,** 71—77

Eliasz A W, Chapman D, Ewing D F (1976) Phospholipid phase transitions Effects of *n*-alcohols, *n*-monocarboxylic acids, phenylalkylalcohols and quaternary ammonium compounds Biochim Biophys Acta **448,** 220—233

Frischleder H, Gleichmann S (1977) Investigation of the influence of alkyl ammonium iodides on the thermotropic phase transition of dipalmitoyl lecithin lamellar systems using differential scanning calorimetry Stud Biophys **64,** 95—100

Gabrielska J, Kuczera J, Oswięcimska M, Przestalski S, Witek S, Żyłka R (1981) Effect of alkyl chain length in alkoxymethylene trimethylammonium chlorides on ion transport across liposome membranes Stud Biophys **82,** 149 155

Grupe R, Menzel G, Sternberg B, Zwanzig M Goring H (1978) Interaction of homologous quaternary trimethylalkylammonium halides (TMMA halides) with lipid membranes III Influence of TMMA halides on the thermal phase transition of lecithin dispersions Stud Biophys **69,** 161 - 173

Helenius A, Simons K (1975) Solubilization of membranes by detergents Biochim Biophys Acta **415,** 29—59

Jain M K, Wu N M (1977) Effect of small molecules on the dipalmitoyl lecithin liposomal bilayer III Phase transition in lipid bilayer J Membrane Biol **34,** 157—201

Kleszczynska H, Matyjasik S Sarapuk J, Grobelny D, Witek S (1981) Interaction of some quaternary ammonium salts with red cell and planar lipid membranes Stud Biophys **84,** 173 -178

Kuczera J, Gabrielska J, Żyłka R, Przestalski S, Witek S, Grobelny D (1983) Influence of the size of the polar part of amphiphile ammonium salts on ion transport across liposome membrane Stud Biophys **90,** 203 211

Lelkes P I, Kapitkovsky A, Eibl H, Miller I R (1979) Head group — dependent modulation of phase transition in dipalmitoyl lecithin anlogs FEBS Lett 181—185

Sarapuk J, Oswięcimska M, Witek S (1981) Alkoxymethylene trimethylammonium chlorides-induced changes in the electrical conductance of bimolecular lipid membranes Stud Biophys **84,** 167—172

Sarapuk J, Przestalski S, Witek S, Vucelic D (1984) Effect of some quaternary ammonium salts on planar lecithin membranes Stud Biophys **100,** 113—117

Sarapuk J, Hendrich A, Przestalski S, Podolak M, Bojko I, Witek S (1985) Interaction of some glycine esters with model membranes Stud Biophys **105,** 121—128

Singleton W S, Gray M S, Brown M L, White I L (1965) Chromatographically homogeneous lecithin from egg phospholipids J Amer Oil Chem Soc **42,** 53—56

Witek S, Grobelny D, Ptaszkowska J, Bielecki A, Bakuniak E, Fulde S, Gorska-Poczopko J (1978a) Belg Pat No 864782

Witek S, Oswięcimska M, Ptaszkowska J, Bakuniak E, Gorska-Poczopko J, Łaszcz E (1978b) Belg Pat No 864781

Final version accepted February 18, 1986