FREE ENERGIES OF ADSORPTION OF DIPEPTIDES AND 2,5-PIPERAZINEDIONES ON SILICA FROM NEUTRAL AQUEOUS SOLUTIONS AS ESTIMATED FROM HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHIC RETENTION DATA

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The equilibrium constants (K) and free energies ($-\Delta G$) of adsorption of dipeptides and their cyclic derivatives, 2,5-piperazinediones, on silica from neutral aqueous solutions were calculated from their HPLC retention data on a silica gel column. For the majority of the dipeptides $-\Delta G$ values were negative, ranging from −1 170 to 430 J mol^{-1}, and $K < 1$, thus indicating a very weak adsorption. The 2,5-piperazinediones under study exhibited higher adsorbabilities ($-\Delta G$ −1 070 + 3 030 J mol^{-1}, $K > 1$) as compared to the respective dipeptides. The effect of the α-substituent structure on the adsorbability is analyzed. A linear dependence of $-\Delta G$ on the number of aliphatic carbon atoms in a sorbate molecule was observed for the series of dipeptides and 2,5-piperazinediones derived from bifunctional aliphatic amino acids.

Adsorption interactions of protein constituents, i.e. amino acids and peptides, with inorganic matrices (clays, alumina, silica, etc.) are of interest from the viewpoints of molecular evolution and the origin of life[1], the formation of biomineralized structures[2], soil chemistry[3], biogeochemistry and racemization dating[4], as well as practical liquid chromatography[5]. The conventional approach to characterization of interaction of a compound with a mineral matrix in aqueous medium consists in measuring its adsorption isotherms and deriving from them the equilibrium constants (K) and free energies ($-\Delta G$) of adsorption. This approach has been applied, for instance, by Greenland et al.[6,7] to the adsorption of amino acids and some short peptides on the aluminosilicates montmorillonite and illite (the process was characterized by non-standard free energies of adsorption). As for pure silica, which is also of great interest owing to its wide

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occurrence in the Earth's crust and living organisms[8], no investigation has been made, to our knowledge, for amino acids and short peptides (except for an unsuccessful attempt to measure the isotherms directly in static conditions, undertaken at our Institute[9], from which it was concluded that these compounds possess extremely low adsorbabilities.

There exists, however, another, chromatographic (or dynamic) approach using high-performance liquid chromatography (HPLC), where the K and $-\Delta G$ values can be estimated from the retention values (k) which are easy to measure, viz. as

$$K = k/\theta \qquad (1)$$

$$-\Delta G = \boldsymbol{R}T \ln K, \qquad (2)$$

where θ is the phase ratio, which is constant for a packed liquid-chromatographic column and depends on the amount of the stationary phase in the column. The simplest, first approximation expression for the phase ratio[10] is

$$\theta = V_s/V_m, \qquad (3)$$

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

$$V_s = V_u - V_m, \qquad (4)$$

where V_u is the geometric volume of the empty, unpacked column. A more rigorous approach to the liquid-chromatographic estimation of the K and $-\Delta G$ values needs to account for the mass (m, in g) and specific surface area (S, in $m^2\ g^{-1}$) of the stationary phase[11], and the phase ratio (in $m^2\ ml^{-1}$) can be calculated as

$$\theta' = mS/V_m = V_s \rho S/V_m, \qquad (5)$$

where ρ (in $g\ cm^{-3}$) is the density of the stationary phase.

Pochlapsky and Gopen[12] reported recently on the use of the dynamic approach to obtain relative free energies of interaction between hydrophobic and amphiphilic amino acid side chains. We applied this approach[13] to estimate the free energies of amino acid

adsorption on pure silica in neutral aqueous medium. This work is a continuation of the study, examining the adsorption of dipeptides and their cyclic derivatives, 2,5-piperazinediones (PD's), using the same chromatographic system.

EXPERIMENTAL

Retention data were measured on a Milikhrom 4UV microcolumn chromatograph (Nauchpribor, Orel, C.I.S.) with UV detection at 190 – 210 nm. A commercially available stainless steel microcolumn 64 × 2 mm i.d. (also from Nauchpribor) was packed with Silasorb 600 silica gel, mean particle size 4 μm, specific surface area (BET) 550 $m^2\ g^{-1}$ (Chemapol, Prague, The Czech Republic). Geometric volume of the unpacked column was V_u = 201 μl (based on the dimensions reported by the supplier); the dead volume of the packed column, estimated as the retention volume of benzene with wet dichloromethane as eluant[14] (after subtracting the volume of connecting tubing), was V_m = 95 μl. Double-distilled deionized water was used as eluant at a flow rate of 50 μl min^{-1}. The temperature was 19 °C. Experimental error of recording the retention volumes was less than 2%.

All dipeptides used in this work were from Reanal (Budapest, Hungary) and were used as received. 2,5-Piperazinediones were synthesized by the gas–solid method as described earlier[15,16].

Equilibrium constants and free energies of adsorption were calculated from Eqs (*1*) – (*4*).

RESULTS AND DISCUSSION

The experimental retention data for the dipeptides and PD's are presented in Table I. The *k* values (as well as the corresponding column efficiencies, typically about 200 – 400 theoretical plates) are rather low, indicating that the chromatographic system applied is not convenient for the separation and analysis of simple peptides and PD's, as well as amino acids[13].

To estimate the equilibrium constants *K* and free energies of adsorption $-\Delta G$ from the above data using Eqs (*1*) and (*2*), one must first determine the phase ratio θ. This can be done in two different ways. The simplest approach takes into account the volume of the unpacked column V_u and the dead volume V_m. From Eqs (*3*) and (*4*) we obtain θ = 1.12 using the data given in Experimental. This approach, however, does not take into account specific properties of the stationary phase, such as its density and surface area, in contrast to the approach based on Eq. (*5*). The latter, which is generally more rigorous, includes two characteristics of the stationary phase, viz. the density ρ and BET specific surface area *S*, which, however, are not so clearly determined as it might seem at first glance. the reason is that in the present case they are measured, applying conventional procedures, for totally dehydrated silica gel. Actually, however, during the packing of the columns and during the liquid-chromatographic process, first, the silica gel is covered by a rather thick layer of adsorbed water; second, the relatively large molecules of the bioorganic solutes cannot penetrate into all narrow pores of the stationary phase. These circumstances can lower the actual ρ and *S* values, although it is not clear to what extent. When adopting ρ = 1.9 g cm^{-3} (for totally dehydrated amorphous silica[17] and *S* = 550 $m^2\ g^{-1}$, the phase ratio is $\theta' = 1.17\ m^2\ ml^{-1}$.

TABLE I
Equilibrium constants (K) and free energies ($-\Delta G$) of adsorption of dipeptides and 2,5-piperazinediones on silica in neutral aqueous medium, derived from experimental retention values (k)

Compound	M.w.[a]	k	K	$-\Delta G$, J mol^{-1}
		Dipeptides		
Gly-Gly	132	0.70	0.63	–1 140
Gly-DL-Ala	146	0.82	0.73	–760
L-Ala-L-Ala	160	0.82	0.73	–760
DL-Ala-DL-Ala	160	0.86	0.77	–630
Gly-L-Val	174	1.10	0.98	–40
Gly-L-Leu	188	1.34	1.19	430
Gly-DL-Asn	189	0.72	0.64	–1 080
DL-Ala-DL-Asn	203	0.74	0.66	–1 020
L-Val-L-Val	216	1.33	1.19	410
Gly-DL-Phe	222	1.10	0.98	–40
L-His-L-Leu	268	0.69	0.62	–1 170
DL-Ala-DL-Trp	275	0.91	0.81	–500
		2,5-Piperazinediones		
cyclo-Gly-Gly	114	0.72	0.64	–1 070
cyclo-Gly-DL-Ala	128	0.84	0.75	–700
cyclo-D-Ala-D-Ala	142	0.91	0.81	–500
cyclo-L-Ala-L-Ala	142	0.93	0.83	–450
cyclo-DL-Ala-DL-Ala	142	0.93	0.83	–450
cyclo-Gly-L-Val	156	1.15	1.02	55
cyclo-Gly-DL-Nva	156	1.22	1.09	200
cyclo-Aib-Aib	170	1.12	1.00	0
cyclo-Gly-DL-Leu	170	1.46	1.31	650
cyclo-Gly-L-Leu	170	1.58	1.41	840
cyclo-L-Pro-L-Pro	194	2.61	2.33	2 050
cyclo-L-Val-L-Val	198	1.99	1.78	1 400
cyclo-DL-Nva-DL-Nva	198	2.13	1.90	1 560
cyclo-L-Leu-L-Leu	226	3.91	3.49	3 030

[a] M.w. Molecular weight.

The equilibrium constants and free energies of adsorption, calculated for $\theta = 1.12$, are presented in Table I. Most of the dipeptides, similar to amino acids[13], are characterized by negative $-\Delta G$ values and $K < 1$; L-Val-L-Val and Gly-L-Leu are exceptions. For a comparison, Greenland et al.[7], who measured adsorption isotherms on clays in static conditions, have obtained for simple glycine peptides considerably higher values for the equilibrium constants and non-standard free energies of adsorption: in particular, for Gly-Gly on calcium montmorillonite they were 3.61 and 3 180 J mol^{-1}, respectively; and on calcium illite, 10.2 and 5 780 J mol^{-1}, respectively. Thus, as compared with clays, silica – which does not exhibit strong cation-exchanging properties – has actually a considerably weaker capability to adsorb dipeptides from diluted aqueous solutions. This capacity is difficult to characterize quantitatively using the standard static approach.

In our previous work[13] we found that no general correlation exists between amino acid molecular weights and the corresponding K and $-\Delta G$ values. However, such correlation has been observed for aliphatic bifunctional amino acids (i.e. those containing no heteroatoms and cyclic fragments in the α-substituent), whose adsorbabilities decreased in order Leu, Ile > Val > Ala > Gly. Taking into account the fact that only the first member of the α-amino acid family, Gly, contains one aliphatic carbon atom whereas the other members, with no exception, contain two or more such atoms, we suggest that adsorbability is well suited correlation with the number of aliphatic carbon atoms (Z); the contribution of other side-chain groupings can also be seen from such comparison. In the amino acid series Gly-Ala-Val-Leu-Ile, the dependence of $-\Delta G$ on Z was found to be nearly linear, with the increment in $-\Delta G$ of about 300 J mol^{-1} per aliphatic C-atom. As a rule, heteroatoms and other non-aliphatic groupings in the α-substituent affect the adsorbability of the amino acid considerably. The imidazole nucleus (for His) and carboxylic groups (for Asp and Glu) cause the sharpest drop of the $-\Delta G$ values; amide (Asn and Gln) functions also reduced the adsorbability, but to a much lesser extent. The presence of a phenyl nucleus brings about increase in $-\Delta G$ (Phe, $\delta(-\Delta G)$ = 1 010 J mol^{-1} as compared with Ala) provided that the nucleus does not contain oxygroups, as in Tyr and 3,4-dioxyphenylalanine.

The situation in the present case is similar. The plot of $-\Delta G$ vs Z is shown in Fig. 1. For dipeptides which are derived from aliphatic bifunctional amino acids only, this dependence tends to be linear, the $-\Delta G$ increment being about 340 J mol^{-1} per aliphatic C-atom, which is close to that for the case of amino acids. Heteroatoms and aromatic nuclei in the α-substituent change the characteristics of peptides substantially. The lowest $-\Delta G$ value (–1 170 J mol^{-1}) was found for L-His-L-Leu, containing an imidazole ring. The amide groupings of Asn moieties also reduce the adsorbability, whereas indolyl and phenyl nuclei increase it. So, for glycine dipeptides (Gly-DL-Ala, Gly-DL-Asn, and Gly-DL-Phe) the transition from Ala to Asn lowers the energy by 320 J mol^{-1}, whereas the transition from Ala to Phe leads to an increase in $-\Delta G$ by 720 J mol^{-1}. 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^{-1}; Ala to Trp, increase by 130 J mol^{-1}.

The set of PD's which we had available involved derivatives of bifunctional aliphatic amino acids only. For this reason we were unable to evaluate the influence of polar and aromatic α-substituents on their adsorbability. Here, in contrast to the previous two

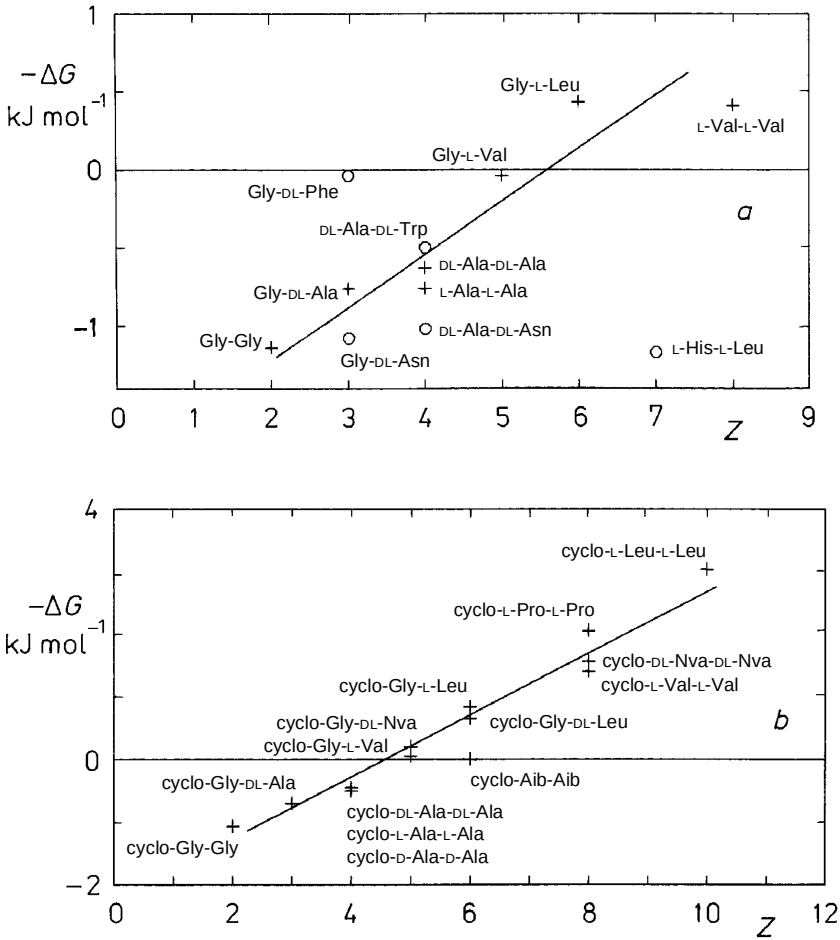

FIG. 1

Dependence of the free energy of adsorption ($-\Delta G$) on silica from neutral aqueous medium on the number of aliphatic carbon atoms (Z) in adsorbate molecules: *a* dipeptides, *b* 2,5-piperazinediones. The compounds are derived from: + aliphatic bifunctional, ❍ other amino acids

classes of compounds, most PD's exhibit $K > 1$ and the corresponding $-\Delta G$ values are $-1\,070 + 3\,030$ J mol^{-1} ($K = 1$ and $-\Delta G = 0$ for *cyclo*-Aib-Aib; Table I). A linear trend of the $-\Delta G(Z)$ dependence (Fig. 1*b*) is still more evident, with an increment of about 510 J mol^{-1} per aliphatic carbon atom, which is considerably than for the related amino acids or dipeptides. In other words, the increase in adsorbability associated with the development of the hydrocarbon side chain reaches its maximum when the adsorbate molecules do not contain terminal amino and carboxylic groups any more and thus lose their zwitterionic structure. So, cyclization to the corresponding PD for Gly-Gly results in an $-\Delta G$ increase by 70 J mol^{-1}; for DL-Ala-DL-Ala, 180 J mol^{-1}; and for L-Val-L-Val, 990 J mol^{-1}.

Rigorous interpretation of the data obtained requires additional detailed thermodynamic studies, particularly at variable temperatures. Now, the reliability of the reported results should be discussed. We feel that so far, none of the existing methods for the determination of the adsorption characteristics gives sufficiently accurate data. The chromatographic approach, used in this work, is no exception due to the great difficulties associated with an accurate experimental determination of the dead volumes as well as the retention volumes of the solutes under study, especially with the very poor efficiencies observed (about 200 – 400 theoretical plates). As was mentioned in the Experimental, the error of recording the retention volumes was less than 2%. Consider an overestimated error of 5% and analyze how it can affect the final $-\Delta G$ value for the case of the dipeptide Gly-Gly ($-1\,140$ J mol^{-1}), which is one of the most weakly retained solutes used in this work. As Eqs (*1*) – (*4*) demonstrate, the highest $-\Delta G$ deviations will be observed if the retention volume is overestimated while the dead volume is underestimated, and vice versa. For a 5% error the calculations give -860 and $-1\,500$ J mol^{-1}, respectively. Although the two results differ significantly (by 25 and 32%, respectively) from the tabulated value, they are substantially lower than the data by Greenland et al. for the adsorption of Gly-Gly on calcium montmorillonite or illite[7]. Evidently, similar deviations would also be observed for the other solutes, shifting the corresponding points in Fig. 1 with respect to *x*-axis, but the overall picture of the $-\Delta G(Z)$ plot would remain the same for both the dipeptides and PD's. Thus, since the chromatographic approach employed can introduce errors (units to tens per cent) in the calculations, the method can hardly be regarded as rigorous for the determination of the adsorption characteristics (apparently like all the other existing methods). The method, however, can be very helpful in estimating the $-\Delta G$ and K values for weakly adsorbed organic and biological compounds, which is difficult (if not impossible) by measuring the adsorption isotherms in static conditions.

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