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Inclusion complex formation of antisense nucleotides with hydroxypropyl-β-cyclodextrin

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Abstract

The interaction between 26 nucleoside derivatives and hyroxypropyl-β-cyclodextrin (HPBCD) was determined by charge-transfer chromatography. HPBCD interacted with each nucleoside decreasing the lipophilicity of the guest molecules. Both sterical (specific hydrophobic surface area) and hydrophobicity parameters of nucleosides influenced the strength of interaction. Substituents with longer alkyl chain considerably increased the strength af interaction whereas the effect of double or triple bond in the chain was negligible.

Keywords: Antisense nucleosides; Hydroxypropyl-β-cyclodextrin; Hydrophobic interaction

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that are well known for their ability to form inclusion complexes with a wide variety of guest molecules (Szejtli, 1988). As sparingly soluble guest molecules drugs can be encapsulated in the hydrophobic cavity of CDs and the hydrophilic outer sphere of the inclusion complex turns towards the water, CDs are good solubilizers for drugs. Complex formation with CDs may improve the bioavailability of drugs: the adsorption promoting effect was proved in oral, as well as in sublingual and percutaneous administration (Uekama et al., 1985; Pitha et al., 1986; Szemán et al., 1987). The properties of CDs can be improved by certain chemical modifiations, modified CDs such as hydroxypropyl derivatives are more soluble in water than the unmodified CDs and their complexes do not precipitate, either. In recent years hydroxypropyl-β-cyclodextrin (HPBCD) derivatives with various average number of substitutions have been extensively applied in pharmaceutical formulations (Loftsson et al., 1991). Erbulozole (Distelmans et al., 1991), the steroid

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anesthetic, alfaxalone for intravenous dosing (Estes et al., 1990), sulbutanol (Marques et al., 1994) naproxen (Blanca et al., 1991), non-steroidal antirheumatics (Backensfeld et al., 1991), methyltestosterone (Müller and Albers, 1991), nitrosothiol (Bauer and Fung, 1991), pancratistatin (Torres-Labanderia et al., 1990), ketoprofen (Orienti et al., 1991), dihydropyridine derivatives (Müller and Albers, 1992), itraconazole and saperconazole (Hostetler et al., 1992) have been successfully complexed with various HPBCD derivatives.

Synthetic nucleosides may have many biological effects (De Clercq, 1980); they can incorporate into DNA (Ötvös et al., 1987) resulting in modification of some enzymatic processes (Sági et al., 1977). According to our knowledge, their interaction with CDs has never been studied in detail.

Charge-transfer chromatography has been frequently applied to study the interaction of CDs and CD polymers with a vide variety of bioactive compounds such as barbiturates (Cserháti et al., 1986; Cserháti et al., 1989) and chlorophenol derivatives (Cserháti et al., 1988; Cserháti et al., 1990).

As the more frequent applications of CDs in the pharmaceutical formulations are expected in the future, studies of the interaction of CDs with drugs is of practical and theoretical importance. The objectives of our study were to determine the relative strength of interaction between some antisense nucleotides and HPBCD and to find relationship between molecular structure and inclusion complex stability.

2. Materials and methods

DC-Fertigplatten Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) plates were impregnated with paraffin oil as described earlier (Cserháti et al., 1983). The chemical structures of nucleosides are listed in Table 1. They were synthesized by the research groups of Dr. Gy. Sàgí at the Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary. The nucleoside derivatives were dissolved separately in methanol to give a concentration of 5 mg/cm^3, and 2 mm^3 of solution was spotted onto the plates. As the aim was to study the complex formation between the nucleosides and hydroxypropyl-β-cyclodextrin (average degree of substitution 2.7) and not to study the effect of HPBCD on the separation of nucleosides, the nucleosides were spotted separately on the plates in each instance; in this manner the HPBCD/nucleoside ratio was always identical for each nucleoside derivative. This experimental design excluded the competition between the various nucleosides for the cavitites of HPBCD and their possible interaction with each other, which may influence the complex formation.

Methanol was chosen as the organic solvent miscible with water because it forms only weak complexes with β-CDs (Buvári et al., 1983–1984; Harada and Takahashi, 1984). Methanol was incorporated in the eluent in the concentration range 0–70 vol.% in steps of 5%. HPBCD concentration in the eluent varied between 0 and 12.5 mM in steps of 2.5 mM. After development the plates were dried at 105°C and the nucleoside spots were detected under UV light. For each experiment five replicate determinations were carried out.

To separate the effect of methanol and HPBCD concentrations on the lipophilicity of nuleosides, the following equation was fitted to the experimental data:

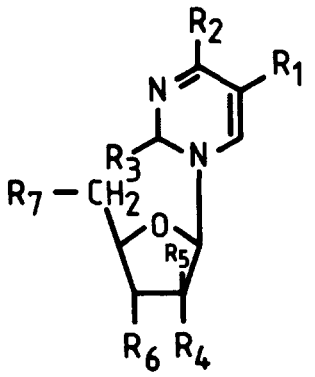
$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 \quad (1)$$

where R_M = actual R_M value of a nucleoside at given methanol and HPBCD concentrations; $R_{M0} = R_M$ value of a nucleoside extrapolated to zero methanol and HPBCD concentrations (related to molecular lipophilicity); b_1 = decrease in the R_M value caused by a 1% increase in the methanol concentration in the eluent (related to the specific hydrophobic surface area of compounds); b_2 = decrease in the R_M value caused by 1 mM increase in the concentration of HPBCD in the eluent (related to the strength of nucleoside-HPBCD interaction); C_1 and C_2 = methanol and HPBCD concentrations, respectively. Eq. (1) was applied separately for each compound.

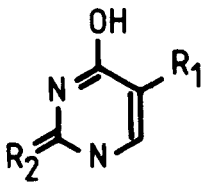
To elucidate the influence of steric and lipophilic parameters on the strength of the nu-

Table 1
Chemical structure of nucleosides

No. of compound



A



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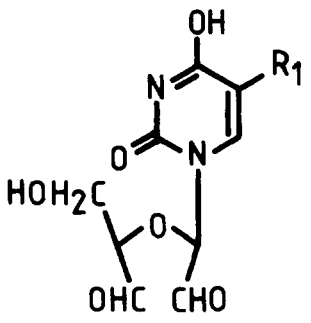

C

	General structure	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	A	C_6H_{13}	OH	O			OH	OH
2	A	C_7H_{15}	OH	O			OH	OH
3	A	C_8H_{17}	OH	O			OH	OH
4	A	$C_{10}H_{21}$	OH	O			OH	OH
5	A	$CH=CH\text{-}C_3H_7$	OH	O			OH	OH
6	A	$CH=CH\text{-}C_4H_9$	OH	O			OH	OH
7	A	$CH=CH\text{-}C_5H_{11}$	OH	O			OH	OH
8	A	$CH=CH\text{-}C_6H_{13}$	OH	O			OH	OH
9	A	$C\ C\text{-}C_4H_9$	OH	O			OH	OH
10	A	$C\ C\text{-}C_5H_{11}$	OH	O			OH	OH
11	A	$C\ C\text{-}C_6H_{13}$	OH	O			OH	OH
12	A	C_6H_{13}	OH	$R_3\text{-O-}R_4$			CH_3COO	CH_3COO
13	A	C_6H_{13}	OH	O Cl			OH	OH
14	A	C_6H_{13}	OH	O			CH_3COO	CH_3COO
15	A	C_6H_{13}	OH	O			C_2H_5COO	C_2H_5COO
16	A	C_6H_{13}	NH_2	O			OH	OH
17	A	C_6H_{13}	OH	O Cl			CH_3COO	OH
18	A	C_6H_{13}	OH	O			$C_5H_{11}COO$	$C_5H_{11}COO$
19	A	C_8H_{17}	OH	O			C_2H_5COO	C_2H_5COO
20	A	C_8H_{17}	OH	Cl			OH	OH
21	A	C_8H_{17}	OH	O	OH		OH	OH
22	A	C_6H_{13}	OH	O		OH	OH	OH
23	B	C_6H_{13}	S					
24	B	C_6H_{13}	O					
25	C	C_6H_{13}						
26	C	C_8H_{17}						

cleoside-HPBCD interaction, stepwise regression analysis was applied (Mager, 1982). The relative strength of interaction (b_2 value of Eq. (1)) was taken as dependent, and the lipophilicity and steric parameters (R_{M0} and b_1 values of Eq. (1) as well as their combination b_1/R_{M0}) as independent

variables. The number of accepted independent variables was not limited and the acceptance limit was set to 95% significance level.

To evaluate the impact of the length of alkyl substitution at position R_1 and that of the presence of double or triple bonds in the alkyl chain, stepwise regression analysis was also applied. Length of alkyl chain, the presence of double and triple bonds in the alkyl chain were the independent, and the b_2 value of Eq. (1) the dependent variable. The other conditions were the same as above.

3. Results and discussion

The simultaneous effects of methanol and HP-BCD concentrations on the R_M values of nucleosides 18 and 26 are shown in Figs. 1 and 2. The R_M value decreases in each instance with increase in methanol concentration, i.e. these compounds do not show any anomalous retention behavior in this concentration range that would invalidate the evaluation using Eq. (1). An increase in HPBCD concentration also caused a decrease in R_M values, indicating the complex (probably inclusion complex) formation. Interaction of the more hydrophilic HPBCD with the nucleosides reduces the lipophilicity of the latter. This finding suggests that the pharmacological

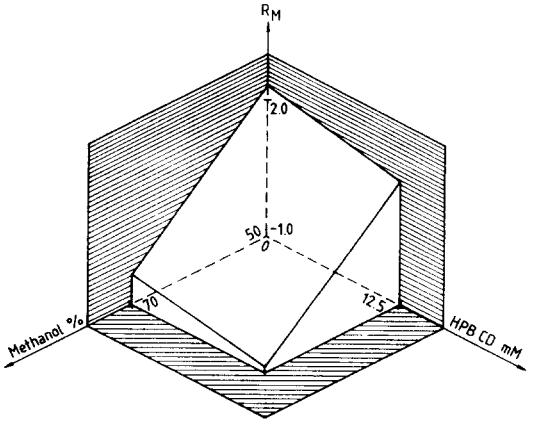

Fig. 1. Effect of methanol and hydroxypropyl-β-cyclodextrin (HPBCD) concentrations on the R_M value of nucleoside 18.

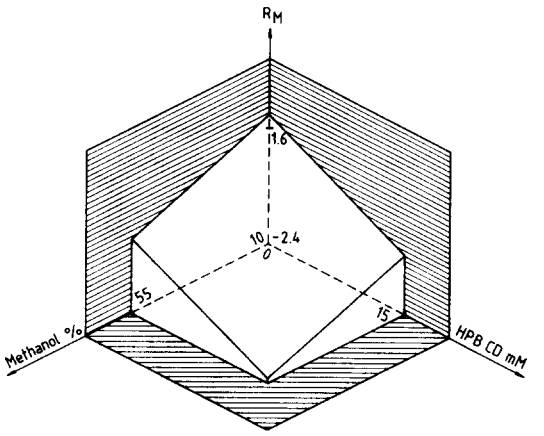

Fig. 2. Effect of methanol and hydroxypropyl-β-cyclodextrin (HPBcD) concentrations on the R_M value of nucleoside 26.

properties (adsorption, uptake, half-life, etc.) of nucleoside-HPBCD complexes may be different from that of uncomplexed nucleosides, resulting in enhanced effectivity. The effect of methanol and HPBCD depends on the type of nucleoside and on the composition of the eluent.

The parameters of Eq. (1) are compiled in Table 2. The equation fits the experimental data well, the significance levels in each instance being over 99.9% (see calculated F values). The ratios of variance explained were about 82–99% (see r^2 values). The parameters of Eq. (1) show high variations between the nucleosides proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity of nucleosides to form inclusion complexes with HPBCD (b_2) differ considerably. This finding suggests also that the inclusion complex formation may influence the biological effect of individual nucleosides differently. The strength of interactions is generally higher than that of chlorophenols (Cserháti et al., 1990). This finding indicates that the heterocyclic ring of nucleosides fits better to the HPBCD cavity than the chlorophenol ones. The path coefficient E; (b, % values) indicates that the HP-BCD concentration has a similar effect on the lipophilicity of nucleosides as the methanol concentration.

Significant correlation was found between the strength of nucleoside-HPBCD interaction and

Table 2
Relationship between the R_M values of nucleosides and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in the eluent, numbers refer to nucleoside derivatives in Table 1

$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$

Parameter	No. of nucleoside							
	1	2	3	4	5	6	7	8
R_{M0}	1.63	2.32	2.45	4.30	1.74	1.67	2.40	1.60
$-b_1 \cdot 10^2$	3.53	4.42	3.96	6.85	5.26	3.68	4.54	4.84
$s_{b1} \cdot 10^3$	2.69	3.23	4.72	3.48	3.10	2.43	3.85	1.73
$-b_2 \cdot 10^3$	7.17	10.51	11.23	11.35	7.78	7.16	10.58	7.30
$s_{b2} \cdot 10^3$	8.29	9.95	14.45	9.17	7.35	7.48	11.87	3.12
b_1%	60.22	56.44	51.94	61.41	61.57	61.26	56.91	54.53
b_2%	39.78	43.56	48.06	38.59	38.43	48.74	43.09	45.47
r^2	0.9347	0.9409	0.8732	0.9653	0.9613	0.9502	0.9214	0.9880
$F_{calc.}$	85.88	95.61	37.89	194.66	186.19	114.5	470.37	411.45
Parameter	No. of nucleoside							
	9	10	11	12	13	14	15	16
R_{M0}	1.60	2.03	2.82	2.67	2.58	3.37	4.21	1.80
$-b_1 \cdot 10^2$	4.84	5.29	6.51	4.95	4.91	5.65	6.73	4.72
$s_{b1} \cdot 10^3$	1.73	4.01	5.23	2.96	3.21	2.82	3.65	1.68
$-b_2 \cdot 10^3$	7.30	8.84	12.31	8.64	8.44	5.86	6.15	6.35
$s_{b2} \cdot 10^3$	3.12	7.25	11.19	8.89	9.70	1.60	9.43	3.04
b_1%	54.53	51.92	48.75	63.26	53.77	72.24	73.88	57.34
b_2%	45.47	48.08	51.25	36.74	36.23	27.76	26.12	42.66
r^2	0.9880	0.9509	0.9329	0.9603	0.9736	0.9645	0.9666	0.9876
$F_{calc.}$	411.45	96.83	69.47	145.13	118.24	204.02	188.26	398.70
Parameter	No. of nucleoside							
	17	18	19	20	21	22	23	
R_{M0}	1.28	9.00	6.77	4.49	2.55	1.30	1.65	
$-b_1 \cdot 10^2$	3.01	13.27	10.47	7.26	4.56	2.98	3.57	
$s_{b1} \cdot 10^3$	1.74	6.73	6.13	2.63	4.50	2.51	4.61	
$-b_2 \cdot 10^3$	6.91	5.01	5.05	5.72	12.92	7.60	9.85	
$s_{b2} \cdot 10^3$	5.27	5.97	7.51	6.98	13.51	7.54	13.84	
b_1%	56.85	73.25	71.73	74.18	51.47	54.05	52.08	
b_2%	43.15	26.75	28.27	25.82	48.53	45.95	47.92	
r^2	0.9593	0.9754	0.9684	0.9826	0.9046	0.9239	0.8440	
$F_{calc.}$	153.20	198.62	168.54	394.83	56.88	72.84	32.46	
Parameter	No. of nucleoside							
	24	25	26					
R_{M0}	1.97	1.51	2.56					
$-b_1 \cdot 10^2$	3.96	3.44	4.30					
$s_{b1} \cdot 10^3$	2.81	2.72	7.49					
$-b_2 \cdot 10^3$	10.11	8.34	16.64					
$s_{b2} \cdot 10^3$	8.43	8.15	22.50					
b_1%	54.05	55.29	43.70					
b_2%	45.95	44.71	56.30					
r^2	0.9451	0.9314	0.8212					
$F_{calc.}$	103.26	81.46	27.55					

the hydrophobicity and steric parameters of nucleosides (Table 3), the significance level being over 99% (see $F_{calc.}$ value). This result indicates that the interaction is of hydrophobic character and also depends on the steric conditions: nucleoside has to be inserted into an HPBCD cavity of given dimensions. This result supports the assumption that nucleosides enter into the lipophilic cyclodextrin cavity, and they are retained by hydrophobic forces. However, these two parameters explain only about 41% of the total variance indicating than other molecular parameters account for the strength of interaction. Unfortunately, other molecular parameters of nucleosides have never been determined, therefore, it was impossible to include them in the calculation.

The presence of a double or that of the triple bond in the alkyl chain of substituents at position R_1 does not exert a significant influence on the strength of nucleoside-HPBCD interaction.

However, the strength of interaction increases linearly with increasing length of the alkyl chain (Fig. 3). This finding emphasizes the considerable contribution of steric parameters to the strength of the interaction. However, in the case of alkyl chains the lipophilicity and the length of alkyl chain is strongly intercorrelated making it impossible to separate the impact of steric and hydrophobic parameters in the inclusion complex formation of nucleosides with HPBCD.

Table 3
Relationship between the strength of nucleoside-hydroxypropyl-β-cyclodextrin interaction (b_2) and the lipophilic parameters (R_{M0} and b_1) of nucleosides. Results of stepwise regression analysis

$b_2 = A + B_1 \cdot R_{M0} + B_2 \cdot b_1 + B_3 \cdot b_1/R_{M0}$			
$A = 0.27$	$r^2 = 0.4121$	$F_{calc.} = 5.37$	
	Independent variables		
	R_{M0}	b_1	b_1/R_{M0}
$B \cdot 10^2$	−6.90	3.95	9.50
$s_B \cdot 10^2$	2.54	1.69	2.94
b, %	48.05	35.43	16.52
Significance level %	98.80	97.25	99.63

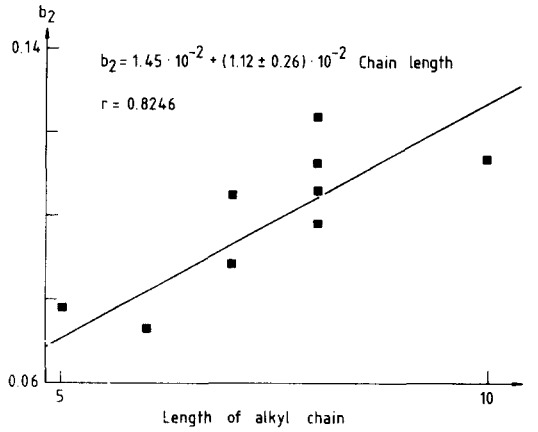

Fig. 3. Relationship between the strength of nucleoside-HPBCD interaction (b_2) and the length of alkyl chain of substituents at position R_1.

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