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SPECIFIC EFFECTS IN THE INTERACTION BETWEEN IONIZED GELS AND AMINO ACIDS

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SUMMARY

The interaction between amino acids and a highly swollen ion-exchange gel are discussed. Effects specific for each of the amino acids are treated separately from purely electrostatic interactions of the cationic or dipolar form of the molecules. The degree of dissociation in the gel is compared to that in solution for the different species. From absorption studies it is found that the specific effects for the cationic amino acids are much more pronounced than those of similar dipolar molecules. The temperature dependence of the specific interactions shows that they are enthalpy effects rather than caused by entropy changes. These results can probably be explained by assuming dispersion forces to be operative between the amino acid side chains or backbone and the polyelectrolyte resin matrix.

INTRODUCTION

Ion and molecule binding in biological systems is recently undergoing much investigation and the field has been thoroughly reviewed[1,2]. Interactions in these systems are often highly specific but the mechanisms of these specific effects are only rarely understood.

In highly dilute polyelectrolyte gels it is possible to estimate purely electrostatic interactions as well as to measure the overall absorption of molecules and ions. We therefore thought a gel-solution system to be suitable for the differentiation between electrostatic and more specific interactions.

The problem is often complicated by various dissociative equilibria of the reactive species, since for example, the distribution of the ionized form of a molecule between gel and solution will most probably differ from that of the unionized form.

Ion-exchange resins of very low cross-linkage were used as a model for a gel. The water content of the swollen resin was over 90 % and its overall electrolyte content 0.36 mequiv/ml. The resin differs from a similar electrolyte solution by its uneven charge distribution, due to the highly charged polymer chains which comprise its matrix. On the other hand such a highly swollen gel is easily penetrable by ordinary solute molecules and behaves therefore in this respect similarly to a dilute polyelectrolyte solution or biological system. Because of the great importance of amino acid binding studies small amounts of the various acids were chosen to play the part of the interacting species. Their distribution between gel and ambient solution was

determined as a function of the surrounding pH. These measurements could then be compared with the results to be anticipated from purely electrostatic interactions between resin and amino acid.

Distribution of dipolar ions between gel and solutions

When the distribution coefficients of different amino acids

$$K_0' = \frac{C_+' + C_\pm'}{C_+'' + C_\pm''}$$

are plotted as a function of pH — pK_a in solution a number of curves result. C's are concentrations and subscripts denote the substance involved. In particular (+) indicates the cationic form of an amino acid RH_+ and (±) the dipolar form of the molecule $R_\pm$. The general characteristics of the curves show that at low pH values the molecules are strongly absorbed in a negatively charged gel and therefore presumably in their cationic form. With increasing pH the degree of dissociation in the gel decreases until the substance is at its isoelectric point wholly in the dipolar form. The latter is usually only very slightly absorbed in the gel.

The equilibrium between gel (') and solution ('') is determined by the following equations

$$RH_+'' \rightleftharpoons RH_+' \qquad K_1 = \frac{[RH_+]' f_+'}{[RH_+]'' f_+''} \tag{1}$$

$$R_\pm'' \rightleftharpoons R_\pm' \qquad K_2 = \frac{[R_\pm]' f_\pm'}{[R_\pm]'' f_\pm''} \tag{2}$$

$$RH_+ \rightleftharpoons R_\pm + H^+ \qquad K_a = \frac{[R_\pm]\,[H^+]}{[RH_+]} \frac{f_\pm f_{H^+}}{f_+} \tag{3}$$

where the K's are true thermodynamic equilibrium constants and f's denote activity coefficients. For purely ionic effects, as shown previousiy[3], differences between the interactions of various cations with a highly swollen polyelectrolyte gel can be attributed to differences in their distance of closest approach to the polymer. They are then expressed as $f_{M^+}'(el)$.

We assume that this distance for an amino acid cation $NH_3^+RCOO^-$ is determined by the NH_3^+-group dimensions only and is therefore independent of the rest of the molecule. It has also been shown that the dipole–polyion contribution to K_0' is generally slight[4]. The previously mentioned curves of pH — pK_a *versus* K_0' should therefore coincide on purely electrostatic grounds for most amino acids. The differences observed for the various species can thus be attributed to specific effects of the cationic or dipolar form of the molecules.

Thermodynamically this is expressed as follows:

The change in the Gibbs free energy when exchanging 1 mole, say, Na^+ ions in the gel for NH_3^+RCOOH is given by combining the appropriate Eqn. 1. At equilibrium

$$\Delta G = \Delta G^0 + RT \ln K_+/K_{Na^+} + RT \ln f_+' f_{Na^+}''/f_+'' f_{Na^+}' = 0 \tag{4}$$

where K_+ and K_{Na^+} are the distribution coefficients of ions NH_3^+RCOOH and Na^+ between gel and solution. PV terms are insignificant in our highly swollen gel and

have therefore been omitted. By choosing the infinitely dilute solution as reference state both for the gel and solution[5], it is seen that $K_{\pm}/K_{Na^+}$, the measurable quantity, equals $(f_{Na^+}'f_{+}'')/(f_{Na^+}''f_{+}')$. In our system the electrochemical properties are determined by one predominant counterion—here Na^+ ions. Its activity-coefficient ratio f_{Na^+}'/f_{Na^+}'' and also K_{Na^+} are not likely to change in the presence of small amounts of different amino acid ions. Changes in $RT\ln K_{\pm}$ are therefore reflected in $RT\ln f_{+}'/f_{+}''$. As already mentioned, the purely electrostatic part of this interaction energy is determined by the size of the $-NH_3^+$ group and is essentially measured by the selectivity of NH_4^+ over Na^+ ions in a Na^+ gel[3].

We divide the activity coefficient f_{+} into an ionic part and into another part which depends upon the particular amino acid and write $f_{+}' = f_{+}'(\mathrm{el}) \cdot f_{+}'(\mathrm{sp})$. The specific part of the interaction energy is

$$RT\ln f_{+}'(\mathrm{sp}) = RT\ln(K_{Na^+}/K_{+})\,(f_{Na^+}'/f'(\mathrm{el})) = RT\ln K_{NH_4^+}/K_{+} \qquad (5)$$

It is here assumed that the activity coefficient ratio f_{+}''/f_{Na^+}'' equals unity in the dilute solution. Since the $-NH_3^+$ groups of all the amino acids are similar the differences in absorption of the various amino acids are attributed to $f_{+}'(\mathrm{sp})$. For the dipolar ions we obtain from Eqn. 2, $K_2 = 1$ and $K_{\pm} = f_{\pm}'/f_{\pm}''$. Again $f_{\pm}' = f_{\pm}'(\mathrm{el}) \cdot f_{\pm}'(\mathrm{sp})$ where $f_{\pm}'(\mathrm{el})$ can be calculated from dipole–polyion interactions[4]. For amino acids of equal dipole moments differences in $K_{\pm}$ are attributed to $f_{\pm}'(\mathrm{sp})$.

Dissociation in the gel

Our expressions allow us also to determine the degree of dissociation of an amino acid in the gel. In the absence of specific interactions we obtain by dividing Eqn. 3 for the primed by the double primed quantities:

$$\frac{C_{H^+}'}{C_{H^+}''}\frac{f_{H^+}'}{f_{+}'}f_{\pm}' = \frac{K_{+}}{K_{\pm}} = \left(\frac{1-\alpha'}{\alpha'}\right)\left(\frac{\alpha''}{1-\alpha''}\right) \qquad (6)$$

where α is the degree of dissociation. Again it is here assumed that the activity coefficients in the dilute solution all equal unity. We see that as long as f_{+}' can be set equal to f_{H^+}' and $f_{\pm}$ does not differ much from unity it is enough to know the concentration ratio of H^+ (or any other representative cation) in order to calculate the degree of dissociation of an amino acid in the gel. In real systems, specific effects do operate and the above mentioned assumptions cannot be made any more. The experimentally obtained quantities K_{+} and $K_{\pm}$ allow us, however, still to find α.

From Eqn. 6 $(1-\alpha')/\alpha' = (K_{+}/K_{\pm})\,[(1-\alpha'')/\alpha'']$. We can therefore draw a "titration curve" of pH against $1-\alpha$ (see Fig. 1).

Distribution experiments show here to what extent a dipolar molecule is in its ionized form in a gel at a given outside concentration and pH. This information might be of value when considering reactivity in gel systems where the reacting species concentration is of interest.

Temperature dependence of equilibria

The causes of interactions between amino acids and polymers can be of various character and measurements of the temperature dependence of the equilibria might help to distinguish between them. By differentiating the specific interaction energy

$$\frac{\partial \ln f_{+}'(\mathrm{sp})}{\partial 1/T} = \frac{\Delta H(\mathrm{sp})}{R}$$

we obtain the enthalpy. Subtracting the latter from $RT\ln f_+'$(sp) we also obtain the entropy change of the relevant interaction.

The adsorption can be caused by dispersion forces. In this case the energy should be approximately proportional to a sum of $\bar{\alpha}_1\bar{\alpha}_2/r$ terms for all the interacting bonds of the polymer (1) and of the molecule (2)[6]. $\bar{\alpha}$ is the polarisability of a bond and r the distance between the mutually attracting bonds. It can be anticipated that these attractions will be accompanied by a negative enthalpy change. If the interactions lead to a configurational change and ordering effects in the gel the corresponding entropy change will also be negative.

Another cause for the above mentioned interactions might be sought in so called hydrophobic bond formation[7] reviewed recently by KAUZMANN[8]. In this case the explanation of the polymer amino acid attraction lies in the ordering effect of amino acids upon the surrounding water molecules ("iceberg" formation). Binding to the polymer matrix with the accompanying organic–water interface decrease diminishes this ordering effect. The interaction would therefore be principally of entropic nature with a large positive entropy change while ΔH(sp) is near zero or even positive.

EXPERIMENTAL

The amino acids investigated were glycine (BDH Analar grade), α-alanine, DL-β-alanine, γ-aminobutyric acid, L-leucine (all supplied by Nutritional Biochemicals Corp.) and DL-phenylalanine (Merck and Co., Inc.). The resin used was a Zeokarb-225 preparation (cross linked polystyrene sulphonate) of 1% nominal divinylbenzene content (Permutit Co., London). It was carefully purified by washing repeatedly with 0.2 N NaOH and 0.2 N HCl and rinsing with triple-distilled water. Very coarse and very fine particles were discarded and beads of about 50 μ diameter size were retained for experiment.

The chromatographic procedure has been described previously[4]. Briefly: the amino acids were chromatographed on 10-mm dia. columns of 15–50 cm length. The volume occupied by the Na^+-resin in the column was carefully marked in each experiment. After equilibration with the appropriate buffer the column was loaded with 0.1 ml 0.05–0.10% amino acid solution. Elution was carried out with buffers containing Na^+ ions in 0.02 N concentration. In the pH range 4.1 to 5.5 acetate buffer was used while at lower pH values formate was employed. The experiments were checked near pH 4.1 with both formate and acetate but no difference whatsoever was observed between the two anions.

Elution was carried out very slowly 5 ml/h/cm^2 column cross section so as not to disturb equilibrium between solution and resin.

The amino acids were determined in the eluate by the ninhydrin method of MOORE AND STEIN[9].

The temperature dependence was measured in similar experiments using columns fitted with water jackets through which water from a thermostat was pumped. At sufficiently high flow rates the temperature difference between the water inflow and outflow was less than 0.1°. At temperatures higher than room temperature the buffer solution tended to discharge air bubbles in the column. It was usually possible to avoid this without elaborate equipment[10], by deaerating the solutions and packing the column at elevated temperatures.

pH measurements were carried out with a Metrohm (Herisau) Präzisionsmodell E-187 pH meter, which was calibrated with standard buffers pH 4.0 (potassium biphthalate) and pH 6.87 (KH_2PO_4–Na_2HPO_4) before and after measurements. The accuracy attained was ± 0.015 pH units. The pK_a values of amino acids were measured with a glass calomel electrode assembly at a Na^+ ion concentration of 0.02 N[11].

RESULTS

The distribution of amino acids between gel and solution was determined by chromatography. The pH dependence of $\Delta V/\Delta x$, the elution volume per unit column volume, was measured for glycine, α-alanine, β-alanine, leucine, γ-aminobutyric acid and phenylalanine. The distribution coefficient K_0^i was calculated was a function of pH from

$$K_0^i = \frac{\Delta V/\Delta x - \delta}{1 - \delta - \delta'}$$

where δ is the column void-volume fraction and δ' the part occupied by the resin matrix (δ' was assumed to equal 0.025)[4]. The pK_a's of the substances were taken from the literature[12] or measured at a Na^+ ion concentration of 0.02 N. The distribution coefficient for amino acids is

$$K_0^i = \frac{C_+' + C_\pm'}{C''} = \frac{C_+'(1-\alpha'')}{C_+''} + \frac{C_\pm'\alpha''}{C_\pm''} = K_+(1-\alpha'') + K_\pm\alpha''$$

By plotting K_0^i as a function of $1-\alpha''$, K_+ and $K_\pm$ were found from the slope and intercept of the straight line[13]. The data are summarized in Table I.

$RT\ln f_+'(sp)$ values were calculated from Eqn. 5. $\bar{\alpha}$ is the average bond polarisability of the $-CH_2RCOOH$ part of the amino acid molecule[14]. The distribution coefficient of NH_4^+ ions was found from column chromatography of NH_4Cl in a resin

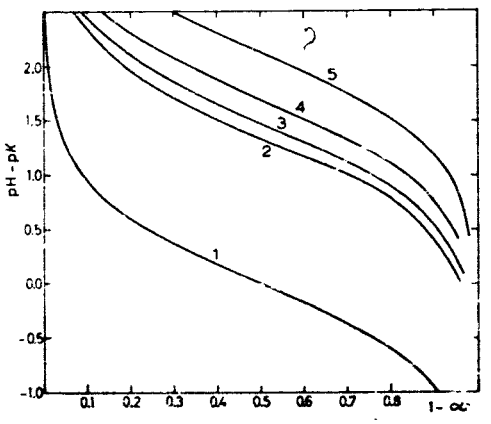

Fig. 1. pH − pK_a of amino acids in solution *versus* 1 − α. Curve 1, 1 − α in solution. Curves 2–5, 1 − α in the gel for glycine, γ-aminobutyric acid, leucine and phenylalanine, respectively.

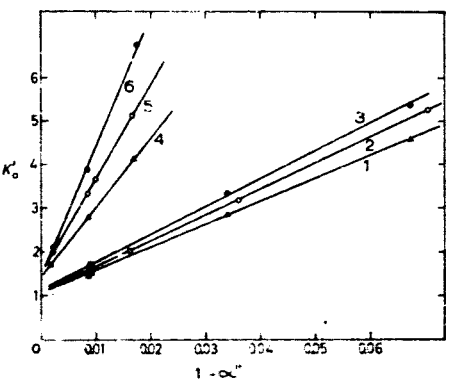

Fig. 2. Temperature dependence of K_0^i as a function of 1 − α in solution. Leucine at 35° (1), 17° (2), 8° (3); phenylalanine at 35° (4), 17° (5), 8° (6).

in the Na^+ ion form eluted by 0.02 N NaCl and checked with 0.02 N sodium acetate.

Fig. 1 shows the degree of dissociation as a function of $pH - pK_a$ for the above mentioned amino acids. α is a single valued function of $pH - pK_a$ in very dilute solution while in the resin pronounced differences between the different amino acids are found.

TABLE I

pK_a VALUES, DISTRIBUTION COEFFICIENTS AND SPECIFIC INTERACTION ENERGIES BETWEEN AMINO ACIDS AND POLYELECTROLYTE GEL AT 17°

	pK_a	K_+	$K_\pm$	$RT \ln f_+ (sp)$ (cal/mole)	$RT \ln f_\pm (sp)$ (cal/mole)	$\bar{a}$ ($\times 10^{23}$ cm^3)
Glycine	2.38	25.9	1.10	95	55	43.1
α-Alanine	2.38	26.9	1.10	115	55	62.5
β-Alanine	3.60	26.5	1.15	105	82	62.5
γ-Aminobutyric acid	4.03	37.0	1.20	305	108	81.9
Leucine	2.36	58.0	1.10	550	55	120.7
Phenylalanine	2.04	220	1.60	1410	275	
NH_4^+ ion		22.0				

TABLE II

ENTHALPY AND ENTROPY CHANGE OF SPECIFIC AMINO ACIDS INTERACTION WITH POLYELECTROLYTE GEL

Temperature	Glycine		Leucine		Phenylalanine	
	K_+	$K_\pm$	K_+	$K_\pm$	K_+	$K_\pm$
8°	25.5 ± 1	1.08 ± 0.05	64	1.10 ± 0.05	300	1.60 ± 0.10
17°			58		220	
35°			52		150	
cal/mole	0		−1100		−4300	
e.u.	+0.3	+0.10	−1.85	+0.2	−9.5	+0.9

The temperature dependence of K_+ and $K_\pm$ was measured for glycine, leucine and phenylalanine. The latter as opposed to the other substances showed a strong effect. K_+ of a phenylalanine decreases from 300 at 8° to 160 at 35° while the corresponding figures for leucine are 64 and 54 and glycine did not show any temperature dependence within the bounds of our experimental error. Table II shows the K_+ and $K_\pm$ values at different temperatures. ΔH and ΔS as derived from the temperature dependence are assumed to be constant in the range 8° to 35°.

The accuracy of our results depends on a number of factors. The volumes $\Delta V/\Delta x$ could be determined from the elution peaks with a precision of ± 1 %. The experiments were reproducible in a range of ± 2 %. The calculated values of K_+ and $K_\pm$ are therefore accurate to ± 4 %. The main error in our determinations lies however in possible inaccuracies in pK_a values. These are very important since especially at strong interactions only a small proportion of the amino acid was in the cationic form in solution. This proportion depends strongly upon the assumed pK_a. The absolute values of the distribution coefficients must therefore be accepted with caution. Relative distribution coefficients at different temperatures, for example, should not be affected by this error.

DISCUSSION

The distribution of micro-amounts of the dipolar molecules mentioned here can be calculated, taking into account purely electrostatic interactions. Therefore deviation from this behaviour is attributed to specific interaction.

It is assumed that in the very dilute gel employed by us the only interactions encountered are those between single solute molecules and fairly stretched polyelectrolyte chains. No effects of the resin structure and pore size were therefore taken into account. It is further assumed that the cationic $-NH_3^+$ group of the amino acids is similar in size to the ammonium ion. No specific effects are attributed to the latter and it served us therefore as reference ion. For the given resin and solution concentrations the distribution coefficient of NH_4^+ in a Na^+ form resin was found to be $K_{NH_4^+} = 22$ and K_+ relative to this value yields the specific attraction. The effects due to the different dipole moments of the $R_\pm$ form have been discussed elsewhere[4], and shown to be comparatively small.

With regard to the cationic form it is seen that even the simple glycine and alanine side chains undergo some interactions with the resin matrix. Lengthening of the methylene chain does not enhance the effect for β-alanine but does so in the case of γ-aminobutyric acid. The effect increases appreciably with the bulkiness of the side chain as shown by leucine. The introduction of a benzene ring in the phenylalanine side chain has a very strong effect as reflected by the great increase in K_+. Although also with the dipolar molecule a similar effect was observed its magnitude for the ionized form is much greater.

Such behaviour seems at first contrary to ordinary experience since it could be imagined that specific group interactions would be an additive effect independent of any electrostatic dipolar or ionic interactions.

We think that the explanation for this behaviour must be sought in the steric configuration of the amino acid in relation to the polystyrene chain. When in the dipolar form the preferred situation of K_+ will be such that its positive $-NH_3^+$ group is attracted by the negatively charged resin while COO^-, being repelled by the polyanion, points into the free solution. In this way there is comparatively little contact between polymer and the organic part of the amino acid. The ionized RH_+ ion, however, is free to have its side chain to assume any direction with respect to the polymer. Therefore there is a better chance of close proximity between the side chain and polymer matrix—an effect which is shown in our experiments by a high sensitivity of K_+ to the character of the side chain.

The kind of forces responsible for specific interactions will now be briefly discussed. By inspecting the last column of Table I it is seen that the molecular polarisabilities, calculated from averaged bond polarisabilities, increase together with the $RT\ln f_+'(sp)$ factors. In order to account for the observed energies by London's dispersion forces the differences in $RT\ln f_+'(sp)$ for different amino acids ought, however, to increase proportionally to $const \cdot \sum \frac{\bar{\alpha}_i \bar{\alpha}_k}{r_{ik}^6}$ where $\bar{\alpha}_i$ is the polarisability of a bond belonging to the dipolar molecule and $\bar{\alpha}_k$ the corresponding quantity for the resin matrix. It is reasonable to assume that enlarging the side chain, as when substituting leucine for alanine, or adding methylene groups along the molecular axis, like in β-alanine and γ-aminobutyric acid, increases the rotational mobility of one part of the molecule with respect to another part. The average r_{ik} distance for leucine

might well be smaller than the corresponding quantity for alanine and the same applies to the flexible γ-aminobutyric acid chain. This means closer fitting of the organic part of the molecule into the resin matrix cavities and could account for the $RT\ln f_{+}(sp)$ value differences.

COULSON AND DAVIS have calculated by a LCAO (Linear Combination of Atomic Orbitals) treatment the dispersion forces for conjugated hydrocarbon chains[15]. They show that the mutual orientation of the molecules is of importance. The 6th-power distance law is correct only at large intermolecular distances and the π-electron contribution to dispersion forces is very significant for large conjugated systems. No complete calculation for benzene–benzene ring interactions have been done but the value for hexatriene interaction from COULSON's data is — 2700 cal/mole at an intermolecular distance of 4 Å. We assume that the phenylalanine side chain interacts with the polymer styrene rings in a somewhat similar fashion. The calculated interaction energies decrease very rapidly with distance so that the experimental value of ΔH — 4300 cal/mole might be explained by a side-chain interaction with the resin rings at a distance somewhat less than 4 Å.

Our temperature-dependence measurements are not very accurate and can yield no information in those cases where the overall interaction energy is small. For leucine, however, and even more so for phenylalanine, appreciable negative enthalpy and also negative entropy effects have been found. These point strongly in the direction of a dispersion force character with some complex formation. The possibility of hydrophobic bonds seems to be limited in those cases since no positive entropy effect has been found. For glycine, on the other hand, no temperature effect was observed and a small positive entropy contribution is possible.

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