Correlations between Structure and Biological Activity of Bis-Quaternary Isosters of 1,5-Pentanediammonium Dibromides Algicidal Activity and Inhibition of Photochemical Activity of Chloroplasts

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Isosteric *N,N'*-bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides (PDDBr) (alkyl = n-hexyl–n-octadecyl) with X = CH_2, NCH_3, O or S inhibit photosynthetic processes in *Chlorella vulgaris* algae and in spinach chloroplasts. The dependence of the inhibitory activity against photosynthesizing organisms on the critical micelle concentration as well as on the number of carbon atoms in the alkyl substituents of PDDBr shows a nonlinear parabolic course. From two theoretical models (parabolic and bilinear) used for the description of correlations between biological activity and the structure of PDDBr the bilinear model gave better parameters of statistical analysis. The isosteric exchange of X = CH_2 to NCH_3, O or S practically did not affect the biological activity of PDDBr and the studied correlations could be expressed by a single regression equation for all 36 investigated compounds.

Long-chain bis-quaternary ammonium salts with alkyls longer than octyl belong to membrane-active compounds showing several biological effects, *e.g.* herbicidal [1], antimicrobial [2—4], antibacterial [5] or fungicidal activity [6, 7], as well as ganglioblocking effects [8]. Recently it was found that compounds from the group of quaternary ammonium salts show algicidal effects as well and they inhibit photosynthetic processes in plant chloroplasts causing the destruction of photosystem II with the subsequent release of Mn^{2+} ions from the manganese-protein complex [9].

The study of aggregation properties of isosteric *N,N'*-bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides (PDDBr) with X = CH_2, NCH_3, O or S in aqueous solutions showed that critical micelle concentration (CMC) of these compounds does not reflect the small differences in lipophilicity of compounds with a very similar structure sensitively enough. From hydrophobicity indices, surface area per molecule and from the surface tension at CMC values of PDDBr the following lipophilicity increase sequence was determined: S, CH_2, NCH_3, O [10]. The comparison of antimicrobial activity of corresponding PDDBr compounds with different X against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans* has shown only very small differences, *i.e.* the isosteric exchange practically does not affect the antimicrobial activity [4].

This paper is aimed to investigate the algicidal activity of PDDBr as well as their inhibitory effect on photochemical activity of spinach chloroplasts and to correlate this biological effect with the structure and CMC of studied compounds using the parabolic model of *Hansch* [11] and *Kubinyi*'s bilinear model [12].

The biological characteristics of PDDBr concerning photosynthesis inhibition in algae *Chlorella vulgaris* (log {1/MIC}) and in plant chloroplasts (log {1/IC_{50}}) and the CMC values of PDDBr are summarized in Table 1. It is evident that the intensity of the biological activity depends on the length of the alkyl substituents (R) of the effector, with alkyl prolongation it increases reaching the maximum approximately at dodecyl derivatives. Quasi-parabolic course of this dependence, the so-called "cut-off" effect can be explained with the free volume theory [9]. From Table 1 it is evident that the isosteric exchange of one atom in the joining bridge causes only a very small difference in biological activity of PDDBr isosters with the same alkyl chain length, *i.e.* the molecule modification does not affect the mode of action. Similar results were obtained by the study of antimicrobial activity of PDDBr isosters against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans* [4].

The regression coefficients of the regression equations for both applied theoretical models (parabolic and bilinear, respectively) as well as the corresponding parameters of the statistical evaluation are shown in Table 2.

In general it can be concluded that both applied models are suitable for the description of the studied correlations. The correlation indices *r* of the regression equations are in the range of 0.858—0.999, the values of *F*-test and *t*-test values of regression coeffi-

Table 1. Biological Characteristics (log (1/(IC_{50}/(mol dm^{-3}))), log (1/(MIC/(mol dm^{-3}))) and Logarithms of Critical Micelle Concentration (log (CMC/(mol dm^{-3}))) of *N,N'*-Bis(alkyldimethyl)-3-X-1,5-pentanediammonium Dibromides

X	*m*	−log {CMC}	log {1/IC_{50}}	log {1/MIC}
CH_2	6	0.7950	0.700	–
CH_2	8	1.3188	2.065	3.901
CH_2	9	1.6021	3.025	4.373
CH_2	10	2.0506	3.827	4.958
CH_2	11	2.5229	4.421	5.455
CH_2	12	2.9586	4.438	5.265
CH_2	13	3.3872	4.147	5.300
CH_2	14	3.7447	4.268	5.204
CH_2	16	4.4949	4.120	4.903
NCH_3	6	–	0.978	2.804
NCH_3	7	–	1.702	3.700
NCH_3	8	1.5086	2.658	3.852
NCH_3	9	1.7448	3.408	4.405
NCH_3	10	2.0757	4.158	4.840
NCH_3	11	2.5229	4.578	5.339
NCH_3	12	2.9586	4.399	5.407
NCH_3	13	3.4815	4.223	5.226
NCH_3	14	3.9208	4.200	5.203
NCH_3	15	4.3665	4.247	5.064
NCH_3	16	4.9208	4.182	4.960
O	6	–	0.704	–
O	8	1.3565	2.322	3.785
O	9	1.6576	3.148	4.497
O	10	2.1135	4.019	5.372
O	11	2.5850	4.198	5.358
O	12	3.0000	4.400	5.402
O	13	3.4202	4.245	5.437
O	14	3.7212	4.171	5.180
O	16	5.1938	4.081	4.952
S	6	0.8861	1.003	3.002
S	8	1.4318	2.200	4.125
S	10	2.2077	4.002	5.473
S	12	3.0177	4.357	5.297
S	14	4.0000	4.172	5.300
S	16	5.0132	3.998	4.700
S	18	6.0000	3.981	4.665

IC_{50} — concentration of effector causing 50 % inhibition of the oxygen evolution rate in spinach chloroplasts; MIC — minimum inhibitory concentration causing total inhibition of chlorophyll production in algae *Chlorella vulgaris*; *m* — number of carbon atoms in the alkyl substituents.

cients *A*, *B*, and *C* show that the significance level corresponds to 99 % (with one exception of 95 %). It has been confirmed that the bilinear model is better for the description of the studied correlations. This is shown by higher *r* and *F*-values obtained with the bilinear model in comparison to the corresponding values obtained for the parabolic one, as well as by high positive values of partial *F*-test (p*F*) (in the range of 10.88—192.87) evaluated according to *Kubinyi* [12]. The calculated theoretical *m* or CMC values showed that the most effective inhibitors for all four investigated PDDBr isoster series with X = CH_2, NCH_3, O or S are undecyl—tridecyl derivatives. The number of members in the set $n = 7$, 8 or 9 can be regarded as insufficient for the more-parameter equation, however the calculated statistical parameters (*r*, *s*, *F*) and the confidence intervals of the regression coefficients show that the obtained results are significant.

With respect to relatively small differences in the inhibitory activity of compounds with different X, but with the same length of alkyl substituents, for the quantitative description of the photosynthesis-inhibiting activity—structure relationship a single correlation equation can be used for all 36 studied derivatives as for algal, so for chloroplast systems (Table 2). Statistical parameters corresponding to these regression equations support the above-mentioned assertion concerning the significance of the results obtained for individual series of isosters with X = CH_2, NCH_3, O or S.

EXPERIMENTAL

Isosteric *N,N'*-bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides $[R(CH_3)_2N^+(CH_2)_2]_2X\ 2Br^-$ (PDDBr) where the linear alkyl chain had 6—18 carbon atoms and X = CH_2, NCH_3, O or S were synthesized according to [4]. CMC values of aqueous PDDBr solutions were determined by conductivity measurements [10].

The effect of PDDBr on chlorophyll production in stationary cultured algae *Chlorella vulgaris* (7 d, 16 h light/8 h dark) was investigated according to the method described in [13]. Their effect on the oxygen evolution rate in spinach chloroplasts was studied spectrophotometrically in the presence of electron-acceptor 2,6-dichlorophenol-indophenol [9]. The biological activity of PDDBr concerning photosynthesis inhibition was expressed by the minimum inhibitory concentration (MIC) for algae and by IC_{50} values for chloroplast systems, *i.e.* by concentrations causing total or 50 % inhibition of the studied parameter.

For the description of correlations between biological activity (log {1/MIC} or log {1/IC_{50}}) and critical micelle concentration (CMC) or the number of carbon atoms (*m*) in the alkyl substituents of PDDBr two theoretical models have been used — the parabolic model of *Hansch* [11] and *Kubinyi*'s bilinear model [12].

Table 2. Regression Coefficients (*A*, *B*, *C*) for Parabolic (P) and Bilinear (B) Relationships between Structure and Inhibitory Activity of *N,N'*-Bis(alkyldimethyl)-3-X-1,5-pentanediammonium Dibromides in Photosynthesizing Organisms Spinach Chloroplasts (I) and *Chlorella vulgaris* (II)

$$\log\{1/Y\} = Ax + Bx^2 + C \quad [P]$$
$$\log\{1/Y\} = A\log x - B\log(\beta x + 1) + C \quad [B_1]$$
$$\log\{1/Y\} = Ax - B\log(\beta \cdot 10^x + 1) + C \quad [B_2]$$

X	*x*	PO	Eqn	*A*	*B*	*C*	log *β*	*n*	*r*	*s*	*F*	Optimum *m* / CMC/(mol dm^{-3})	p*F*
CH_2	CMC	I	P	−3.797 (±0.417)	−0.564 (±0.078)	−1.819 (±0.491)		9	0.981	0.290	76.46	4.31×10^{-4}	
	CMC	I	B	0.342 (±0.082)	3.750 (±0.227)	5.585 (±0.281)	2.015	9	0.996	0.133	376.20	9.67×10^{-4}	18.86
	m	I	P	1.892 (±0.219)	−0.071 (±0.010)	−8.224 (±1.162)		9	0.983	0.273	86.14	13.44	
	m	I	B	0.807 (±0.031)	0.893 (±0.053)	−4.226 (±0.279)	−10.835	9	0.997	0.121	453.47	11.81	20.60
	CMC	II	P	−2.490 (±0.328)	−3.844 (±0.057)	1.379 (±0.431)	–	8	0.969	0.155	38.44	5.77×10^{-4}	
	CMC	II	B	0.407 (±0.059)	2.681 (±0.198)	6.736 (±0.211)	2.118	8	0.992	0.080	149.76	1.36×10^{-3}	10.88
	m	II	P	1.575 (±0.225)	−0.061 (±0.010)	−4.761 (±1.301)	–	8	0.966	0.162	34.84	12.90	
	m	II	B	0.576 (±0.036)	0.697 (±0.052)	−0.749 (±0.345)	−10.901	8	0.992	0.082	144.89	11.58	11.79
NCH_3	CMC	I	P	−2.412 (±0.712)	−0.336 (±0.111)	0.204 (±1.028)	–	9	0.857	0.355	8.28	2.59×10^{-4}	
	CMC	I	B	0.212 (±0.057)	1.433×10^{11} ($\pm 1.281 \times 10^{10}$)	5.139 (±0.214)	−8.945	9	0.985	0.121	94.91	1.34×10^{-3}	38.40
	m	I	P	1.896 (±0.189)	−0.073 (±0.009)	−7.814 (±0.982)	–	11	0.983	0.249	114.92	13.05	
	m	I	B	0.845 (±0.023)	0.922 (±0.036)	−4.139 (±0.195)	−10.293	11	0.998	0.087	976.20	11.34	50.70
	CMC	II	P	−2.325 (±0.409)	−0.330 (±0.064)	1.316 (±0.590)	–	9	0.936	0.204	21.33	2.97×10^{-4}	
	CMC	II	B	0.291 (±0.031)	3.861 (±0.192)	6.362 (±0.121)	1.895	9	0.995	0.057	305.14	1.04×10^{-3}	58.33
	m	II	P	1.318 (±0.116)	−0.051 (±0.005)	−3.248 (±0.603)	–	11	0.987	0.153	145.33	13.02	
	m	II	B	0.492 (±0.025)	0.609 (±0.047)	−0.005 (±0.223)	−11.208	11	0.992	0.116	153.85	11.83	5.09
O	CMC	I	P	−2.309 (±0.489)	−0.301 (±0.075)	0.079 (±0.724)	–	8	0.929	0.313	15.85	1.47×10^{-4}	
	CMC	I	B	0.151 (±0.036)	6.780 (±0.360)	4.834 (±0.132)	1.436	8	0.996	0.076	307.75	8.37×10^{-4}	63.63
	m	I	P	1.895 (±0.171)	−0.072 (±0.008)	−8.090 (±0.905)	–	9	0.989	0.232	19.71	12.78	
	m	I	B	0.840 (±0.020)	0.904 (±0.031)	−4.361 (±0.174)	−10.456	9	0.999	0.070	1234.46	11.57	40.86
	CMC	II	P	−2.016 (±0.486)	−0.279 (±0.074)	1.886 (±0.720)	–	8	0.893	0.311	9.83	2.44×10^{-3}	
	CMC	II	B	0.258 (±0.049)	7.668 (±0.596)	6.263 (±0.176)	1.317	8	0.989	0.104	107.16	1.68×10^{-3}	31.59
	m	II	P	1.759 (±0.320)	−0.069 (±0.013)	−5.718 (±1.855)	–	8	0.942	0.232	19.71	12.76	
	m	II	B	0.860 (±0.061)	0.970 (±0.078)	−3.125 (±0.570)	−10.173	8	0.989	0.103	110.05	11.06	16.27
S	CMC	I	P	−2.520 (±0.512)	−0.299 (±0.073)	−7.232 (±0.738)	–	7	0.951	0.485	18.72	6.05×10^{-5}	
	CMC	I	B	0.203 (±0.058)	3.175 (±0.220)	5.106 (±0.257)	2.120	7	0.995	0.151	211.21	5.19×10^{-4}	27.87
	m	I	P	1.443 (±0.243)	−0.051 (±9.010)	−5.821 (±1.357)	–	7	0.972	0.368	34.06	14.24	
	m	I	B	0.758 (±0.042)	0.830 (±0.062)	−3.646 (±0.365)	−10.679	7	0.996	0.147	224.99	11.70	15.88

CMC	II	P	−1.910 (±0.492)	−0.249 (±0.071)	1.840 (±0.710)	–	7	0.899	0.466	8.37	1.47×10^{-4}	
CMC	II	B	0.324 (±0.063)	3.241 (±0.307)	6.503 (±0.277)	1.845	7	0.986	0.176	70.73	1.59×10^{-3}	18.03
m	II	P	1.190 (±0.234)	−0.045 (±0.010)	−2.407 (±1.306)	–	7	0.943	0.354	16.01	13.18	
m	II	B	0.645 (±0.050)	0.781 (±0.069)	−0.921 (±0.423)	−10.185	7	0.989	0.157	90.01	10.87	12.33
CMC	I	P	−2.610 (±0.237)	−0.334 (±0.036)	−0.503 (±0.344)	–	33	0.922	0.397	85.42	1.25×10^{-4}	
CMC	I	B	0.221 (±0.031)	3.914 (±0.135)	5.164 (±0.117)	1.885	33	0.990	0.144	753.31	7.79×10^{-4}	192.87
m	II	P	1.685 (±0.096)	−0.062 (±0.004)	−7.009 (±0.519)	–	36	0.975	0.275	314.89	13.65	
m	II	B	0.805 (±0.018)	0.881 (±0.029)	−4.023 (±0.164)	−10.579	36	0.994	0.140	1255.26	11.61	90.81
CMC	II	P	−1.864 (±0.185)	−0.253 (±0.028)	1.992 (±0.277)	–	33	0.893	0.282	57.27	2.05×10^{-4}	
CMC	II	B	−2.217 (±0.033)	−0.347 (±0.139)	1.662 (±0.130)	2.052	33	0.973	0.144	258.87	1.15×10^{-3}	78.66
m	II	P	1.288 (±0.085)	−0.049 (±0.004)	−3.064 (±0.475)	–	34	0.957	0.214	167.18	13.12	
m	II	B	0.550 (±0.020)	0.678 (±0.033)	−0.410 (±0.198)	−10.864	34	0.979	0.149	364.11	11.50	32.34

β — nonlinear parameter of the bilinear equation; n — number of members in the set; r — correlation index; s — standard deviation; F — F-test value; Y — MIC or IC_{50}; optimum m (or CMC) — theoretical value of m (or CMC) corresponding to the highest activity; CMC and m are defined in Table 1.

REFERENCES

1. Supin, G. S., Sidenko, Z. S., Stonov, L. D., Bakunenko, L. A.,and Dziomko, V. M., *Zh. Obshch. Khim. 39*, 2651 (1969).
2. Imam, T., Devínsky, F., Lacko, I., Mlynarčík, D., and Krasnec, Ľ., *Pharmazie 38*, 308 (1983).
3. Devínsky, F., Lacko, I., Mlynarčík, D., Račanský, V., and Krasnec, Ľ., *Tenside Deterg. 22*, 10 (1985).
4. Devínsky, F., Lacko, I., Bittererová, F., and Mlynarčík, D., *Chem. Papers 41*, 803 (1987).
5. Cigáneková, V., Kallová, J., Devínsky, F., and Lacko, I., *Folia Microbiol. 34*, 202 (1989).
6. Sumiki, Y., Yamamoto, K., and Takeda, K., *J. Agric. Chem. Soc. Jpn. 26*, 325 (1952).
7. Yamamoto, K. and Udagawa, S., *J. Agric. Chem. Soc. Jpn. 26*, 589 (1952).
8. Fakstorp, J., Pedersen, J. G. A., Poulsen, E., and Schiling, M., *Acta Pharmacol. Toxicol. 13*, 52 (1957).
9. Kráľová, K., Šeršeň, F., Mitterhauszerová, Ľ., Devínsky, F., and Krempaská, E., *Photosynthetica 26*, 181 (1992).
10. Devínsky, F., Lacko, I., Bittererová, F., and Tomečková, L., *J. Colloid Interface Sci. 114*, 314 (1986).
11. Hansch, C. and Leo, A., *Substituent Constants for Correlation Analysis in Chemistry and Biology*. Wiley, New York, 1979.
12. Kubinyi, H. and Kehrhahn, O. H., *Arzneim.-Forsch. / Drug Res. 28*, 598 (1978).
13. Kráľová, K., Šeršeň, F., and Gregáň, F., *Photosynthetica 25*, 455 (1991).

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