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EFFECT OF WATER-SOLUBLE β-CYCLODEXTRIN POLYMER ON THE LIPOPHILICITY OF POLYMYXINE EXAMINED BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

Thin-layer chromatography has shown that the water-soluble β-cyclodextrin polymer forms inclusion complexes with the antibiotic polymyxine, reducing its lipophilicity and its adsorption energy on silica gel. A lower dielectric constant and increasing salt concentration of the solution as well as greater cation radii counteract the complex formation.

INTRODUCTION

Due to the favourable physico-chemical characteristics of cyclodextrin inclusion complexes of bioactive compounds, their rapidly increasing application is expected in human therapy[1]. β-Cyclodextrin forms inclusion complexes with aromatic amino acids[1–5]; it was therefore of interest to study the formation of inclusion complexes with bioactive peptides, *e.g.*, polymyxine.

The biological activity of a compound depends strongly on its lipophilicity and on its adsorption energy on different surfaces because the first contact between the compound and the target organism is generally of adsorptive character, however its penetration through membranes is governed chiefly by the molecular lipophilicity. As the environmental salt concentration and the charge and radii of cations can modify the effect of polymyxine[6,7], we also examined the influence of some salts on the inclusion complex formation of polymyxine.

MATERIALS AND METHODS

The determination of lipophilicity was carried out by reversed-phase thin-layer chromatography (RP-TLC)[8,9] on Kieselgel G plates impregnated with 5% paraffin oil in *n*-hexane. The impregnation was carried out in a sandwich TLC chamber: 30 cm^3 of solvent were poured into the chamber, the Kieselgel G plates were inserted

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and left overnight. Then the plates were taken out and the *n*-hexane was evaporated at room temperature. The samples were always spotted on the lower part of a plate. It is to be stressed that impregnated plates should be used as soon as possible because the thin layer of paraffin oil is liable to decomposition.

The adsorption strength (adsorptivity) was measured on non-impregnated plates because recent observations had indicated that the results obtained on such plates showed a better correlation with biological activity than those determined on impregnated ones[10]. In some cases the adsorption of polymyxine was studied on cellulose layers. The standard deviation of R_F values measured on different plates is significantly higher than that of values measured on the same plate, therefore the determination of R_F value differences was carried out by dividing one plate into identical parts[11].

Polymyxine was from Pfizer (New York, NY, U.S.A.). A volume of 1 μl of a solution of 5 mg polymyxine per cm^3 distilled water was spotted on the plates. All determinations were run in quadruplicate. Because of the fairly low solubility of the β-cyclodextrin monomer in the eluents, its water-soluble polymer (weight-average molecular weight 5300) was prepared by cross-linking with epichlorohydrin[12]. This polymer was soluble even in the eluent water–ethanol (2:3). Ethanol was chosen as the organic solvent miscible with water because it does not form stable inclusion complexes with β-cyclodextrin[13] and thus does not modify the character of the interaction between β-cyclodextrin polymer and polymyxine. The eluents applied are listed in Table I.

TABLE I

ELUENT SYSTEMS EMPLOYED

Layer	*Ethanol (%)*	*Salt concentration (N)*	*β-Cyclodextrin polymer concentration (mg/cm^3)*
Reversed phase	20–60	0.025–0.20	0–10
Silica gel	20–80	0.025–0.20	0–25

After development the plates were dried at 105°C, and the polymyxine was detected by ninhydrin reagent[14]. According to our observations, higher quantities of the β-cyclodextrin polymer decrease the sensitivity of the otherwise very sensitive chlorotoluidine reaction (plates were placed in a gaseous chlorine atmosphere for 20 min, the chlorine adsorbed on the background was removed by aeration and the plates were sprayed with *o*-toluidine solution[14]), increasing the detection limit of polymyxine and lowering the accuracy of the determination of R_F value by video-densitometry. This can easily be explained by the fact that gaseous chlorine adsorbs more strongly on the β-cyclodextrin polymer surface than on silica gel and on silica gel impregnated with paraffin oil. When the chlorine evaporates from the polymer surface the polymyxine also loses a considerable part of the adsorbed chlorine.

To avoid the uncertainty of visual evaluation, the exact R_F values were determined by a video-densitometer (telechrom OE 976; Chinoin, Budapest, Hungary).

RESULTS AND DISCUSSION

Lipophilicity

Some characteristic R_F values measured are listed in Table II. From the data it is obvious that at constant organic solvent ratio and salt nature the interaction between β-cyclodextrin polymer and polymyxine becomes weaker with increasing salt concentration. Probably the ions inhibit entry of the polymyxine into the cavity of the β-cyclodextrin polymer (Table II, lines 1–4). At constant organic solvent ratio and salt concentration the polymyxine–β-cyclodextrin interaction weakens also with increasing ionic radius (Table II, lines 3–6).

TABLE II

R_F VALUES OF POLYMYXINE IN VARIOUS ELUENT SYSTEMS

Line no.	*Ethanol (%)*	*Cation*		*β-Cyclodextrin polymer concentration (mg/cm³)*	R_F	ΔR_F
		Type	*Normality*			
Reversed phase						
1	50	K^+	0.10	0	0.05[c]	0.17
2	50	K^+	0.10	10	0.22	
3	50	K^+	0.20	0	0.47[c]	0.03
4	50	K^+	0.20	10	0.50	
5	50	Li^+	0.20	0	0.13[c]	0.12
6	50	Li^+	0.20	10	0.25	
Silica gel						
7	50	Mg^{2+}	0.05	0	0.28[c]	0.12
8	50	Mg^{2+}	0.05	10	0.40	
9	50	Mg^{2+}	0.10	0	0.38[c]	0.05
10	50	Mg^{2+}	0.10	10	0.43	
11	50	Li^+	0.10	0	0.07[c]	0.05
12	50	Li^+	0.10	10	0.12	
13	50	K^+	0.10	0	0.58[c]	0.02
14	50	K^+	0.10	10	0.60	
15	20	Na^+	0.15	0	0.29[c]	0.16
16	20	Na^+	0.15	10	0.47	
17	60	Na^+	0.15	0	0.64[c]	0.03
18	60	Na^+	0.15	10	0.67	

From the foregoing it might have been concluded that the extent of inclusion complex formation can be affected also by a change in the dielectric constant, *i.e.*, organic solvent concentration, of the eluent. Our results did not support this hypothesis. It is supposed that a change in the organic solvent ratio can modify the solvation shell not only around the ions but also around the interacting host and guest molecules, and that these effects are counterbalanced within the standard deviation of the method.

Adsorptivity

The effect of β-cyclodextrin polymer on the adsorption of polymyxine is illustrated in Fig. 1. It is that the effect is of saturation character and can therefore be described by logarithmic functions.

At constant salt nature and organic solvent ratio, increasing salt concentration reduces the stability of the inclusion complex (Table II, lines 7–10). This observation was somewhat unexpected because the ions present in the eluent are bound to the

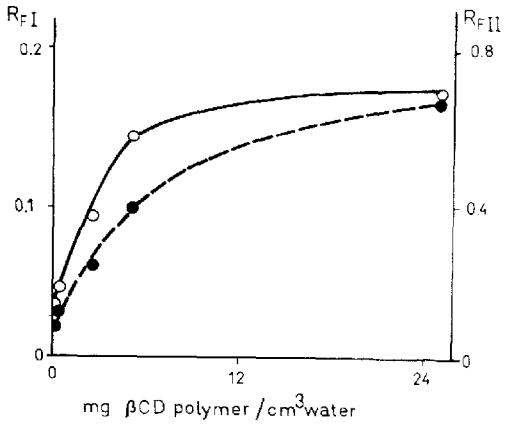

Fig. 1. Effect of β-cyclodextrin polymer on the R_F of polymyxine. R_{FI} obtained on Polygram Sil G (Macherey, Nagel & Co.) plates with water as eluent; R_{FII} obtained on cellulose (Merck 5552) plates with water as eluent.

available silanol groups of the silica gel surface thus reducing its adsorption capacity. Consequently the competitive equilibrium between the dissolved β-cyclodextrin polymer and the silanol groups of the solid phase should be shifted in favour of the complexation of polymyxine by the polymer. Our data however strongly contradict this supposition. The effect of ions on the stability of inclusion complex may be higher than that which follows from our data because we could measure only the resultant of two effects: the diminution of the adsorption strength of the silica gel and the decrease of the stability of the inclusion complex. At constant salt and organic solvent concentration the ions with greater ion radii result in greater reduction in stability of the inclusion complex (Table II, lines 11–14).

Although lipophilicity studies gave no proof of the effect of organic solvent ratio on the stability of the polymyxine–β-cyclodextrin polymer inclusion complex, adsorptivity studies clearly show that an increasing organic solvent ratio (lower dielectric constant of the medium) reduces the stability of the complex at constant ion nature, ion and β-cyclodextrin polymer concentrations (table II, lines 15–18).

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