Hydrophilic Interactions between Charged Amino Acids and the Effect of Ions on the Strength of Interaction

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The interaction between oppositely charged amino acids was studied by charge-transfer reversed-phase thin-layer chromatography. The dependence of the lipophilicity of Arg, Lys and Orn on the concentration of Glu, Asp, Gln and Asn in the eluent was considered to be related to the strength of interaction. The interaction of dibasic amino acids with Glu and Asp was stronger than with Gln and Asn. Mono- (Li^+, Na^+, K^+, Rb^+ and Cs^+) and divalent (Mg^{2+} and Ca^{2+}) cations decreased the strength of interaction suggesting the electrostatic character of the interaction. Their inhibitory effect mainly depended on their concentration and to a lesser extent on the ion charge and hydrated ion radii. Stepwise regression analysis proved that the strength of interaction depends on the polarity parameters of amino acids and is independent of their chemical structure.

Introduction

Recent research indicates that protein structure and association capacity are influenced by the various interactions between amino acid pairs [1].

Lysine and cysteine residues were directly involved in the aggregation between ovalbumin and lysosyme, the aggregation is partially due to electrostatic attraction [2]. Tyrosine can interact with other amino acids in bovine alpha-lactalbumin [3]. The edge-to-face interaction of two aromatic side chains makes an enthalpic contribution of between −1 and −2 kcal/mol to the energy stabilization of a protein [4, 5].

The hydrophobic, hydrophilic or mixed character of interactions between amino acid pairs in proteins has been vigorously discussed. Many studies suppose that hydrophobic interactions are the most important physical forces that guide a polypeptide chain to its specific folded form in an aqueous environment [6–9]. In peptides the Trp, Phe, Leu and Val have been involved in the formation of hydrophobic interactions [10]. In some cases the hydrophilic interactions between polar amino acid residues have also been observed. The electrostatic interactions influence the association and conformation of proteins [11]. The magnitude of electrostatic interactions between small aromatic rings in peptides and proteins was calculated to be −0.82 kcal/mol for Phe–Phe and −1.19 kcal/mol for Phe–His [12]. Charge-charge interactions was observed between His 64 and Asp 99 in subtilisin BPN [13] and between the Trp and Tyr in peptides [14]. The existence of intermolecular hydrogen bonding between the carboxyl and amino groups was proved by ^{13}C NMR [15].

Charge-transfer thin-layer chromatography (TLC) has been applied to study the interaction between adenine and amino acids [16].

The objectives of our work were to study the interaction between oppositely charged amino acids, to elucidate the effect of various ions on the strength of interaction and to determine the physico-chemical parameters of amino acids accounting for the interaction.

Materials and Methods

Amino acids were purchased from REANAL Fine Chemicals (Budapest, Hungary). Each amino acid was of L-configuration and of analytical purity. DC Fertigplatten Cellulose (Merck, Darmstadt, F.R.G.) plates were used as reversed-phase

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support without impregnation. Arg, Lys and Orn were separately dissolved in water:2-propanol 4:1 (v/v) at a concentration of 1 mg/ml, 5 µl of these solutions was spotted onto the plates. Distilled water was used as eluent containing separately 0–150 mM Gln or Asn, 0–12.5 mM Glu or Asp, 0–3 mM $CaCl_2$ or $MgCl_2$, 0–10 mM LiCl, NaCl, KCl, RbCl or CsCl and their various combinations. After development the plates were dried at 105 °C and the amino acid spots were detected with the traditional ninhydrin reagent. The detection has to be carried out very carefully and under continuous supervision because the spots of dibasic amino acids appear only for some minutes then they become invisible on the rapidly reddening amino acid background. Each experiment was run in quadruplicate.

The R_M values of Arg, Lys and Orn which characterize the molecular lipophilicity in reversed-phase TLC, were calculated according to Eqn. (1) [17].

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

To elucidate the individual effects of the hydrophilic physico-chemical parameters on the strength of interaction, stepwise regression analysis [18] was applied to select the independent variables influencing the interaction significantly. In the common multivariate regression analysis the presence of independent variables exerting no significant influence on the dependent variable lessens the significance level of the independent variables significantly influencing the dependent variable. To overcome this difficulty the stepwise regression analysis automatically eliminates of the selected equation the insignificant independent variables increasing in this manner the information power of the calculation.

The R_M value in Eqn. (1) was taken as dependent variable. The concentration and the polarity parameters ($pK\alpha_{NH_2}$, $pk\alpha_{COOH}$, pK of tertiary polar group and the pI-value taken from ref. [19]) of amino acids and the concentration, charge and hydrated radii of ions [20] served as independent variables. As the nature of the correlation (linear or quadratic) between the above variables had not been established previously both first- and second-order forms of the seven independent variables (overall 14 variables) were included in the stepwise regression analysis. The partial F-value of the independent variables was set to $F = 2$, and the number of accepted variables was not limited. The calculation was separately carried out for Arg, Lys and Orn.

Results and Discussion

Each amino acid (Glu, Asp, Gln and Asn) decreased the lipophilicity of Arg, Lys and Orn (Fig. 1) which unambiguously proved the existence of interaction between amino acid pairs. The interaction of dibasic amino acids with the more mobile Glu, Asp, Gln and Asn enhances their mobility (lessens their lipophilicity). The higher lipophilicity decrease indicates stronger interaction. The effect of dicarboxylic acids is much more higher than that of the corresponding amides. This phenomenon is probably due to the fact that the higher difference between the polarity of dibasic and dicarboxylic amino acids facilitate the formation of charge transfer complexes.

The R_M value of dibasic amino acids non-linearly depended on the concentration of the other amino acid. We assume that the non-linearity may be caused by the different stoichiometry of charge-transfer complexes formed at various molar ratios. At low concentration of dicarboxylic amino acids the complex may contain more dibasic amino acid (1:2 stoichiometry) than at higher concentration (2:1 stoichiometry). We have to emphasize that the explication outlined above is only a hypothetical one because the study of complex formation by charge transfer chromatography does not give any

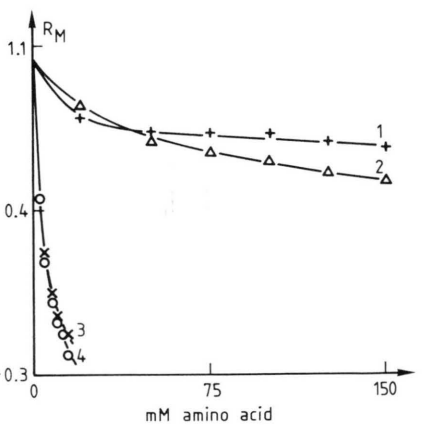

Fig. 1. Dependence of R_M-value of lysine on the amino acid concentration in the eluent 1. glutamine, 2. asparagine, 3. glutamic acid, 4. aspartic acid.

information about the stoichiometry of the complexation.

Ions markedly lessen the strength of interaction between the charged amino acids (Fig. 2–4). The ions probably bind to the polar groups of amino acid changing the polarity and the interactive capacity. This phenomenon can be considered as a competitive inhibition of the complex formation. The inhibitory effect of ions prove the electrostatic character of interaction, the potassium ion causes stronger inhibition than Li^+ and Rb^+ (Fig. 2). The

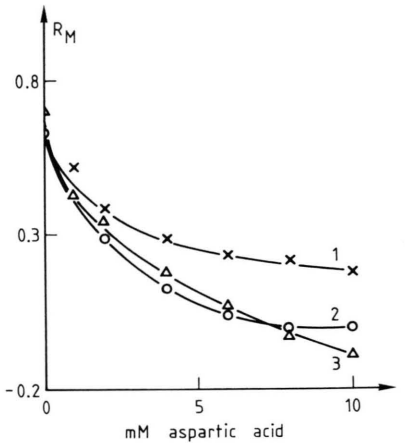

Fig. 2. Effect of monovalent cations on the interaction between ornithine and aspartic acid at 1 mM ion concentration 1. K^+, 2. Li^+, 3. Rb^+.

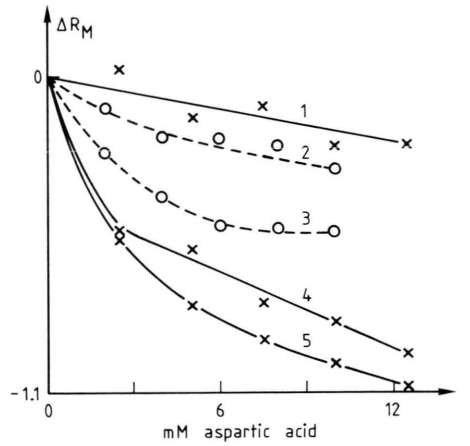

Fig. 3. Effect of various Na^+ concentrations on the interaction between arginine and aspartic acid 1. 10 mM, 2. 5 mM, 3. 2 mM, 4. 1 mM, 5. control, $R_M = 0$ positions corresponds to the original R_M-value of arginine ($R_M = 1.18$).

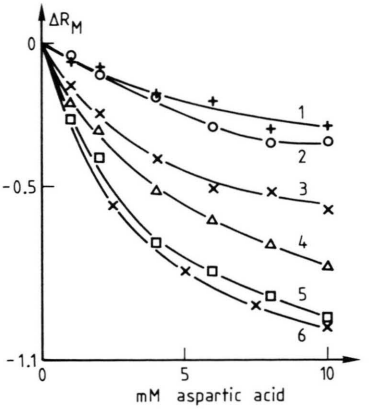

Fig. 4. Effect of various Ca^{2+} concentrations on the interaction between arginine and aspartic acid 1. 3 mM, 2. 2 mM, 3. 1 mM, 4. 0.5 mM, 5. 0.25 mM, 6. control, $R_M = 0$ position corresponds to the original R_M-value of arginine ($R_M = 1.18$).

effect of ions increases with the increasing ion concentration, however the Na^+ has a lower impact on the interaction (Fig. 3) than the Ca^{2+} does (Fig. 4).

The result of stepwise regression analysis are listed in Tables I–III. The equation selected by the stepwise regression analysis fits well to the experimental data, the significance level was in each case over 99.9% (see *F*-values). Depending on the character of dibasic amino acid 7–9 of the 14 independent variables influence the lipophilicity of the amino acid significantly (see (*t*-values). These in-

Table I. Dependence of the R_M-value of arginine on the physico-chemical parameters of amino acids and ions. Results of stepwise regression analysis ($n = 149$).
$R_M = a + b_1 \cdot x_1 + b_2 \cdot x_1^2 + b_3 \cdot x_2^2 + b_4 \cdot x_4^2 + b_5 \cdot x_5 + b_6 \cdot x_5^2 + b_7 \cdot x_6 + b_8 \cdot x_6^2$
s_b = Standard deviation of the slope *(b)*-values.
$a = 0.74$ $F = 62.00$ $r^2 = 0.7806$

Independent variable	Parameters $b \times 10^3$	$s_b \times 10^4$	Path coeff. %	t
x_1	−24.4	31.9	23.2	7.63
x_1^2	0.1	0.2	15.4	6.80
x_2^2	63.5	53.9	16.4	11.77
x_4^2	−228.6	144.7	9.5	15.80
x_5	−160.9	234.6	14.1	6.86
x_5^2	10.5	22.3	9.4	4.72
x_6	248.4	800.6	5.6	3.10
x_6^2	−125.7	321.4	6.4	3.91

x_1 = Amino acid concentration (mM); x_2 = pI; x_3 = pK of alpha amino group; x_5 = ion concentration (mM); x_6 = ion charge; x_7 = ion radii.

Table III. Dependence of R_M-value of ornithine on the physico-chemical parameters of amino acids and ions. Results of stepwise regression analysis (n = 146).
$R_M = a + b_1 \cdot x_1 + b_2 \cdot x_1^2 + b_3 \cdot x_2^2 + b_4 \cdot x_3 + b_5 \cdot x_3^2 + b_6 \cdot x_5 + b_7 \cdot x_5^2 + b_8 \cdot x_6 + b_9 \cdot x_6^2$
s_b = Standard deviation of the slope *(b)*-values.
$a = 0.62$ $F = 58.2$ $r^2 = 0.7939$
For symbols see Table I.

Independent variable	Parameters $b \times 10^3$	$s_b \times 10^4$	Path coeff. %	t
x_1	−28.6	34.3	12.1	8.35
x_1^2	0.2	0.2	8.0	7.39
x_2^2	40.6	76.0	5.1	5.34
x_3	470.0	2050.0	25.9	2.29
x_3^2	−56.1	270.6	29.8	2.70
x_5	−155.1	234.3	7.5	6.62
x_5^2	9.2	22.4	4.5	4.10
x_6	277.6	810.6	3.3	3.42
x_6^2	−131.7	322.9	3.7	4.08

Table II. Dependence of R_M-value of lysine on the physico-chemical parameters of amino acids and ions. Results of stepwise regression analysis (n = 150).
$R_M = a + b_1 \cdot x_1 + b_2 \cdot x_1^2 + b_3 \cdot x_2^2 + b_4 \cdot x_4^2 + b_5 \cdot x_5 + b_6 \cdot x_5^2 + b_7 \cdot x_7^2$
s_b = Standard deviation of the slope *(b)*-values.
$a = 0.62$ $F = 70.00$ $r^2 = 0.7752$
For symbols see Table I.

Independent variable	Parameters $b \times 10^3$	$s_b \times 10^4$	Path coeff. %	t
x_1	−24.5	33.8	26.2	7.23
x_1^2	0.1	0.2	17.4	6.41
x_2^2	69.5	56.6	20.3	12.28
x_4^2	−241.8	154.7	11.4	15.63
x_5	−141.9	225.1	14.0	6.30
x_5^2	9.1	21.9	9.1	4.14
x_7^2	66.2	232.3	1.6	2.85

dependent variables account for about 77−79% of the total variance (see r^2-values). The calculations proved the non-linear dependence of R_M-value of dibasic amino acids on the concentrations of amino acid and ion concentration. In many cases the quadratic forms exert a significant influence. The strength of interaction of each dibasic amino acid with the other amino acids is very similar, the differences between b-values of amino acid concentration (x_1 and x_1^2) are negligible. This results suggests that the interaction negligibly depends on the chemical structure of amino acid, it is mainly governed by the electrostatic interaction between the polar groups. It further implicates that similar interaction of commensurable strength may exist in proteins between any oppositely charged amino acid pairs.

The overall polarity of amino acids (pI) value in each case, the pK-value of α-carboxyl and α-amino groups in some cases influenced significantly the interaction. Their impact is commensurable with that of the amino acid concentration (see path coefficients). This finding lends support to the assumption that the interaction is really governed by electrostatic forces, where both the concentration and the polarity of the interaction agent exert a similar impact on the complex formation.

The ion concentration considerably influence the lipophilicity of the dibasic amino acid, this result is in good accordance with our previous qualitative conclusions. The role of physico-chemical parameters of ions (charge and hydrated radii), however significant, is of secondary importance.

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