Biophysical Chemistry, 32 (1988) 15–20
Elsevier

BPC 01301

A comparative study of the permeation of dihexadecyl phosphate vesicles by various carboxylic acids and some of their tetrazole analogues

Philippe Pernice [a], Madeleine Castaing [b], Pierre Ménassa [c] and Jean Louis Kraus [a]

[a] Département de Chimie, Faculté des Sciences de Luminy, Case 901, 13288 Marseille Cedex 9, [b] Collège de France, Chimie des interactions moléculaires, 75231 Paris Cedex 05, France and [c] Chemistry Department, Concordia University, Montreal, Quebec H3G IM8, Canada

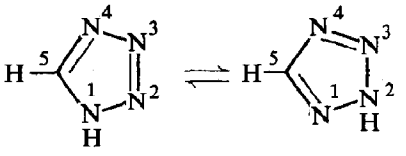
Received 5 April 1988
Accepted 26 May 1988

Tetrazole; Dihexadecyl phosphate vesicle; Permeation; Carboxylic acid; Model membrane

The evaluation of the effect that perturbers may have on a membrane model is described. The model was made from dihexadecyl phosphate bilayers. The perturbers used were carboxylic acids and some of their tetrazole analogues. The results show a good correlation between the permeation properties of the carboxylic acids and tetrazole analogues. Moreover, this study reveals that membrane-perturbing effects are mainly under the control of conformational parameters and dependent on the carbon chain length of the permeants.

1. Introduction

The medicinal chemistry of tetrazoles, as recently reviewed by Singh et al. [1], reveals that even if the tetrazole function has no pharmacological activity by itself, many of its derivatives exhibit interesting biological properties. As this chemical group is metabolically stable [2,3] and behaves like an acid, it has inspired the synthesis of various potential medicinal agents [2]. Tetrazole is an aromatic azapyrrole which can be visualized as existing in the tautomeric forms a and b. The combined inductive effect of the three tertiary nitrogen atoms of the ring confers to the tetrazole group an acidic strength similar to that of aliphatic carboxylic acids.



a b

Since the absorption and distribution of drugs incorporating carboxyl groups are affected by various types of membranes [4], it appeared of interest for medicinal chemists to compare the permeation properties of tetrazoles with those of carboxylic acids.

The aim of the present work was therefore to quantify, and then to compare, leakage of the ionic marker tris(2,2′-bispyridyl) ruthenium(II)

Correspondence address: J.L. Kraus, Département de Chimie, Faculté des Sciences de Luminy, Case 901, 13288 Marseille Cedex 9, France.

chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot H_2O$) induced by various carboxylic acids and some of their tetrazole analogues through the membrane of vesicles composed of dihexadecyl phosphate (DHP) [5]. These single-compartment vesicles represent a realistic model for permeability investigations, since, like living cells, their membrane is made of bilayers. The results presented here are based on the monitoring of leakage of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ across the bilayers by electronic absorption spectroscopy. The results are discussed in terms of the hydrophilicity and conformational structure of the permeating agents and of their interaction with the membranes.

2. Materials and methods

2.1. Materials

2.1.1. Carboxylic acids

The purity of most of the carboxylic acids was stated to be 99% or greater, the sources being as follows: chloroacetic, butanoic, caprylic, lauric and palmitic acids (Aldrich); linoleic and arachidonic acids (Sigma); valproic acid (Abbott Labs). Undecanoic, heptadecanoic, stearic, arachidic, behinic and lignoceric acids were generous gifts from Dr. Ucciani, Aix-Marseille III University.

2.1.2. Tetrazole analogues

All tetrazole analogues have been synthesized in our laboratory according to methods previously published. The syntheses of valproyl tetrazole [6], and linoleyl and arachidonyl tetrazole [7] have been described. Chloromethyl tetrazole, hexyl tetrazole and lauryl tetrazole were synthesized for this work according to the method of Arnold and Thatcher [8], as follows: a mixture of 1 equiv. of the corresponding nitrile, 1 equiv. anhydrous $AlCl_3$ and 4.4 equiv. dry powdered NaN_3 suspended in dry tetrahydrofuran (THF) was refluxed for 24 h under nitrogen. The reaction mixture was then acidified by addition of 15% HCl and the excess hydrazoic acid was removed by means of an aspirator. The THF layer was separated from water and dried over $MgSO_4$. After evaporation of the solvents, and suitable recrystallization, the corresponding tetrazole analogues were obtained.

The structures of these tetrazoles were determined by ^{1}H-NMR in C^2HCl_3 or $(C^2H_3)_2SO$ using a Bruker CW80 spectrometer. Micro-analytical analyses were performed by Guelph Laboratories (Ontario, Canada). Analyses indicated by the symbols of the elements were within $\pm 0.4\%$ of theoretical values.

Infrared spectra were measured in nujol mull using a Perkin Elmer 1310 spectrometer.

Chloromethyl tetrazole: yield, 83%; m.p., 64°C; infrared spectrum, 3.6–4.7 μm (NH tetrazole); ^{1}H-NMR (C^2HCl_3), δ (ppm), 4.20 (s, 1H); $C_2H_3ClN_4$ (C, H, N).

Hexyl tetrazole: yield, 72%; m.p., 42°C; infrared spectrum (nujol mull), 3.6–4.7 μm (NH tetrazole); ^{1}H-NMR (C^2HCl_3), δ (ppm), 0.8–2.2 (m, 11H), 3.2 (t,2H); $C_6H_{12}N_4$ (C, H, N).

Lauryl tetrazole: yield, 75%; m.p., 66°C; infrared spectrum (nujol mull), 3.6–4.7 μm (NH tetrazole); ^{1}H-NMR (C^2HCl_3), δ (ppm), 0.8–2.2 (m, 21H); $C_{12}H_{24}N_4$ (C, H, N).

2.1.3. Other chemicals

Doubled-distilled deionized water was used. The analytical grade cation-exchange resin AG 50W-X_2 (100–200 mesh, hydrogen form) was purchased from Biorad. $(CH_3)_2$ SO, $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and DHP were purchased from Aldrich.

2.2. Methods

2.2.1. Preparation of DHP vesicles

The present experiments were performed on vesicles composed of DHP as these are known to be of a very high stability (a few months). The entrapment of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions in such vesicles has been studied extensively by Tricot et al. [9]. Based on the data reported by these authors, the optimal concentration of DHP was estimated to be 2×10^{-3} M l^{-1}, while that of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions would reach a value of 3.3×10^{-4} M l^{-1}, and the pH at sonication a value of 5.7.

Vesicles were prepared by heating the required amount of DHP dissolved in double-distilled deionized water for 60 min at 80°C. The solution was then alkalinized to the required pH by ad-

dition of NaOH and $Ru(bpy)_3Cl_2 \cdot 6H_2O$ injected into the mixture. Sonication was then performed for approx. 45 min at 80 °C. After cooling, the vesicle suspension was filtered in order to remove the titanium particules released by the sonicator probe. This suspension contained single-compartment vesicles with $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions adsorbed within the outer and inner surfaces of their membrane. Further details on preparation of DHP vesicles are available the data reported by Tricot and Fendler [10].

2.2.2. Calculation of PD_{50}

The absorbance (A) of $Ru(bpy)_3Cl_2 \cdot 6H_2O$, at approx. 455 nm, was determined by recording the visible absorption spectra of the samples with a Kontron 860 spectrometer (Kontron/Instrument Division). The experimental procedure was as follows. Firstly, the DHP vesicle suspension was passed over a cation-exchange resin and, therefore, free $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions in the sample as well as those adsorbed at the outer surface of the membranes were exchanged with the H^+ of the resin. The A value of this suspension, when corrected for the contribution of vesicles containing no entrapped marker molecules, was considered as the 'standard A' (A_S). Secondly, the perturbing agent (i.e., carboxylic acids and their tetrazole analogues) was added to the suspension of eluted vesicles. This induced membrane perturbation and $Ru(bpy)_3Cl_2 \cdot 6H_2O$ leakage from the inside of the vesicles. These ions were removed from the sample, as explained above, using a cation-exchange resin, and the absorption spectrum was recorded. The A value now determined, when corrected for the contribution from vesicles, was considered as the 'measurement A' (A_M). The value of the A_M/A_S ratio was thus equal to the percentage of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions remaining entrapped in the vesicles after the action of a given perturbing agent, at a given concentration. The permeation dose at 50%, i.e., PD_{50}, was defined as the concentration of the perturbing agent required to induce a 50% decrease in the value of the initial $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ion concentrations. In other words, the PD_{50} value corresponded to A_M/A_S ratios equal to 0.50. This provided a useful means of comparison of the action of the various perturbing agents.

It should be stressed that, owing to their low hydrophilicity, perturbing agents of chain length greater than eight carbons were dissolved in

Table 1

PD_{50} values for permeation of Ru ions induced by various saturated and unsaturated carboxylic acids through the membrane of DHP vesicles

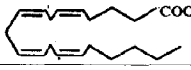
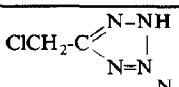
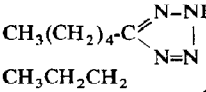
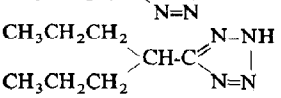
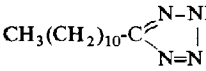
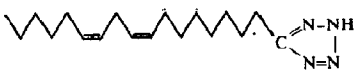
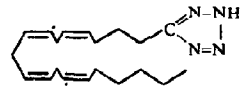
Number of carbons	Name	Formula	PD_{50} ($M\,l^{-1}$)
2	acetic acid	CH_3COOH	104.00×10^{-2}
2	chloroacetic acid	$ClCH_2COOH$	65.00×10^{-2}
6	caproic acid	$CH_3CH_2CH_2CH_2CH_2COOH$	7.30×10^{-2}
8	caprylic acid	$CH_3(CH_2)_6COOH$	0.80×10^{-2}
8	valproic acid	$CH_3CH_2CH_2$ >CH-COOH $CH_3CH_2CH_2$	3.00×10^{-2}
11	undecanoic acid	$CH_3(CH_2)_9COOH$	0.10×10^{-2}
12	lauric acid	$CH_3(CH_2)_{10}COOH$	0.20×10^{-2}
16	palmitic acid	$CH_3(CH_2)_{14}COOH$	0.81×10^{-2}
17	heptadecanoic acid	$CH_3(CH_2)_{15}COOH$	0.77×10^{-2}
18	stearic acid	$CH_3(CH_2)_{16}COOH$	0.90×10^{-2}
20	arachidic acid	$CH_3(CH_2)_{18}COOH$	0.90×10^{-2}
22	behenic acid	$CH_3(CH_2)_{20}COOH$	0.90×10^{-2}
24	lignoceric acid	$CH_3(CH_2)_{22}COOH$	0.90×10^{-2}
18	linoleic acid		0.04×10^{-2}
20	arachidonic acid		0.02×10^{-2}

Table 2

PD_{50} values for the permeation of Ru ions induced by the tetrazole analogues of some of the carboxylic acids through the membrane of DHP vesicles

Number of carbons	Name	Formula	PD_{50} ($M\,l^{-1}$)
2	chloroacetic acid		53.00×10^{-2}
6	caproic acid		5.20×10^{-2}
8	valproic acid		1.50×10^{-2}
12	lauric acid		0.05×10^{-2}
18	linoleic acid		0.14×10^{-2}
20	arachidonic acid		0.023×10^{-2}

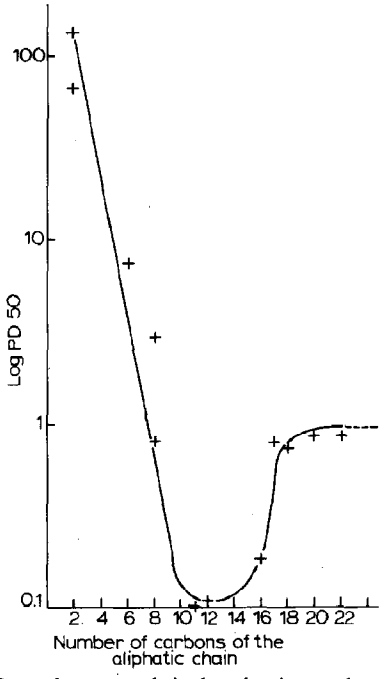


Fig. 1. Dependence on chain lengths, in number of carbon atoms, of $-\log_{10} PD_{50}$ values. The standard deviation was found to be $\pm 1\%$ of the PD_{50} values.

$(CH_3)_2SO$. In these cases, an equivalent amount of $(CH_3)_2SO$ was added to the standard vesicle suspensions prior to the recording of their absorption spectra allowing the calculation of the standard A, i.e., A_S. Moreover, we ascertained that the perturbing effect of $(CH_3)_2SO$ on the vesicle membrane could be neglected as the PD_{50} determined for this solvent was higher than 7 M.

3. Results

Tables 1 and 2, and fig. 1 show that the magnitude of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ leakage across the membrane of DPH vesicles, represented by the PD_{50} value, is under the control of four major structural parameters, to each of which can be assigned a particular perturbing effect: (1) functional group effect, (2) carbon chain length effect, (3) carbon chain unsaturation effect, and (4) carbon chain ramification effect. The additive contribution of each of these effects gives the resulting PD_{50} value. All of these perturbing effects are dependent on each other, but differ significantly in their intrinsic action.

4. Discussion

4.1. Functional group effect

The experimental data (tables 1 and 2) presented here clearly show that both carboxylic acids and their tetrazole analogues permeate the bilayer membrane of DHP vesicles. This perturbing effect seems to be slightly more pronounced for the tetrazole analogues having a carbon chain length of less than 12 carbons. This can be interpreted in terms of the hydrophilicity of the perturbing agents and/or their ability to form hydrogen bonds. The hydrophilicity of the tetrazole ring is slightly lower than that of the carboxylic group as has been observed through thin-layer chromatography [11]. For example, the R_F value for the simplest amino acid glycine is 0.46, while that corresponding to its tetrazole analogue is 0.56 for a solvent of methanol/water (7 : 3) and an adsorbent of silica gel TLC plates.

Now, if one considers compounds with a chain length of 12 or more carbon atoms, the observed difference in PD_{50} values determined for carboxylic acids and their analogues becomes very small and, in addition, the observed PD_{50} values for both functions decrease significantly. Consequently, the carbon chain length effect appears to exert the greatest influence.

4.2. Carbon chain length effect

The longer the aliphatic chain length, the lower is the corresponding PD_{50} value. PD_{50} values ranged between 10^{-3} and 2×10^{-2} M l^{-1} for compounds of chain length greater than 11 carbons. This result can be understood in terms of the membrane partition coefficients of the permeants [12]. A longer aliphatic chain corresponds to a larger partition coefficient, which leads to a lower PD_{50} value. Moreover, the amount of permeating molecules entering the membrane indeed increases with the partition coefficient of the permeant. The curve shown in fig. 1 presents the minimum PD_{50} value for compounds having a carbon chain length ranging from 12 to 14 atoms. Compounds with a longer carbon chain length of 16–24 carbon atoms exhibit significantly higher PD_{50} values. This result is probably directly related to specific conformations adopted by the aliphatic chains under the present experimental conditions, conformations which directly affect the outer structure of the vesicle. One argument related to conformational roles played by the carbon side chains in the membrane, is found in the specfic cases of unsaturated fatty acids.

4.3. Carbon chain unsaturation effect

Linoleic and arachidonic acids, and their tetrazole analogues, which possess 18 and 20 carbon atoms are respectively about 20–40-times more perturbing than the corresponding saturated analogues. Such a result resembles previous data reported for a lipid dispersion as a result of the effect of gramicidin A compared to that of cholesterol [13]. Gramicidin A which is a larger molecule and more rigid along its length than cholesterol has the greatest effect.

4.4. Carbon chain ramification effect

In addition to the other effects, it is of interest to emphasize the fact that the perturbing effect of valproic acid is about 4-times less than that of its linear analogue, i.e., caprylic acid. Moreover, the substitution of a hydrogen of the methyl group of acetic acid by a chlorine atom affects the perturbing properties by a factor of 2.

Due to the high velocity of the perturbing reaction, no kinetic studies have been attempted thus far and the mechanism by which the leakage of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions occurs is still unknown. One possibility already mentioned by Menassa and Sandorfy [5] is that bulky $Ru(bpy)_3Cl_2 \cdot 6H_2O$ ions bind electrostatically to anionic groups of the vesicles via the hydratation water molecules. The perturbers would slightly affect the bridging of these water molecules, but until now it has not been possible to measure the effect that perturbers may have on the outer surface of vesicles.

5. Conclusions

This work describes the use of a simple membrane model system for the comparative study of vesicle permeation by various carboxylic acid and some of their tetrazole analogues. The results obtained show a close correspondence between the membrane-perturbing properties of the carboxyl and tetrazole functions. Moreover, the perturbations induced by the carboxylic acids are under the control of the conformational and hydrophobicity parameters of the carbon chain. The perturbing effect is maximum for unsaturated compounds, while compounds of carbon chain length 11–14 carbon atoms seem to be the most perturbing inducers.

Acknowledgements

We are most grateful to Professor B. Belleau (McGill University) for help and advice in the preparation of the tetrazoles. Mr. K. Raoui is gratefully acknowledged for technical assistance.

References

1 H. Singh, A.S. Chawla, V.K. Kapoor, D. Paul and R.K. Malhotra, Prog. Med. Chem. 17 (1980) 151.
2 R.N. Butler, Adv. Heterocycl. Chem. 21 (1977) 323.
3 S.K. Figdor and M.S. von Wittenau, J. Med. Chem. 10 (1967) 1158.
4 R.M. Julien, A primer drug action (W.H. Freeman, San Francisco, 1975) p. 12.
5 P.E. Menassa and C. Sandorfy, Biophys. Chem. 25 (1986) 175.
6 J.L. Kraus, Pharm. Res. Commun. 15 (1983) 119.
7 J.L. Kraus and G. Lajoie, Jr, C.R. Acad. Sci. Paris 295 (1982) 761.
8 C. Arnold and D.N. Thatcher, J. Org. Chem. 34 (1969) 1141.
9 Y.M. Tricot, D.N. Furlong, A.W.H. Mau and W.H.F. Sasse, Aust. J. Chem. 38 (1985) 527.
10 Y.M. Tricot and J.H. Fendler, J. Am. Chem. Soc. 106 (1984) 735.
11 Z. Grzonka, J. Chromatogr. 51 (1970) 310.
12 P. Seeman, Pharmacol. Rev. 24 (1972) 583.
13 D. Chapman, W.E. Peel and P.J. Quinn, Ann. N.Y. Acad. Sci. (1978) 67.