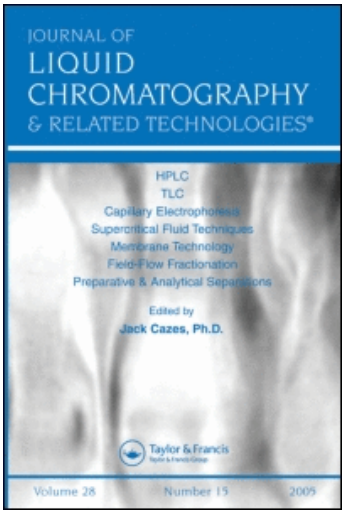

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ADENINE - AMINO ACID INTERACTIONS STUDIED BY CHARGE-TRANSFER CHROMATOGRAPHY

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ABSTRACT

The interactions of adenine with 13 amino acids were studied by charge-transfer chromatography, using distilled water as eluent. The strength of each interaction was calculated from the linear dependence of the lipophilicity of adenine on the amino acid concentration. Stepwise regression analysis proved that the strength of interaction is influenced only by the lipophilicity of the amino acid side-chain and the pK value of the second carboxyl group. This finding indicates that the amino acid side-chain is directed towards the adenine, and the polar head group towards the water. The

character of the interaction can be hydrophobic or hydrophilic, depending on the polarity of the amino acid side-chain.

INTRODUCTION

Nucleotides can bind to various proteins (1), a phenomenon which may result in changed inter- and intramolecular ligation to enzymes (2). The efficiency of the in vitro correction of five: "mismatch analogues" (guanine and adenine analogues) incorporated into M13 mp 9 DNA was earlier studied in an attempt to elucidate the structural determinants required for mismatch recognition by the repair machinery of **E.coli** (3). It has been established that beef heart F_1-ATP-ase has two types of binding sites for adenine nucleotides (4,5), but the character of the interactive forces (hydrophobic or hydrophilic) and the impact of the individual molecular substructures of the nucleotides on the strengths of the interaction have not been studied in detail.

Charge-transfer chromatography is frequently used to study complex formation between various organic molecules of low molecular weight (6,7). The theory and practice relating to the determination of relative complex stabilities by charge-transfer chromatography on reversed-phase thin-layer chromatographic plates was published recently (8). This method has been applied successfully to study the for-

mation of inclusion complexes between cyclodextrin and polymyxin (9), substituted s-triazine (10), triphenylmethane (11) and barbituric acid derivatives (12). Besides the study of inclusion complex formation, the method has been applied to investigate the interactions between non-ionic tensides and dioleoylphosphatidylcholine (13) and between amino acids and some nitrostyrene derivatives (14).

The aims of our study were to determine the relative strength of the interactions between adenine and some amino acids, and to correlate these with the physico-chemical parameters of the amino acids.

We are well aware that our data do not prove that the interactions between adenine and amino acids are due to charge-transfer phenomena, and therefore the expression "charge--transfer chromatography" is perhaps misleading here. We consider charge-transfer chromatography in a general sense, as a method suitable for the detection of any type of weak interaction between two molecular species.

MATERIAL AND METHODS

Adenine and amino acids (alanine, aspartic acid, asparagine, citrulline, glycine, glutamic acid, leucine, iso-leucine, nor-leucine, phenylalanine, serine, tryptophan and valine) were of analytical purity.

Silufol UV_{254} (Kavalier, Czechoslovakia) plates were impregnated with paraffin oil as described in ref.9. Adenine was dissolved in distilled water at a concentration of 0.5 mg/cm^3;

5 mm^3 of this solution was spotted on the plates. Distilled water was used as eluent. Amino acids were dissolved in the eluent in the concentration range 5 - 60 mM. After development, the plates were dried at 105°C and the position of the adenine spot and the amino acid front were detected with the traditional ninhydrin reagent. The chromatograms were evaluated with a Shimadzu CS-930 Dual-Wavelength TLC Scanner at 600 nm since higher concentrations of the amino acids reacted with ninhydrin give a colour too strong to be measured at 470 nm. In each experiment five replicate determinations were carried out.

The R_M values of adenine which characterize its molecular lipophilicity in reversed-phase thin-layer chromatography, were calculated according to eq.1. (15):

$$R_M = \log\left(\frac{1}{R_f} - 1\right) \qquad (1)$$

As the mobilities of the amino acids differed from that of the eluent, the R_M values of adenine were corrected for the different mobilities of the amino acids via the following equation:

$$R_{MC} = R_{MO} - (R_{MO} - R_M)\frac{1}{R_f} \qquad (2)$$

where R_{MC} = R_M value of adenine corrected for amino acid mobility,

R_{MO} = R_M value of adenine determined in amino acid free eluent (distilled water),

R_M = R_M value of adenine determined in eluent containing amino acid,

R_f = R_f value of amino acid front.

Linear correlations were calculated separately for each amino acid, between the lipophilicity (R_{MC}) of adenine and the concentration of amino acid in the eluent:

$$R_{MC} = R_{MO} + b.C \qquad (3)$$

where C = concentration of amino acid in eluent (mM),

b = change caused in adenine lipophilicity by 1mM change in concentration of amino acid in eluent (considered to be related to complex stability).

As mentioned in the introduction, the character of amino acid adenine interactions has not yet been adequately clarified. When the interaction is hydrophobic in nature, the strength of the interaction must depend on the lipophilicity of the amino acid side-chain. In the event of a hydrophilic interaction, the effect must depend on the polarity parameters of the amino acid (the pK values of the alpha-amino, alpha-carboxyl and other polar groups, and the pI value of the amino acid). To elucidate the individual effects of the above physico-chemical parameters on the strength of interaction, stepwise regression analysis (16) was applied to select the independent variables influencing the interaction significantly. The b value in eq.3. (an indicator of the interactive strength) was taken as dependent variable. The polarity parameters (the pK values of the alpha-amino, alpha-

-carboxyl and second carboxyl group, and the pI value taken from ref.17), and the lipophilicity of the amino acid side--chain taken from ref.18, served as independent variables. As the natur of the correlation (linear or quadratic) between the above variables had not been established previously, both first and second-order forms of the five independent variables (overall 10 variables) were included in the stepwise regression analysis. The partial F value of the independent variables was set to F = 1, and the number of accepted variables was not limited. As glycine was ineffective and no lipophilicity value was found for nor-leucine or citrulline, these compounds were omitted from the calculations.

RESULTS AND DISCUSSION

Some densitograms are shown in Fig.1. Adenine can readily be detected with ninhydrin, even in the presence of 60 mM amino acid. The presence of amino acids did not change the peak shape or peak symmetry, i.e. the presumed interaction did not impair the reproducibility of determination of the R_M values. The amino acids cover the plate surface evenly, and their front is steep, which means that in eq.2. the R_f value of the amino acid front can be determined exactly and the R_M values can be corrected accurately.

Except for glycine, each amino acid decreased the lipophilicity of adenine (Fig.2.), which unambiguously proved the existence of an amino acid - adenine interaction. Its inter-

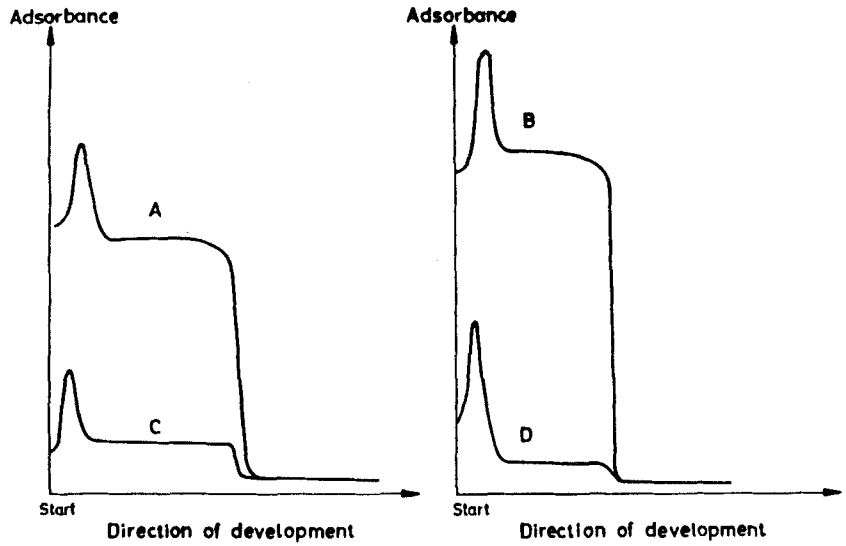

FIGURE 1. Densitogramms of amino acid - adenine interactions.
A = 60mM citrullin B = 60 mM valin
C = 5mM citrullin D = 5mM valin

actions with the more hydrophilic amino acids make adenine less lipophilic. The fact that the R_M values of adenine extrapolated to zero amino acid concentration differ in the various cases needs some explanation. We assume that the fluctuation of secondary experimental conditions (relative humidity of plates before impregnation, temperature of development, etc.) may be responsible for the observed deviations. This finding also indicates that the determination of interactive strength at only one concentration of interacting agent may result in considerable errors. We strongly advocate that only the slope value (b) in eq.3. is an adequate indicator of the strength of interaction, and that the

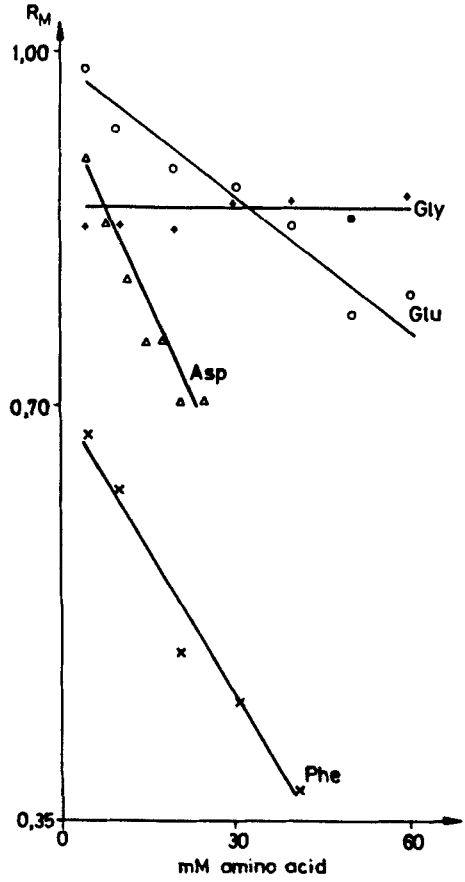

FIGURE 2. Effect of amino acids on the lipophilicity of adenine.

application of individual R_M values to assess interactive strength may lead to inadequate results.

The parameters of the linear correlations between the lipophilicity of adenine and the concentrations of the amino acids (eq.3.) are compiled in Table 1.

Table 1.

Parameters of Linear Correlation between Lipophilicity of Adenine (R_{MC}) and Concentrations of Amino Acids (C).

$$R_{MC} = R_{MO} + b.C$$

n = 8 $r_{99\%} = 0.8343$ $r_{99.9\%} = 0.9249$

Amino acid	$R_{MO}.10^2$	-b	$s_b.10^2$	r
Ala	79	0.19	4.4	0.8665
Asn	100	0.22	2.9	0.9519
Asp	95	1.06	9.3	0.9774
Citr	84	0.18	3.9	0.8810
Glu	95	0.29	6.1	0.8876
iLeu	78	0.22	4.8	0.8791
Leu	103	0.36	6.5	0.9135
nLeu	104	0.29	4.9	0.9250
Phe	69	0.67	9.1	0.9497
Ser	81	0.13	1.7	0.9479
Trp	88	0.97	2.4	0.8601
Val	85	0.17	3.0	0.9214

In all cases the significance level of the linear correlation was over 99 %. This proves the validity and applicability of eq.3. The strengths of the interactions varied considerably, ranging from 1.06 for aspartic acid to 0.13 for serine. However, these values are lower than the strengths of other reported interactions (8,13) and thus the amino acid - adenine interactions are fairly weak ones. Aspartic acid influences the adenine modibility most strongly, but the amino acids with a bulky lipophilic side-chain (Phe and Trp) also display a considerable strength of interaction.

Table 2.

Dependence of Strength of Amino Acid - Adenine Interaction (b) on pK Value (x_1) and Lipophilicity (x_2) of Amino Acid Side-Chain. Results of Stepwise Regression Analysis

$$b = a + b_1.x_1 + b_2.x_2^2$$

$n = 10$	$F_{95\%} = 4.74$	$t_{95\%} = 2.37$
$a = 1.61$	$F_{calc.} = 5.41$	$r^2 = 0.6057$

Independent variables	Parameters			
	b	s_b	b'%	$t_{calc.}$
x_1	-0.23	0.08	52.49	3.00
x_2	0.12	0.04	47.51	2.72

The results of stepwise regression analysis are listed in Table 2.

Only 2 of the 10 independent variables (the pK value and the square of the lipophilicity of the amino acid side-chain) influence the strength of interaction significantly. The path coefficients (b'% values) reveal that their relative impacts on the strength of interaction is very similar. The overall polarities of the amino acids (pI) and the pK values of the alpha-amino and alpha-carboxyl groups do not exert a significant influence on the interaction, which means that the amino acid side-chain is responsible for the observed effect. The results of stepwise regression analysis lend support to the assumption that the amino acid side-chain is directed towards the adenine molecule, and the polar head group towards the water. The side--chain can interact with the adenine molecule in two different ways:

hydrophile-hydrophile interactions between the second carboxyl group of amino acid and the alkaline substructures of adenine (probably hydrogen-bond formation);

hydrophobe-hydrophobe interactions between the apolar side-chain of the amino acid and the corresponding adenine substructures.

The observed effect may be the resultant of these interactions. Our data lead us to suppose that adenine can also interact (though weakly) with the amino acid side-chains in proteins. As the role of the other molecular moieties of nucleotides in nucleotide-protein binding is not known, the biological consequences of our finding cannot be evaluated.

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