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Note

"Charge transfer" thin-layer chromatography of nucleic acid bases and polycyclic aromatic hydrocarbons

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The use of weak interactions between different classes of molecules has been used by several authors to obtain better separation of similar compounds during chromatography[1–4]. One class of interaction which has been studied is the so called charge transfer interaction[5–7]. Although this form of interaction is somewhat controversial, some authors holding the view that they do not exist, nevertheless, there seems good evidence from thin-layer chromatography (TLC) and column chromatographic studies that these interactions exist and have useful consequences in chromatography.

In a previous study[4] we have looked at the interaction of various biochemical electron donors with moderate to strong electron acceptors. In this paper we present the results of a TLC study of the interaction of nucleic acid bases with polycyclic aromatic hydrocarbon. In addition we have studied the effect of masking of silica gel by the bases. The polycyclic aromatic hydrocarbons are of interest on several counts. They form coloured charge transfer complexes acting as electron donors[5]. There is no reason why they should not act as weak electron acceptors as has been suggested by Rosenthal[8]. Analogues of some of the hydrocarbons studied herein are highly carcinogenic and the whole class of compounds have been the object of much research. Finally these compounds are strongly fluorescent which makes their use in chromatography highly attractive as they can be detected quite simply under UV light.

EXPERIMENTAL

Chemicals were obtained from a variety of commercial sources and were the purest available. Silica gel was Kieselgel G from E. Merck. All the plates were 20 × 20 cm glass squares cleaned before use by immersion in a non-ionic surfactant and then well rinsed in distilled water before drying.

A slurry was made up of 40 g of silica gel mixed with 92 ml of distilled water and 8 ml of methanol to which the nucleic acid base was added. The concentrations of the bases used are indicated in the tables. The slurry was spread onto the plates using a Shandon Uniplan apparatus to a thickness of 2 mm.

The polycyclic aromatic hydrocarbons were made up to 0.1 *M* in chloroform and 2 μl were spotted on the plates using a micropipette. The hydrocarbons were observed on the plates with a UV lamp (254 nm).

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TABLE I

R_F VALUES OF POLYCYCLIC AROMATIC HYDROCARBONS FROM TLC IMPREGNATED PLATES

Solvent: chloroform–heptane (1:99). All results are the mean of 6 determinations.

Impregnant	*Pyrene*	*Phenanthrene*	*Anthracene*	*Naphthalene*
–	76	77	79	81
Adenine	71	68	69	69
Adenosine	74	72	74	76
AMP	61	65	58	52
cAMP	56	65	56	58
ADP	50	50	51	43
ATP	41	43	43	41
Cytosine	51	55	54	55
Thymine	85	89	87	89
Uracil	80	81	81	84
Uric acid	49	50	50	52
Hypoxanthine	55	60	58	58
Guanine	64	69	67	70

RESULTS AND DISCUSSION

In Table I are presented the R_F values for the TLC of four polycyclic aromatic hydrocarbons with a selection of purines and pyrimidines using chloroform–heptane (1:99) as solvent. The corresponding B values, a measure of interaction defined by Harvey and Halonen[1] as

$$B = \frac{R_F - R'_F}{R_F} \times 100$$

where R_F is the value on plain silica gel and R'_F is the value in the presence of impregnant, are presented in Table II.

There is a very clear correlation between the strength of interaction and number of phosphate groups in the adenosine nucleotides which echoes a similar correlation in the interaction with the electron acceptor riboflavin[4]. This indicates that the interaction is between the phosphate group and the hydrocarbon rather than a charge transfer interaction.

A study has also been made of the quenching of pyrene fluorescence by the adenosine nucleotides. Stern-Volmer plots are shown in Fig. 1 and the quenching coefficient derived therefrom are shown in Table III. The correlation is different from that occurring in TLC but again echoes the results with riboflavin[4]. Thus it is demonstrated that the forces observed in charge transfer TLC are not necessarily those observed using other techniques.

There is no obvious correlation between either the ionization potentials or electron affinities of the hydrocarbons and the B values. There is however a very definite order of interaction for the base namely uric acid > cytosine hypoxanthine > guanine > adenine > uracil > thymine. Of these compounds adenine, uric acid, guanine, and hypoxanthine are purines whereas the other are pyrimidines. Experi-

TABLE II

B VALUES DERIVED FROM DATA IN TABLE I

Impregnant	*Pyrene*	*Phenanthrene*	*Anthracene*	*Naphthalene*
Adenine	7	12	13	15
Adenosine	3	6	6	6
AMP	20	16	27	36
cAMP	26	16	29	28
ADP	34	35	35	47
ATP	46	44	46	49
Cytosine	33	29	32	32
Thymine	−12	−16	−10	−10
Uracil	− 5	− 5	− 3	− 4
Uric acid	36	35	37	36
Hypoxanthine	28	22	26	28
Guanine	16	11	15	13

mentally determined ionization potentials and electron affinities of these compounds are unknown although there are theoretical estimates of some of these molecules based on molecular orbital theory[9]. Unfortunately these are based on rather simplified premises and are probably not relevant to our problem. It is interesting to note that the order of interaction can be correlated with expected electron acceptor ability of the purines. Thus uric acid with its three carbonyl groups would be expected to be the strongest electron acceptor of this group. Cytosine with its one electron withdrawing carbonyl group would be expected to be the second best acceptor. Adenine has no electron withdrawing group and has the very strong electron donating amino group. This would be expected to be the weakest of the electron acceptors. Whilst one would expect guanine and hypoxanthine to lie somewhere in between, their order of interaction is more difficult to assess. Although guanine has the strong electron withdrawing carbonyl group, it has the very strong electron donating amino group. Conversely whilst hypoxanthine has no electron withdrawing group, it only has the weaker donating hydroxyl group. There does therefore seem to be a very good correlation between interaction strength and the electron accepting ability of the purines. This is quite contrary to the behaviour of the purines with riboflavin[4] and strongly points to the aromatic hydrocarbons acting as electron donors in this situation, as do of course in conventional charge transfer complexes[5,6].

In the case of the pyrimidines, the situation is less obvious. All three might be expected to show good acceptor properties although being much smaller molecules than either the purines or the aromatic hydrocarbons, the interaction may effectively

TABLE III

DISSOCIATION COEFFICIENTS OBTAINED FROM THE QUENCHING OF PYRENE BY ADENOSINE AND ITS NUCLEOTIDES

Derived from the Stern-Volmer plots shown in Fig. 1.

	Adenosine	*AMP*	*ADP*	*ATP*
K ($\times 10^3$)	9	18	21.3	8

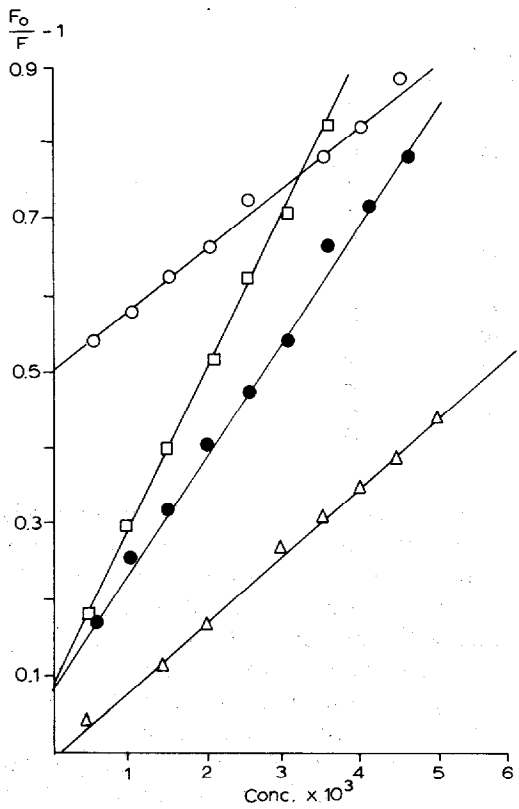

Fig. 1. Stern-Volmer plot for fluorescence quenching of pyrene by adenosine and its nucleotides. Adenosine (△), AMP (●), ADP (□) and ATP (○).

be smaller due to the smaller overlap of molecular orbitals. The negative B values of the two pyrimidines are capable of being interpreted in two different ways. Either these molecules can mask the silica gel on the plate so that the interaction with the stationary phase is lowered and R_F values increased or alternatively as previously suggested by one of us (M.A.S.)[4] the impregnant itself might be moving with the solvent and if there is some binding to the hydrocarbon drags it up the plate with it. In the latter case the more negative the B value the larger the interaction.

The effect of masking has therefore been examined by measuring B values of pyrene with different concentrations of impregnant. The results are shown in Table IV. The trend is quite clear, maximum interaction occurs when the impregnants are in the concentration range 0.125–0.5% (w/w). This finding introduces an element of uncertainty into this topic. Previous workers have tended not to consider the effect of impregnant concentration and masking.

The negative results obtained with thymine and uracil appear to be due to masking rather than their migration with the solvent. The decrease in B values at higher impregnant concentration is surely due to the masking of the active hydroxyl sites on the silica gel. The order of interaction with these sites is thus thymine > uracil > cytosine. The former two compounds with their two carbonyl groups would

TABLE IV

B VALUES FOR PYRENE WITH VARYING CONCENTRATION OF IMPREGNANTS

w/w (%)	*Impregnants*					
	Adenosine	*Adenine*	*Uracil*	*AMP*	*ADP*	*ATP*
0.025	4	8	1	−4	29	62
0.125	7	14	3	3	32	61
0.5	5	4	0	0	36	57
1.0	3	7	−5	20	34	46
1.5	0	9	−1	9	24	45
2	−3	13	−3	13	16	34

be expected to show stronger interaction with hydroxyl groups than cytosine with its single carbonyl group.

In conclusion there appears to be a good indication that these polycyclic aromatic hydrocarbons interact with purines. This interaction correlates very well with the electron accepting ability of the purines and could arise from the formation of charge transfer complexes. The nature of the interaction if any with the pyrimidines is less clear as a major part of the interaction is due to the masking of the silica gel by the pyrimidines.

The interaction of the polycyclic aromatic hydrocarbons with adenosine nucleotides is directly correlated with the number of phosphate groups and this interaction can be presumed to arise from electrostatic forces in addition to any charge transfer forces which may be present.

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