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Research paper

Quantitative relationship between the chemical structure of antisense nucleosides and their capacity to interact with cyclomalto-octaose

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Abstract

The interaction of twelve 8-substituted-2′-deoxyadenosine and seventeen 5-substituted-2′-deoxyuridine derivatives (antisense nucleosides) with cyclomalto-octaose (GCD) was determined by charge-transfer chromatography and the relative strength of the interaction was calculated. The majority of antisense nucleosides (14 deoxyuridine and 11 deoxyadenosine derivatives) interacted with GCD, which probably led to inclusion complex formation. Stepwise regression analysis proved that the strength of interaction was related to the length of the apolar alkyl chain of substituents and the bulkiness of the nucleoside ring structure. The effect of double or triple bonds in the chain was negligible.

Keywords: Gamma cyclodextrin; Antisense nucleotides; Stepwise regression analysis

1. Introduction

Cyclomalto-oligosaccharides (cyclodextrins, CDs) can form inclusion complexes with a wide variety of inorganic and organic compounds [1]. CD complexation enhanced the stability of peptides in nasal enzymatic systems [2], modified the degradation of cortisone acetate and oestradiol benzoate in aqueous solution [3] and the release of hydrocortisone from ointments [4], improved the delivery of acitrecin through hairless mouse skin [5], enhanced the penetration of hydrocortisone into excised human skin [6], decreased the irritation potential of pilocarpine prodrug [7], enhanced the solubility of sparingly soluble drugs [8], etc. Several methods have been developed and successfully employed for the study of the formation of inclusion complexes such as spectrophotometry [9], thermogravimetry [10], nuclear magnetic resonance [11], calorimetry [12], freezing point depression [13], etc. Various chromatographic methods such as high-performance liquid chromatography [14], free solution capillary electrophoresis [15], gas-liquid chromatography [16] and reversed-phase thin-layer chromatography (RP-TLC) [17] have also been used for the study of the interaction of CDs with drugs.

The character of interactive forces involved in host - guest interactions has been vigorously discussed. The preponderant role of hydrophobic forces [18], the importance of hydrogen bonds [19] and electrostatic interactions [20] have been discussed in detail.

Due to its considerable practical and theoretical importance many compounds were tested as carriers for antisense nucleosides. The use of an amphiphilic peptide [21], cationic lipid particles [22] and cationic polyhexylcyanoacrylate nanoparticles [23] was recently reported. CDs and CD derivatives were also applied as carriers for oligonucleotides [24] and it has been established that their interaction with hydroxypropyl-β-cyclodextrin is governed by the sterical and hydrophobic parameters of nucleotides [25].

The objectives of the study were determination of the interaction of some antisense nucleosides with cyclomalto-octaose (further GCD), and assessment of the relationship between molecular structure and complex forming capacity.

2. Materials and methods

Reversed-phase RP-18W/UV254 plates (Macherey-Nagel, Dürren, Germany) were used for the determination of the relative strength of interaction without pretreatment. Cyclomalto-octaose (GCD) was purchased from CYCLOLAB Research and Development Laboratory (Budapest, Hungary) and was used as received. The IUPAC names for nucleosides are compiled in Table 1. The solutes were

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Table 1
IUPAC Names of Nucleosides

No of compound	IUPAC name
1	2′-Deoxyuridine
2	Thymidine
3	2′-Deoxy-5-ethyluridine
4	2′-Deoxy-5-n-propyluridine
5	2′-Deoxy-5-isopropyluridine
6	2′-Deoxy-5-n-butyluridine
7	2′-Deoxy-5-n-pentyluridine
8	2′-Deoxy-5-n-hexyluridine
9	2′-Deoxy-5-n-heptyluridine
10	2′-Deoxy-5-n-octyluridine
11	2′-Deoxy-5-n-tetradecyluridine
12	2′-Deoxy-5-ethynyluridine
13	2′-Deoxy-5-(1-pentyn-1-yl)-uridine
14	2′-Deoxy-5-(1-hexyn-1-yl)-uridine
15	2′-Deoxy-5-(1-heptyn-1-yl)-uridine
16	2′-Deoxy-5-(1-octyn-1-yl)-uridine
17	2′-Deoxy-5-(1-decy-1-yl)-uridine
18	2′-Deoxyadenosine
19	2′-Deoxy-8-ethyladenosine
20	2′-Deoxy-8-n-propyladenosine
21	2′-Deoxy-8-n-pentyladenosine
22	2′-Deoxy-8-n-heptyladenosine
23	(Z)-2′-Deoxy-8-(propen-1-yl)-adenosine
24	(Z)-2′-Deoxy-8-(1-penten-1-yl)-adenosine
25	(Z)-2′-Deoxy-8-(1-hepten-1-yl)-adenosine
26	2′-Deoxy-8-ethynyladenosine
27	2′-Deoxy-8-(propyn-1-yl)-adenosine
28	2′-Deoxy-8-(1-pentyn-1-yl)-adenosine
29	2′-Deoxy-8-(1-heptyn-1-yl)-adenosine

dissolved in methanol in 5 mg/ml concentration, and 4 μl of the solutions were spotted separately on the plates. As our aim was to study the interaction between nucleosides and GCD and not the elucidation of the effect of GCD on their separation, the nucleosides were separately spotted on the plates. Mobile phases were water: methanol mixtures, with methanol concentration varying between 0–95 vol.%. in steps of 5 vol.%. Employment of such a wide range of methanol concentration was motivated by the highly different lipophilicity of antisense nucleosides. Methanol was chosen as the organic modifier because it forms only weak complexes with CDs [26,27]. The concentration of GCD in the mobile phase varied between 0 and 50 mg/ml in steps of 12.5 mg/ml. Since the biological activity of the complexes occurs in ionic environment, each mobile phase contained NaCl at 0.16 M end concentration. Developments were carried out in sandwich chambers ($22 \times 22 \times 3$ cm) at room temperature, with the distance of development at about 16 cm. After development the plates were dried at 105°C and the spots of solutes were revealed by their UV absorption spectra. Each experiment was run in quadruplicate. The R_M value characterizing molecular hydrophobicity in reversed-phase thin-layer chromatography was calculated for each solute in each eluent

$$R_m = \log(1/R_f - 1) \quad (1)$$

When the coefficient of variation of the parallel determinations was higher than 5% the R_M value was omitted from subsequent calculations. In order to separate the effects of methanol and GCD on the lipophilicity of the nucleosides, we fitted the experimental data to the following equation

$$R_M = R_{M0} + b_1 \times C_1 + B_2 \times C_2 \quad (2)$$

where R_M represents the R_M value of the nucleoside determined at given methanol and GCD concentrations; R_{M0} is the R_M value extrapolated to zero methanol and GCD concentrations; b_1 stands for the decrease in the R_M value caused by 1% increase in the methanol concentration of the eluent (related to the specific hydrophobic surface area of the nucleotides) [28]; b_2 is the decrease in the R_M value caused by a 1 mg/ml concentration change of GCD in the eluent (related to the relative strength of interaction); C_1 and C_2 are the concentrations of methanol and GCD, respectively. Eq.2 was applied separately for each nucleoside. As previously indicated, in the case of homogenous solutes lipophilicity (R_{M0}) and the specific hydrophobic surface area (b_1) are strongly intercorrelated [29], therefore, linear correlation was calculated between these two hydrophobicity parameters. The relationship between the structural characteristics of antisense nucleosides and their capacity to form inclusion complexes with GCD was elucidated by stepwise regression analysis [30]. In traditional multivariate regression analysis the presence of the independent variables that exert no significant influence on the dependent variable lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, we performed stepwise regression analysis, which automatically eliminates the insignificant independent variables from the selected equation and increases thereby the information power of the calculation. The dependent variable was the relative strength of GCD - nucleoside interaction, the independent variables were the type of the heterocyclic ring (variable A), the length of the hydrophobic alkyl substituent (variable B), the presence of double or triple bonds in the substituent (C) and the branching of the alkyl chain (D). The numerical values of structural characteristics used in the calculations are compiled in Table 2. The number of accepted independent variables was not limited, the acceptance level was set to 95% significance.

The software for stepwise regression analysis was purchased from CompuDrug Ltd, Budapest, Hungary.

3. Results and discussion

Compounds 1, 2, 3 and 12 were very close to the eluent front even in aqueous 0.16 M NaCl, indicating that these antisense nucleosides are highly hydrophilic and their interaction with GCD cannot be determined under these experimental conditions. Simultaneous effects of methanol and GCD concentrations on the R_M values of nucleosides 4

Table 2
Numerical values of structural characteristics of antisense nucleosides included in the calculation of the relationship between the complex forming capacity and molecular structure[a]

No. of compounds	A	B	C	D
1	10	0	0	0
2	1	0	1	0
3	1	0	2	0
4	1	0	3	0
5	1	0	3	1
6	1	0	4	0
7	1	0	5	0
8	1	0	6	0
9	1	0	7	0
10	1	0	8	0
11	1	0	13	0
12	1	2	2	0
13	1	2	5	0
14	1	2	6	0
15	1	2	7	0
16	1	2	8	0
17	1	2	10	0
18	0	0	0	0
19	0	0	2	0
20	0	0	3	0
21	0	0	5	0
22	0	0	6	0
23	0	1	5	0
24	0	1	7	0
25	0	1	3	0
26	0	2	2	0
27	0	2	3	0
28	0	2	5	0
29	0	2	7	0

[a] A = Presence (1) or absence (0) of uridine base; B = saturated alkyl chain (0), double bond in the alkyl chain (1), triple bond in the alkyl chain (2); C = number of carbon atoms in the alkyl chain; D = number of branching in the alkyl chain.

and 17 are shown in Fig. 1. The R_M values of each compound decreased with the increasing concentration of methanol in the mobile phase, i.e. nucleosides do not show any anomalous retention behaviour, which would invalidate the evaluation using Eq. (2). An increase in GCD concentration also caused a decrease in the R_M values, which points to complex (probably inclusion complex) formation. Interaction between the more hydrophilic GCD and antisense nucleosides reduces the lipophilicity of the latter. The parameters of Eq. (2) are compiled in Table 3. There is good fitting between the parameters of the equation and the experimental data, with significance levels over 99.9% in each case (see calculated F values). The ratios of variance explained varied between 82.61 and 97.21% (see r^2 values). The parameters in Table 3 show marked variations verifying the great differences in lipophilicity, specific hydrophobic surface areas and the complex-forming capacity of nucleosides. The path coefficients (b'% values) indicate that the impact of changes in methanol and GCD concentrations exerts a similar effect on the mobility of nucleosides under reversed-phase chromatographic conditions, which means that the retention can be equally modified by changing either the methanol or the GCD concentration in the mobile phase.

Significant linear relationship was found between the lipophilicity (R_{M0}) and specific hydrophobic surface area (b_1) of antisense nucleosides (Fig. 2). This finding indicates that from the aspect of chromatography these compounds behave like a homologous series of solutes, in spite of their different chemical structures.

Stepwise regression analysis has proven that the capacity of antisense nucleosides to interact with GCD (b_2) depends significantly on the type of the heterocyclic ring (variable A) and on the length of the hydrophobic alkyl chain (variable B), with the significance level over 99.9%

$$b_2 = 0.38 - (0.24 \pm 0.10) \times A + (0.12 \pm 0.02) \times B \qquad (3)$$

where $r^2 = 0.5996; F = 18.72; b'_A\% = 28.30; b'_B\% = 71.70$.

This result can be tentatively explained by the presump-

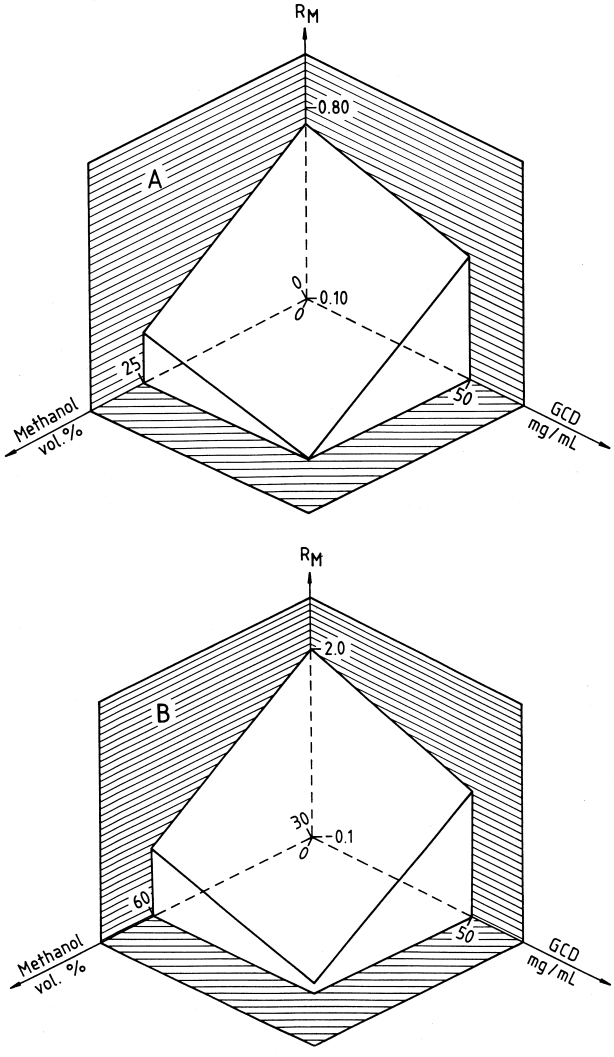

Fig. 1. Effect of methanol and cyclomalto-octaose concentrations on the R_M value of nucleosides 4 (A) and 17 (B).

Table 3
Parameters of linear correlations between the RM values of anti-sense nucleosides and the concentrations of methanol (C1) and cyclomalto-octaose (C2) in the eluent[a]

Parameter	No. of antisense nucleosides																								
	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
R_{M0}	0.74	0.73	1.02	1.36	1.75	2.19	2.62	5.15	1.22	1.46	1.86	2.15	3.34	0.93	1.65	1.79	2.07	3.13	2.06	2.98	1.71	1.48	1.55	2.20	3.03
$-b1.10^2$	1.83	1.94	1.81	2.09	2.08	2.23	3.50	5.90	2.42	2.29	2.35	2.15	4.49	2.38	3.13	2.91	2.17	4.08	2.22	3.86	2.91	2.92	2.34	2.81	4.11
$sb1.10^3$	2.30	2.23	1.78	1.69	1.64	2.80	2.68	3.51	2.21	1.59	1.92	2.70	2.63	2.23	2.13	1.76	3.22	2.34	2.96	3.27	2.39	2.05	2.13	2.93	2.06
$-b2.10^2$	0.36	0.32	0.82	1.00	1.03	1.18	1.03	0.75	1.00	1.00	1.17	1.19	1.32	0.48	0.97	0.91	0.89	0.70	0.77	1.13	1.04	0.98	0.99	0.66	0.75
$sb2.10^3$	0.13	0.12	0.10	0.09	0.09	1.58	3.80	0.24	1.21	8.70	1.05	1.52	3.09	1.22	1.17	0.10	0.18	0.26	0.17	0.45	1.31	0.11	0.12	0.15	0.24
b_{r1}%	73.41	76.88	54.66	53.32	52.51	51.44	82.86	84.41	57.02	55.52	52.32	50.51	80.03	73.12	63.90	63.43	57.91	86.55	61.86	82.35	60.51	62.06	56.43	68.48	86.19
b_{r2}%	26.59	23.12	45.34	46.68	47.49	48.56	17.14	15.59	42.98	44.48	47.68	49.49	19.97	26.88	36.10	36.57	42.09	13.45	38.14	17.65	39.49	37.94	43.57	31.52	13.81
r_2	0.8261	0.8438	0.9210	0.9476	0.9511	0.8811	0.9086	0.9596	0.9261	0.9579	0.9478	0.8850	0.9639	0.8962	0.9500	0.9593	0.8134	0.9721	0.8322	0.9177	0.9340	0.9491	0.9279	0.8886	0.9710
$F_{calc.}$	35.62	40.53	87.43	135.55	145.99	48.15	114.30	178.29	94.03	170.48	136.18	50.01	200.55	64.73	124.39	165.15	28.34	208.86	32.24	85.12	106.14	139.97	96.59	47.85	250.94

[a] Numbers refer to antisense nucleosides in Table 1 ($R_M = R_{M0} + b_1 \times C_1 + b_2 \times C_2$).

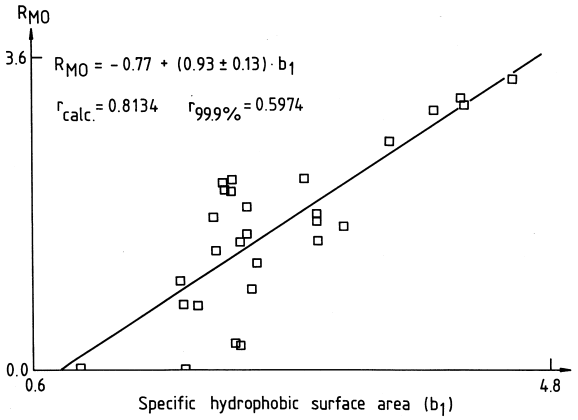

Fig. 2. Relationship between the lipophilicity (R_{M0}) and specific hydrophobic surface area (b_1) of antisense nucleosides.

tion that the bulky adenosine structure fits into the large cavity of GCD better than the uridine molecule. This finding also underlines the important role of sterical compatibility in nucleoside–GCD interaction. Eq. (3) further indicates that the apolar alkyl substituents are involved in the hydrophobic interaction between the apolar inner wall of the GCD cavity and nucleotide molecules. The presence of double or triple bonds in the alkyl chain of substituents and the branching of the alkyl chain did not have a significant influence on the capacity of antisense nucleosides in forming complexes with GCD. It can be assumed that branching alkyl chain especially in the case of longer alkyl chains may lead to the modification of the steric hindrance. Unfortunately, the set of antisense nucleosides involved in the investigations contained only one branched alkyl chain, therefore, the effect of branching on the formation of inclusion complexes could not be established.

It may be concluded from the data above that nucleosides readily form complexes with GCD. Stepwise regression analysis indicated that the length of the apolar alkyl chain and the sterical compatibility of the nucleoside ring structure are related to the strength of the inclusion complexes. Complex formation may modify the physicochemical parameters of the guest nucleoside molecule, which results in increased biological efficiency.

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