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Free Energies of Adsorption of Amino Acids, Short Linear Peptides and 2,5-Piperazinediones on Silica from Water as Estimated from High-Performance Liquid-Chromatographic Retention Data

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Abstract. Equilibrium constants (K) and free energies ($-\Delta G$) of adsorption of amino acids, short linear peptides and cyclic dipeptides (2,5-piperazinediones) on silica from neutral aqueous solutions were calculated from the retention values measured by means of high-performance liquid chromatography on a silica gel column. For most amino acids and linear peptides $-\Delta G$ values were negative and $K < 1$, thus showing very weak adsorption. 2,5-Piperazinediones exhibited higher adsorbability (for most of them, $-\Delta G > 0$ and $K > 1$) as compared to related dipeptides. Influence of the structure of α-substituent on the adsorbability is analyzed. A linear dependence of $-\Delta G$ on the number of aliphatic carbon atoms in a sorbate molecule was found for the series of aliphatic bifunctional amino acids, related dipeptides and 2,5-piperazinediones, as well as for the group from glycine to triglycyl glycine.

Keywords: equilibrium - experimental data, liquid phase, measurement techniques

Introduction

Adsorption interactions of protein constituents, i.e., amino acids and peptides, with inorganic matrices (clays, alumina, silica, etc.) are of interest from view points of molecular evolution and the Life's origins (Cairns-Smith and Hartman, 1986), the formation of biomineralized structures (Lowenstam and Weiner, 1989), soil chemistry (Greenland and Hayes, 1978), biogeochemistry and racemization dating (Hare et al., 1980), as well as practical liquid chromatography (Fallon et al., 1987). Usual approach to the characterization of interaction of a compound with a mineral matrix in aqueous media is based on measuring adsorption isotherms, from which one can determine equilibrium constants (K) and free energies ($-\Delta G$) of adsorption. This approach has been applied, for instance, by Greenland et al. (1965a, b) to the adsorption of amino acids and some short peptides on aluminosilicates montmorillonite and illite. As to pure silica, which is also of great interest due to its wide occurence in the Earth crust and living organisms (Iler, 1979), we are not aware of such works for amino acids and short peptides.

At the same time, there is another, chromatographic (or dynamic) approach with the use of high-performance liquid chromatographic technique, when K and $-\Delta G$ values can be estimated from easily measured retention values k' (Karger, 1971; Salo et al., 1992; Lork et al., 1989; Purcell et al., 1989, 1992; Balmer et al., 1992; Vazquez et al., 1992; Colin and

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Guiochon, 1978; Melander et al., 1978; Scott, 1989; Pochapsky and Gopen, 1992):

$$K = k'/\theta, \tag{1}$$

$$-\Delta G = RT \ln K, \tag{2}$$

where θ is phase ratio, which is constant for each packed liquid-chromatographic column and depends on amount of a stationary phase in the column. The simplest, first-approach expression for the phase ratio (Karger, 1971) is

$$\theta = V_s/V_m, \tag{3}$$

where V_m is volume of mobile phase in the column (i.e., dead volume); V_s, the volume of stationary phase, which can be in turn calculated as

$$V_s = V_u - V_m, \tag{4}$$

where V_u is geometric volume of the empty, unpacked column.

Here we apply this approach (using the simplest expression (3) for the phase ratio) to the estimation of free energies of amino acid, short linear peptide and cyclic dipeptide (2,5-piperazinedione, PD) adsorption on pure silica in neutral aqueous medium.

Experimental

Retention measurements were performed using microcolumn chromatograph Milikhrom 4 UV from Nauchpribor (Orel, Russian Federation) with UV detection at 190–210 nm. Commercially available stainless steel microcolumn 64 × 2 mm I.D. (also from Nauchpribor) has been packed with Silasorb 600 silica gel, mean particle size of 4 μm, specific surface area (BET) of 550 m^2g^{-1} (Chemapol, Prague, Czechoslovakia). Geometric volume of the unpacked column was $V_u = 201$ μl; dead volume of the packed column, $V_m = 95$ μl, measured as the retenton volume of benzene with wet dichloromethane as eluant (Engelhardt, 1979). Doubly-distilled deionized water was used as eluant at flow rate of 50 μl min^{-1}. The temperature was 19°C.

All amino acids and linear peptides, mentioned in the work, were from Reanal (Budapest, Hungary) and were used as received. 2,5-Piperazinediones were synthesized by the gas-solid-phase method as described earlier (Basiuk et al., 1992; Basiuk and Gromovoy, 1994). The solute concentrations were of the order of 10^{-6} g ml^{-1}. The injection volumes were typically 1–2 μl.

Equilibrium constants and free energies of adsorption were calculated according to the Eqs. (1–4).

Results and Discussion

The experimental retention data for the amino acids, linear peptides and PD's are presented in Tables 1 and 2. The k' values are rather low and, thus, the described

Table 1. Equilibrium constants (K) and free energies ($-\Delta G$) of amino acid adsorption on silica in neutral aqueous medium, obtained from experimental retention values (k')*, and the data by Greenland et al. (1965b)** for calcium montmorillonite (CaM) and illite (CaI), for comparison.

Amino acid***	k'	K			$-\Delta G$, J/mol		
		Silica	CaM	CaI	Silica	CaM	CaI
Gly	0.64	0.57	1.77	6.6	−1370	1420	4690
DL-Ala	0.70	0.63	4.1	10.1	−1140	3510	5700
L-Pro	1.32	1.18			400		
L-Val	0.92	0.82			−480		
DL-Ile	1.05	0.93			−170		
L-Leu	1.05	0.93	1.8	4.9	−170	1430	3940
L-Ser	0.61	0.54	2.4	7.3	−1480	2130	4940
DL-Thr	0.68	0.61			−1200		
L-Cys	0.91	0.81			−500		
L-Asn	0.70	0.63			−1140		
DL-Asp	0.25	0.22			−3640		
L-Gln	0.72	0.64			−1070		
L-Glu	0.32	0.28			−3050		
L-Met	0.96	0.86			−370		
L-His	0.32	0.28			−3050		
DL-Phe	1.06	0.95			−130		
D-Tyr	0.60	0.54			−1500		
L-DOPA	0.70	0.63			−1140		
DL-Trp	0.86	0.77			−630		

*19°C.

**25°C.

***Abbreviations of amino acids are given according to the recommendations of IUPAC-IUB Commission on Biochemical Nomenclature [J. Biol. Chem. 264: 668 (1989)]: Gly, glycine; Ala, alanine; Pro, proline; Val, valine; Nva, norvaline; Leu, leucine; Ile, isoleucine; Ser, serine; Thr, threonine; Cys, cysteine; Asp, aspartic acid; Asn, asparagine; Glu, glutamic acid; Gln, glutamine; Met, methionine; His, histidine; Phe, phenylalanine; Tyr, tyrosine; Trp, tryptophan; Aib, α-amino isobutyric acid; DOPA, 3,4-dioxyphenylalanine.

Table 2. Equilibrium constants (K) and free energies ($-\Delta G$) of dipeptide and 2,5-piperazinedione adsorption on silica in neutral aqueous medium, obtained from experimental retention values (k').

Compound	k'	K	$-\Delta G$, J/mol
Dipeptides			
Gly-Gly	0.70	0.63	−1140
Gly-DL-Ala	0.82	0.73	−760
L-Ala-L-Ala	0.82	0.73	−760
DL-Ala-DL-Ala	0.86	0.77	−630
Gly-L-Val	1.10	0.98	−40
Gly-L-Leu	1.34	1.19	430
Gly-DL-Asn	0.72	0.64	−1080
DL-Ala-DL-Asn	0.74	0.66	−1020
L-Val-L-Val	1.33	1.19	410
Gly-DL-Phe	1.10	0.98	−40
L-His-L-Leu	0.69	0.62	−1170
DL-Ala-DL-Trp	0.91	0.81	−500
2,5-Piperazinediones			
cyclo-Gly-Gly	0.72	0.64	−1070
cyclo-Gly-DL-Ala	0.84	0.75	−700
cyclo-D-Ala-D-Ala	0.91	0.81	−500
cyclo-L-Ala-L-Ala	0.93	0.83	−450
cyclo-DL-Ala-DL-Ala	0.93	0.83	−450
cyclo-Gly-L-Val	1.15	1.02	55
cyclo-Gly-DL-Nva	1.22	1.09	200
cyclo-Aib-Aib	1.12	1.00	0
cyclo-Gly-DL-Leu	1.46	1.31	650
cyclo-Gly-L-Leu	1.58	1.41	840
cyclo-L-Pro-L-Pro	2.61	2.33	2050
cyclo-L-Val-L-Val	1.99	1.78	1400
cyclo-DL-Nva-DL-Nva	2.13	1.90	1560
cyclo-L-Leu-L-Leu	3.91	3.49	3030
Glycine tri- and tetrapeptide			
Gly-Gly-Gly	0.77	0.69	−900
Gly-Gly-Gly-Gly	0.84	0.75	−700

chromatographic system is not suitable for the separation and analysis of amino acids, simple peptides and PD's.

To estimate according to the Eqs. (1) and (2) equilibrium constants K and free energies of adsorption $-\Delta G$ from the above data, first of all one should determine the phase ratio θ. The simplest approach accounts only for easily measurable volume of the unpacked column V_u and the dead volume V_m (the values, see Experimental section); from the Eqs. (3) and (4) we obtain $\theta = 1.12$. This value we used in all our calculations.

Amino Acids

The experimental retention data, calculated equilibrium constants and free energies of amino acid adsorption on silica are summarized in Table 1. The most bright feature is negative values of $-\Delta G$ and $K < 1$ for overwhelming majority of amino acids (Pro is the only exception). The data by Greenland et al. (1965b), obtained under static conditions, gave much higher values for the adsorption of Gly, Ala, Leu, and Ser on clays (Table 1): K of 1.77 to 10.1, and $-\Delta G$ values were always positive. Thus, as compared with clays silica, which does not possess strong cation-exchanging properties, has much weaker capability to adsorb amino acids, at least from very diluted aqueous solutions.

There was no correlation between amino acid molecular weights and K and $-\Delta G$ values in the case of clays. For Ca montmorillonite adsorbability decreased in the order Ala > Ser > Leu > Gly; for Ca illite, Ala > Ser > Gly > Leu. In the case of silica, generally the same picture is observed. However, if to select amino acids containing no heteroatoms and cyclic fragments in α-substituent, we obtain the row Leu, Ile > Val > Ala > Gly.

The first member of the α-amino acid family is Gly, containing only one aliphatic carbon atom; and all others, without an exception, contain two or more aliphatic carbons. Therefore, it seems appropriate to estimate the influence just of their number (n_c) on free energies of adsorption, as well as the contribution of other groupings of the side-chain. The plot of $-\Delta G$ vs. n_c is presented in figure 1(a). It is clearly seen that this dependence for the row Gly - Ala - Val - Leu - Ile is nearly linear. From the slope an increment in $-\Delta G$ for each aliphatic C-atom is obtained to be about 300 J/mol. However, Pro is adsorbed much stronger than its open-chain analogue, Val, that should be apparently associated with cyclic structure of the former. There are other amino acids differing by one aliphatic C-atom, namely, Asp - Glu, Asn - Gln, and Ser - Thr; the C-increments in $-\Delta G$ for them were calculated to be 590, 70, and 280 J/mol, respectively.

As a rule, heteroatoms and other non-aliphatic groupings in the α-substituent considerably influence amino acid adsorbability. Imidazole nucleus (for His) and carboxylic groups (for Asp and Glu) cause the most

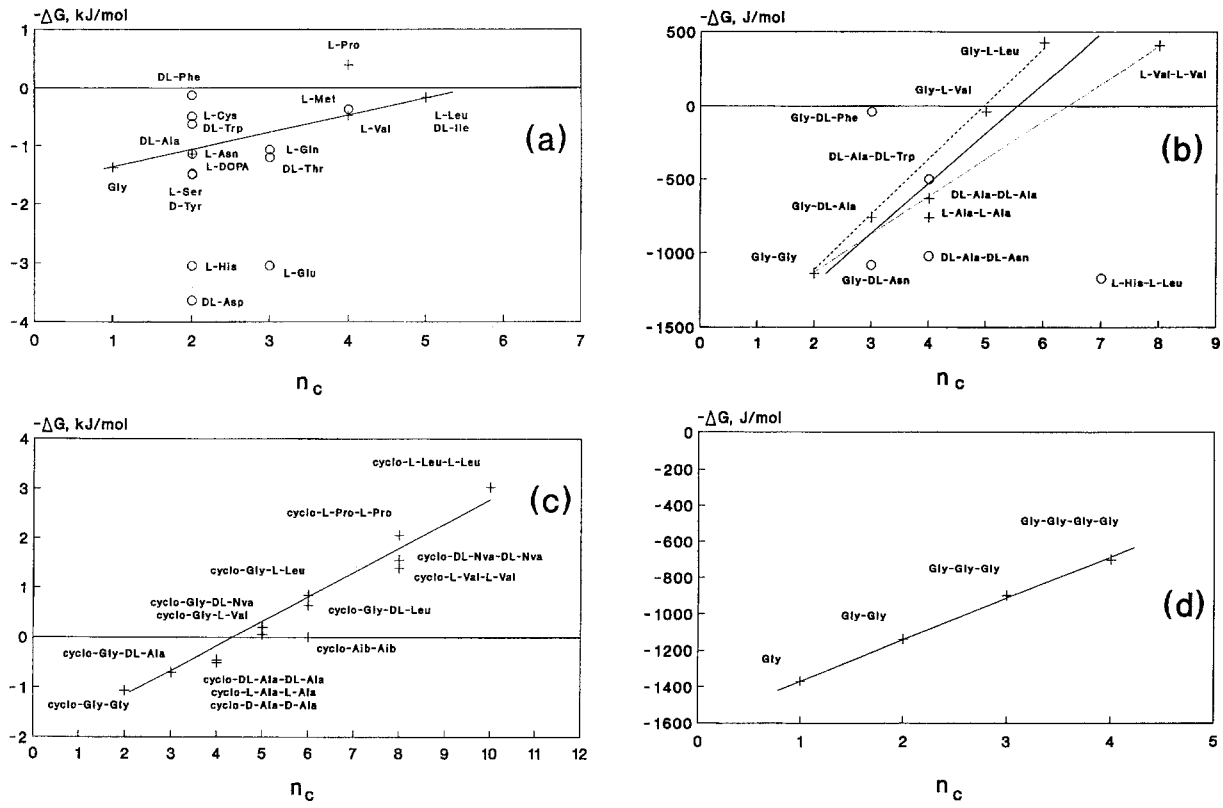

Figure 1. The plot of free energy ($-\Delta G$) of adsorption of silica from neutral aqueous medium vs. the number of aliphatic carbon atoms (n_c) in adsorbate molecules: (a) amino acids; (b) dipeptides; (c) 2,5-piperazinediones; and (d) the series glycine to triglycyl glycine. Aliphatic bifunctional (+) and other amino acids (○), and, by analogy, the related peptides.

sharp reduction in $-\Delta G$ values; amide (Asn and Gln) and alcohol (Ser and Thr) functions also reduce the adsorbability, but to a much lesser extent. The sulfur atom noticeably increases $-\Delta G$ in the case of Cys (as compared with Ala), but only slightly contribute in the case of Met (vs. Val). The appearance of phenyl nucleus results in $-\Delta G$ increase (Phe, $\delta(-\Delta G) = 1010$ J/mol as compared with Ala), but only if the nucleus does not contain oxy-groups, as in Tyr and DOPA.

Dipeptides

The equilibrium constants and free energies of peptide and PD adsorption, calculated for $\theta = 1.12$, are presented in Table 2. Most dipeptides, similarly to amino acids, are characterized by negative $-\Delta G$ values and $K < 1$; the exceptions are L-Val-L-Val and Gly-L-Leu. For comparison, Greenland et al. (1965b), measuring adsorption isotherms on clays under static conditions, have obtained for simple glycine peptides much higher K and $-\Delta G$ values: particularly, for Gly-Gly on calcium montmorillonite they were 3.61 and 3180 J/mol, respectively; and on calcium illite, 10.2 and 5780 J/mol, respectively. Thus, silica exhibits slight capability to adsorb dipeptides from diluted aqueous solutions, similarly to the case of amino acids, that hardly can be characterized quantitatively using the standard static approach.

The plot of $-\Delta G$ vs. n_c is presented in figure 1(b). For dipeptides derived only from aliphatic bifunctional amino acids this dependence tends to be linear (figure 1(b), solid line), and the increment in $-\Delta G$ is about 340 J/mol per each aliphatic C-atom, i.e., close to that for the case of amino acids. One should note, however, that the solid line badly comprises the corresponding points. Detailed consideration reveals that the dipeptides may be in turn subdivided into two following groups: (1) dipeptides derived from two different amino acids, Gly being one of them (the dashed line); (2) homo-amino acid dipeptides (the dotted line). The C-increments in $-\Delta G$ are 390 and 260 J/mol,

respectively; and thus, dipeptides of the first group possess better adsorbability than those of the second group.

The appearance of heteroatoms and aromatic nuclei in the α-substituent substantially changes the characteristics of dipeptides. The lowest $-\Delta G$ value (-1170 J/mol) was found for L-His-L-Leu, containing imidazole ring. The amide groupings of Asn moieties also decrease adsorbability, whereas indolyl and phenyl nuclei increase it. So, for glycine dipeptides (Gly-DL-Ala, Gly-DL-Asn, and Gly-DL-Phe) the change of Ala to Asn lowers the energy by 320 J/mol, and the change of Ala to Phe leads to increase in $-\Delta G$ by 720 J/mol. For alanine dipeptides (DL-Ala-DL-Ala, DL-Ala-DL-Asn, and DL-Ala-DL-Trp), change of the C-terminal Ala residue to Asn causes $-\Delta G$ decrease by 390 J/mol; Ala to Trp, the increase by 130 J/mol.

2,5-Piperazinediones

The set of PD's available involved only the derivatives of bifunctional aliphatic amino acids. For this reason we were not able to evaluate the influence of polar and aromatic α-substituents on the adsorbability. Here, on the contrary to the previous two classes of compounds, most PD's exhibit values $K > 1$ and $-\Delta G > 0$ ($K = 1$ and $-\Delta G = 0$ for cyclo-Aib-Aib; Table 2). A linear trend of the $-\Delta G(n_c)$ dependence (figure 1(c)) is still more evident, with the increment of about 510 J/mol for one aliphatic carbon atom, i.e., much higher value than those for related amino acids and dipeptides. In other words, the enhance in adsorbability during developing the hydrocarbon side-chain becomes maximum when the adsorbate molecules do not contain anymore terminal amino and carboxylic groups and, therefore, lose their zwitterionic structure. So, the cyclization to corresponding PD for Gly-Gly results in $-\Delta G$ increase by 70 J/mol; for DL-Ala-DL-Ala, by 180 J/mol; and for L-Val-L-Val, by 990 J/mol.

Influence of Peptide-Chain Length

As the last, but not least, aspect of adsorption behavior of the studied compounds one should discuss the influence of peptide-chain length. From comparison of the data for amino acids and related dipeptides it is seen that the latter possess better adsorbability. So, when transferring from Gly to Gly-Gly the $-\Delta G$ value increases by 230 J/mol; from DL-Ala to DL-Ala-DL-Ala, by 510 J/mol; and from L-Val to L-Val-L-Val, by 890 J/mol. These values are, as a matter of fact, the increments in free energies of adsorption falling on one amino acid fragment and, as is seen from their comparison, they increase as n_c in amino acid fragment increases. If to attribute these increments to the corresponding n_c values, we obtain the same value $\delta(-\Delta G) = 230$ J/mol for the pair Gly – Gly-Gly (since here $n_c = 1$); 255 J/mol for DL-Ala – DL-Ala-DL-Ala; and 220 J/mol for L-Val – L-Val-L-Val. In other words, these C-increments appear rather close both to each other and to the value obtained above for the series of aliphatic bifunctional amino acids (about 300 J/mol).

Further lengthening peptide chain must influence the adsorbability in a similar way (at least to a certain limit). It can be illustrated for the series from glycine to triglycyl glycine. From figure 1(d) it is evident that in these limits of chain-length the adsorption energy increases linearly; the slope gives the increment in $-\Delta G$ of 220 J/mol per one amino acid fragment (= per one aliphatic carbon atom). In the case of the glycine series adsorption on clay minerals the increments were 2280 J/mol for calcium montmorillonite and 1360 J/mol for calcium illite (Greenland et al., 1965b); thus, the adsorption characteristics (K and $-\Delta G$) of the studied compounds in the silica system are not only much lower as compared with the case of clays, but also change to a much lesser extent during the chain lengthening.

Most likely, some limit exists for the length of peptide, when the observed dependence becomes no more linear as a result of the transfer from almost straight molecule to globular, helix-twisted one, etc. For instance, from the studies on reversed-phase high-performance liquid-chromatographic resolution of amino acids and simple linear peptides (Hearn, 1983) it is known that lg k' values show uniform incremental changes for a homologous peptide series up to *ca.* $n_a = 10$ (where n_a is the number of amino acids in peptide chain; $n_a = 1$ means an amino acid itself). This regularity was observed for mobile phases containing phosphate buffer solutions of pH < 3, both without and with organic additives. Particularly, in the case of alanine and its homopeptides (Molnar and Horvath, 1977) the plot of lg k' vs. the number of alanine residues showed linear dependence with a uniform lg k' increment up to at least $n_a = 6$ (stationary phase: LiChrosorb RP18; mobile phase: 100 mM phosphate buffer, pH 0.2 and 2.1). For the phenylalanine series, similar picture is evident up to at least $n_a = 5$ (stationary phase: Zorbax octadecyl-silica; mobile phase: acetonitrile-water (30 : 70) - 50 mM KH_2PO_4 - 15 mM

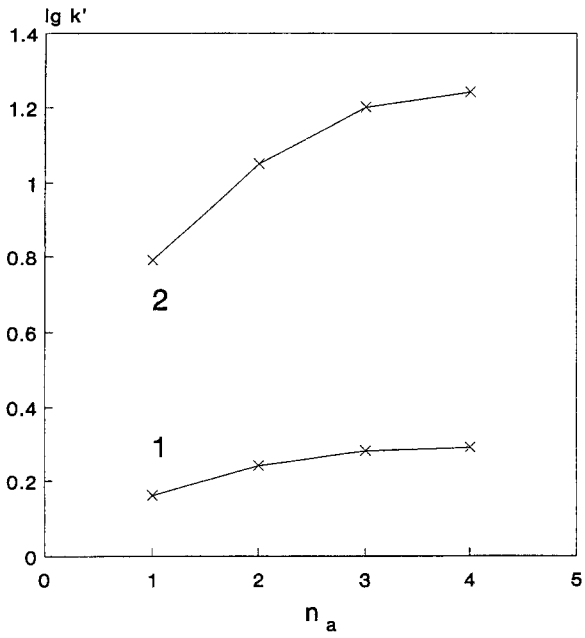

Figure 2. The plot of lg k' vs. the number of amino acid residues, n_a, for homologous glycine series using acetonitrile-containing mobile phases. Mobile phase: acetate buffer solution pH 5.21—acetonitrile with volume ratio of (1) 45 : 55 and (2) 25 : 75; flow rate, 50 μl min^{-1}; temperature, 20°C.

H_3PO_4, pH 2.3; Hearn and Grego (1984)). Nevertheless, in the case of "bare" (underivatized) silica gel, used in the present study, we did not observe the linearity for the glycine series when mobile phase was composed of slightly acidic acetate buffer solution and large amounts of acetonitrile (at least if the eluant contained more than 50% of acetonitrile by volume; two examples are given in figure 2). Apparently, significant content of an organic modifier in a mobile phase causes folding the linear peptide chain so that only a limited number of the molecules' polar groups (external groups in globular molecules) are able to interact with silica surface during the adsorption process, and this number increases more slowly than the chain length. Thus the observed linearity should not be necessarily observed in any adsorption system, depending strongly on solution composition.

The chain lengthening may be imagined as increase in the number not only of amino acid fragments, but also of amide groupings in the molecules. From such point of view, it is interesting to notice the following two pairs of compounds containing the same number of amide groupings and n_c: Gly-Gly – DL-Asn (n_c = 2, one amide bond) and Gly-Gly-Gly – Gly-DL-Asn (n_c = 3, two amide bonds). For the latter we observe nearness (−900 and −1080 J/mol, respectively) and for the former, coincidence (−1140 J/mol) in $-\Delta G$ values. It could evidence for simple additivity of the adsorption characteristics when introducing the main structural fragments into peptide molecules; however, regretfully, this regularity is not observed for the pair Gly-DL-Ala – L-Gln (n_c = 3, one amide bond; $-\Delta G$ of −760 and −1070 J/mol, respectively).

Qualitative Explanation of the Compounds' Behavior

The observed rather low values of K and $-\Delta G$ can be qualitatively explained as follows. Linear peptide molecules (as well as amino acid ones), existing as zwitterions in neutral polar media, are surrounded by a hydrate shell. Their adsorption on silica must be accompanied with a partial destruction of the shell and with desorption of water molecules from the surface, but this process causes a large decrease of entropy. According to Greenland et al. (1965b), in the series Gly – Gly-Gly – Gly-Gly-Gly – Gly-Gly-Gly-Gly the entropy becomes positive only for the latter peptide. For amino acids and dipeptides the entropy factor is generally unfavorable and results in obtained negative values of $-\Delta G$. As new polar groups (besides α-amino and α-carboxylic ones) appear in the molecules, the hydrate shell becomes more large and dense, and the entropy effect is enhanced; and *vice versa*, when the hydrophobic hydrocarbon moiety developes or new hydrophobic amino acid fragments are added into the growing chain. For non-zwitterionic PD's, due to their comparatively high hydrophobicity, lower degree of hydration and poor solubility in aqueous solvents, the action of entropy factor is weakened and, thus, the adsorption process becomes more favorable. In general, the more bulky hydrocarbon α-substituents, the more their number (= number of amino acid fragments) and the less number of heteroatoms in molecules of the compounds, the better their adsorbability on silica in neutral aqueous medium. Cumulation of these factors leads finally to the strong adsorbability observed for long-chain polypeptides and proteins on silica (Iler, 1979).

Conclusions

Despite amino acids and linear dipeptides are slightly adsorbed on hydroxylated silica surface, so that the determination of equilibrium constants K and free

energies of adsorption $-\Delta G$ is hardly possible by measuring the isotherms under static conditions, the use of chromatographic (dynamic) approach allows the K and $-\Delta G$ values to be estimated from amino acids' and peptides' retention values k' on a silica gel column. For most proteinogenic amino acids and derived short peptides, $-\Delta G$ values are negative and $K < 1$, showing indeed very weak adsorption from neutral aqueous solutions. Cyclic dipeptides (or 2,5-piperazinediones) exhibit higher adsorbability (for most of them, $K > 1$; $-\Delta G$ up to about 3 kJ/mol) as compared to related linear dipeptides. The constants depend linearly on the number of aliphatic carbon atoms in the molecules of aliphatic bifunctional amino acids (Gly, Ala, Val, Leu and Ile), related dipeptides and 2,5-piperazinediones, as well as for the homologous series from glycine to triglycyl glycine. The appearance of heteroatoms and aromatic nuclei in the α-substituent changes the adsorption characteristics.

Nomenclature

K	adsorption equilibrium constant	
k'	retention (capacity factor)	
$-\Delta G$	free energy of adsorption	J mol^{-1}
V_m	volume of mobile phase in the column (dead volume)	μl
V_s	volume of stationary phase	μl
V_u	geometric volume of the empty, unpacked column	μl
θ	phase ratio	
n_c	number of aliphatic carbon atoms in the molecules	
n_a	number of amino acid residues in peptide molecules	

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