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Note

Quantitative structure–retention relationships for dinucleoside monophosphates as a function of pH

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Quantitative structure–retention relationships (QSRRs) serve to estimate retention from chemical structure and may aid eluite identification on the chromatogram in high-performance liquid chromatography (HPLC). Recently we formulated QSRR for oligonucleotides in reversed-phase chromatography with fixed mobile phase composition[1]. In the present study, the scope of QSRR is extended to take into account the effect of mobile phase pH on the retention of dinucleoside monophosphates in reversed-phase chromatography.

EXPERIMENTAL

The retention data of El Rassi and Horváth[2] were statistically analyzed by using an approach similar to that described previously[1,3]. Experimental data were obtained[2] with an octadecyl-silica column and the mobile phase was 0.1 *M* sodium acetate or 0.1 *M* sodium phosphate buffer containing 5% (v/v) acetonitrile. The ionic strength of the eluent was maintained at 0.34 by addition of sodium sulfate. The stationary phase was prepared from 5-μm Spherisorb by the method of Kováts and Boksányi[4] and had a carbon load of 14% (w/w). Experiments were carried out at pH 2.12, 3.0, 4.0, 5.0, 5.5, 6.5 and 7.0 with the sixteen diribonucleoside-3′,5′-monophosphates listed in Fig. 1.

RESULTS AND DISCUSSION

In order to express the logarithmic retention factor, κ, of dinucleoside monophosphates, the molecules were first decomposed into a mononucleotide and mononucleoside residue[1]. The pH dependence of the appropriate retention increment of the residues, τ, was expressed as a polynomial function. It was found that for any of the residues in the pH range investigated the retention increment as a function of pH could satisfactorily be represented by the quadratic polynomial as

$$\tau_j(\mathrm{pH}) = v_{j,0} + v_{j,1}[\mathrm{pH}] + v_{j,2}[\mathrm{pH}]^2 \quad (1)$$

where $v_{j,0}$, $v_{j,1}$ and $v_{j,2}$ are the regression parameters for residue j. As usual[1,3], the

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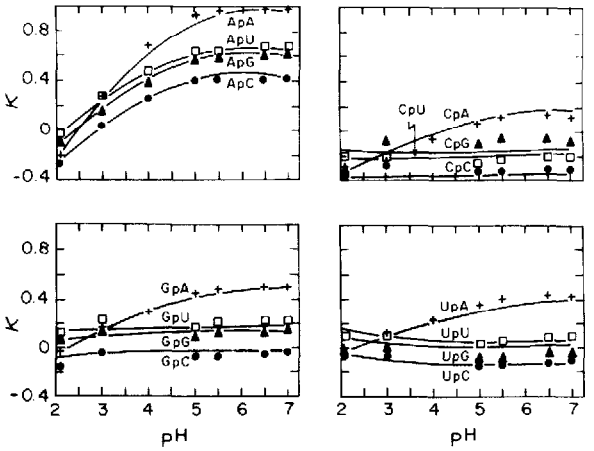

Fig. 1. Plots of the logarithmic retention factors for dinucleoside monophosphates against the pH of the mobile phase. Eluent: 0.1 *M* sodium phosphate or acetate buffer containing 5% (v/v) acetonitrile. Experimental data points and the results of QSRR analysis are shown by symbols and curves, respectively. The dinucleoside monophosphates investigated are: ApA = adenylyl-(3′-5′)-adenosine; ApC = adenylyl-(3′-5′)-cytidine; ApG = adenylyl-(3′-5′)-guanosine; ApU = adenylyl-(3′-5′)-uridine; CpA = cytidylyl-(3′-5′)-adenosine; CpC = cytidylyl-(3′-5′)-cytidine; CpG = cytidylyl-(3′-5′)-guanosine; CpU = cytidylyl-(3′-5′)-uridine; GpA = guanylyl-(3′-5′)-adenosine; GpC, guanylyl-(3′-5′)-cytidine; GpG = guanylyl-(3′-5′)-guanosine; GpU = guanylyl-(3′-5′)-uridine; UpA = uridylyl-(3′-5′)-adenosine: UpC = uridylyl-(3′-5′)-cytidine; UpG = uridylyl-(3′-5′)-guanosine; and UpU = uridylyl-(3′-5′)-uridine.

logarithmic retention factor of dinucleoside monophosphate i is obtained as the sum of the retention increments of all residues. Thus,

$$\kappa_i = \sum_{j=1}^{m} I_j \tau_j(\mathrm{pH}) \tag{2}$$

where m is the number of possible residues in the molecule and I_j is the indicator variable that is unity when residue j is present in the molecules and zero when it is absent. As seen in Table I, there are eight residues in the dinucleoside monophosphates investigated (see Fig. 1). For each eluent pH, the experimental κ_i values of all the dinucleoside monophosphates were analyzed by a combination of eqns. 1 and 2 in order to determine the values of $v_{j,0}$, $v_{j,1}$ and $v_{j,2}$ and the results are listed in Table I.

TABLE I

PARAMETERS OF EQN. 1 FOR pH DEPENDENCE OF THE RETENTION INCREMENT OF VARIOUS RESIDUES IN THE DINUCLEOSIDE MONOPHOSPHATE MOLECULES

Residue	v_0	v_1	v_2
A	−0.926	0.513	−0.041
C	−0.103	−0.037	0.005
G	0.122	0.009	−0.001
U	0.353	−0.120	0.012
pA	−0.762	0.331	−0.025
pC	−0.284	0.039	−0.004
pG	−0.136	0.037	−0.003
pU	0.000	0.000	0.000

The pertinent statistical measures are as follows:

Sample size	103
Number of parameters	24
R^2	0.9781
F value	174
Significance probability	0.0001

Fig. 1 shows the experimental data points obtained for the dependence of the logarithmic retention factor of the dinucleoside monophosphates on the pH of the eluent as well as curves representing the predicted values. It is seen that the conformation between the results of QSRR analysis and experiment is satisfactory.

ACKNOWLEDGEMENTS

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