Short communication

Partition of Piperidinoethylesters of 2-Alkyloxyphenylcarbamic Acid in Unilamellar Phosphatidylcholine Liposomes*

P. BALGAVÝ, I. BENEDIKOVIČ, B. KOPECKÁ and J. GALLOVÁ

Faculty of Pharmacy, J. A. Comenius University, Odbojárov 10, 832 32 Bratislava, Czechoslovakia

Abstract. Molar partition coefficients for amphiphilic N-[2-(2-alkyl-oxyphenyl-carbamoyloxy)-ethyl]-piperidinium chlorides (PAA) between small unilamellar egg yolk phosphatidyl choline liposomes and saline, as determined by ultraviolet difference spectroscopy at 22 °C, pH 5–6, $\bar{\nu} = 34640$ cm^{-1}, and at 100 μmol/l PAA concentration, were 149, 1990, and 7474 for PAA with 5, 7, and 9 carbon atoms in the alkyloxy substituent, respectively. At the PAA concentration used, the cut-off in biological activities of PAAs with long alkyloxy substituents could not be caused by the self-association of PAA molecules in the aqueous phase.

Key words: N-[2-(2-Alkyloxyphenylcarbamoyloxy)-ethyl]-piperidinium chlorides — Unilamellar liposomes — Membranes — Partition coefficient

Kráľová et al. (1992) reported that piperidinoethylesters of 2-, 3-, and 4-alkyloxy-substituted phenylcarbamic acids (PAAs) inhibit photosynthetic processes in algae and plant chloroplasts. The inhibitory activity was dependent on the alkyloxy substituent length n (n = number of carbon atoms in the alkyloxy substituent) showing a maximum effect at $n = 6 - 8$. In analogy with the findings of Pešák et al. (1980) that for homologs with $n = 8 - 10$ the partition coefficient of PAA between n-octanol and phosphate buffer decreases with the increasing n, probably due to self-association in the aqueous phase, Kráľová et al. (1992) suggested that the cut-off in PAAs activities is caused by a decrease in the effective concentration of PAA monomers in solution. In this case the partition coefficient K_p between the site of action (photosynthetic membrane) and the aqueous phase increases less rapidly with the chain length n than does the water solubility (i. e., concentration of monomers) until the point is reached at which maximum achievable concentration

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at the site of action is significantly smaller than that required to cause maximal biological effect.

Since among the 2-substituted homologous series of PAA the most efficient compounds are those with $n = 6 - 8$, and the effective algicidal concentrations are 10–15 μmol/l, we determined molar partition coefficients K_p for homologs with $n = 5 - 9$ at 100 μmol/l concentration. If the supposed self-association was responsible for the cut-off in the PAA activity at 10 μmol/l concentration, we should observe an even more pronounced effect on K_p at concentrations one order of magnitude higher. As model membranes, unilamellar liposomes were chosen to exclude effects caused by PAA diffusion through several lipid bilayers separated by aqueous layers at equilibration.

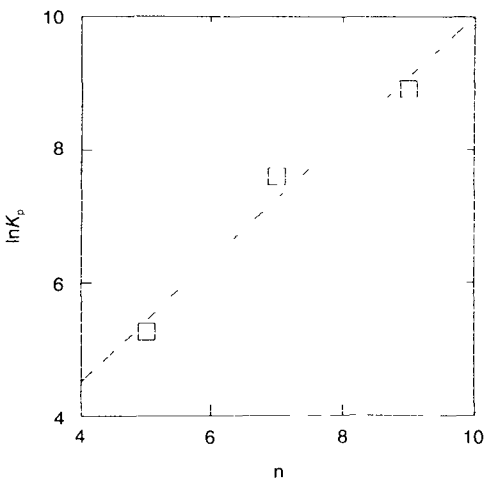

Figure 1. The dependence of the molar partition coefficients K_p for amphiphilic N-[2- (2-alkyl-oxy-phenylcarbamoyloxy)-ethyl]-piperidinium chlorides (100 μmol/l) between small unilamellar egg yolk phosphatidylcholine (60 μmol/l – 1.2 mmol/l) liposomes and saline solution (pH 5–6; t = 22 °C) on the number of carbon atoms n in the 2-alkyloxy substituent. Egg yolk phosphatidylcholine was isolated and purified according to Singleton et al. (1965), unilamellar liposomes were prepared according to Barenholz et al. (1977), and K_p was determined by ultraviolet difference spectroscopy at $\bar{\nu} = 34640$ cm^{-1} according to Welti et al. (1984).

As can clearly be seen from Fig. 1 , the values of $\ln K_p$ are linearly dependent on the number of carbon atoms n in the alkyloxy substituent. The value of the incremental free energy of transfer from the aqueous phase to the lipid phase for the PAA alkyloxy substituent methylene group as calculated from the data in Fig. 1 is $\Delta(\Delta G^0)_{CH_2} = -0.91 \pm 0.14$ RT: this value corresponds to those found for micellization and for partition of different amphiphilic substances to lipid bilayers (Devínsky et al. 1990). Two conclusions follow from these results. First, the value of $\Delta(\Delta G^0)_{CH_2}$ indicates that the main driving force for the binding of PAA molecules to the lipid bilayer is hydrophobic interaction, and that the binding environment of the alkyloxy substituent chains of PAAs in the lipid bilayer is

very similar to that of the hydrocarbon chains of different amphiphiles. Second, contrary to what has been assumed, the PAA partitioning into the lipid bilayer is not influenced by self-association even at concentrations exceeding the range of the cut-off effect; thus the limited solubility cannot explain the cut-off effect in PAAs activities.

There are other possibilities how to explain the observed cut-off effect (for discussion and references see Devínsky et al. 1990, and Baláž et al. 1988). For example, the PAA molecules might diffuse through several membranes separated by aqueous regions to gain access to their site of action. Short chain PAAs would be unable to cross the hydrophobic bilayers because of low K_p whereas long chain PAAs would be unable to penetrate the aqueous regions because of high K_p. PAAs with optimal alkyloxy substituent chain lengths $n = 6-8$ probably possess optimal properties for the transport to their site of action, and this is why they show maximal activity. Not excluding this possibility, we should like to mention also a correlation of the inhibition effect of amphiphilic amines on photosystem II activity with a decrease of the gel-liquid crystal phase transition temperature in synthetic phosphatidylcholine bilayers (Šeršeň et al. 1990). This finding strongly suggests that the inhibition effects of PAAs on photosynthesis might be due to a membrane structure perturbation.

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