Amino Acids (1994) 7: 305–309

Amino
Acids

Free energies of amino acid adsorption on silica in neutral aqueous medium as estimated from high-performance liquid-chromatographic retention data

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Accepted December 18, 1993

Summary. Equilibrium constants (K) and free energies ($-\Delta G$) of amino acid adsorption on silica in a neutral aqueous medium were calculated from the retention values measured by means of high-performance liquid chromatography on a silica gel column. For most amino acids (with the exception of proline) $-\Delta G$ values were negative and $K < 1$, thus showing very low adsorption. Influence of the structure of the α-substituent on adsorbability is analyzed. A linear dependence of $-\Delta G$ on the number of aliphatic carbon atoms was shown for the series: glycine-alanine-valine-leucine-isoleucine.

Keywords: Amino acids – Silica – Adsorption – Free energies – Retention – High-performance liquid chromatography

Abbreviations: Gly: glycine; Ala: alanine; Pro: proline; Val: valine; Ile: isoleucine; Leu: leucine; Ser: serine; Thr: threonine; Cys: cysteine; Asn: asparagine; Gln: glutamine; Asp: aspartic acid; Glu: glutamic acid; Met: methionine; His: histidine; Phe: phenylalanine; Tyr: tyrosine; DOPA: 3,4-dioxyphenylalanine; Trp: tryptophan

Introduction

Adsorption interactions of amino acids and related biological compounds with inorganic matrices (clays, alumina, silica, etc.) are of interest from various view points. The most significant implications comprise adsorption and catalytic aspects of molecular evolution, the formation of biomineralized structures, soil chemistry, biogeochemistry and racemization dating, as well as practical liquid chromatography. The usual approach to the characterization of amino acid interaction 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 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 biota, we are not aware of such works (except for several unsuccessful attempts to obtain the isotherms under static conditions undertaken in our Institute: the only result was conclusion that amino acids are not adsorbed by silica).

At the same time, there is another, dynamic, approach which uses the high-performance liquid chromatographic technique, where K and $-\Delta G$ values can be estimated from easily measured retention values (k'):

$$K = k'V_m/V_s \qquad (1); \qquad -\Delta G = RT \ln K \qquad (2);$$

where V_m is volume of mobile phase in the column; V_s, the volume of stationary phase; V_s/V_m, phase ratio, which is constant for each packed column (Karger, 1971). Particularly, Pochapsky and Gopen (1992) used recently this approach to obtain relative free energies of interaction between hydrophobic and amphiphilic amino acid side chains.

Here we report on the liquid-chromatographic estimation of free energies of amino acid adsorption on pure silica in a neutral aqueous medium.

Materials and method

Amino acids Gly, DL-Ala, L-Val, DL-Ile, L-Leu, L-Cys, L-Met, L-Pro, DL-Phe, D-Tyr, L-DOPA, L-Ser, DL-Thr, DL-Asp, L-Glu, L-Asn, L-Gln, L-His, and DL-Trp from Reanal (Budapest, Hungary) were used as received.

Retention measurements were performed using microcolumn chromatograph Milikhrom 4UV 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 (Chemapol, Prague, Czechoslovakia). The phase ratio for this column (calculated as $V_s/V_m = (V_u - V_m)/V_m$, where $V_u = 201$ μl, volume of the unpacked column; and $V_m = 95$ μl, dead volume of the packed column) was 1.12. Doubly-distilled deionized water was used as eluant at flow rate of 50 μl min^{-1}. The temperature was 19°C. Equilibrium constants and free energies of amino acid adsorption were calculated according to the equations (1) and (2).

Results and discussion

The experimental retention data, calculated equilibrium constants and free energies of amino acid adsorption on silica are summarized in Table 1. The most interesting feature is the negative values of $-\Delta G$ and $K < 1$ for the 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 values varied from 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 a much weaker capability to adsorb amino acids, at least from very dilute 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 >

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. MW, molecular weight

			K			$-\Delta G$, J/mol		
Amino acid	MW	k'	silica	CaM	CaI	silica	CaM	CaI
Gly	75	0.64	0.57	1.77	6.6	−1370	1420	4690
DL-Ala	89	0.70	0.63	4.1	10.1	−1140	3510	5700
L-Pro	115	1.32	1.18			400		
L-Val	117	0.92	0.82			−480		
DL-Ile	131	1.05	0.93			−170		
L-Leu	131	1.05	0.93	1.8	4.9	−170	1430	3940
L-Ser	105	0.61	0.54	2.4	7.3	−1480	2130	4940
DL-Thr	119	0.68	0.61			−1200		
L-Cys	121	0.91	0.81			−500		
L-Asn	132	0.70	0.63			−1140		
DL-Asp	133	0.25	0.22			−3640		
L-Gln	146	0.72	0.64			−1070		
L-Glu	147	0.32	0.28			−3050		
L-Met	149	0.96	0.86			−370		
L-His	155	0.32	0.28			−3050		
DL-Phe	165	1.06	0.95			−130		
D-Tyr	181	0.60	0.54			−1500		
L-DOPA	197	0.70	0.63			−1140		
DL-Trp	204	0.86	0.77			−630		

* 19°C; ** 25°C.

Leu. In the case of silica, generally the same picture is observed. However, we chose to focus on amino acids containing no heteroatoms and cyclic fragments in the α-substituent (the series 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 exception, contain two or more aliphatic carbons. Therefore, it seems appropriate to estimate their numerical influence (n_c) on the 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 Fig. 1. 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 more strongly than its open-chain analogue, Val. There are other amino acid pairs that differ 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 moieties contained in the α-substituent considerably influence amino acid adsorbability. The imidazole nucleus (for His) and carboxylic groups (for Asp and Glu) cause the most sharp reduction in $-\Delta G$ values; amide (Asn and Gln) and alcohol functions (Ser and Thr) 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

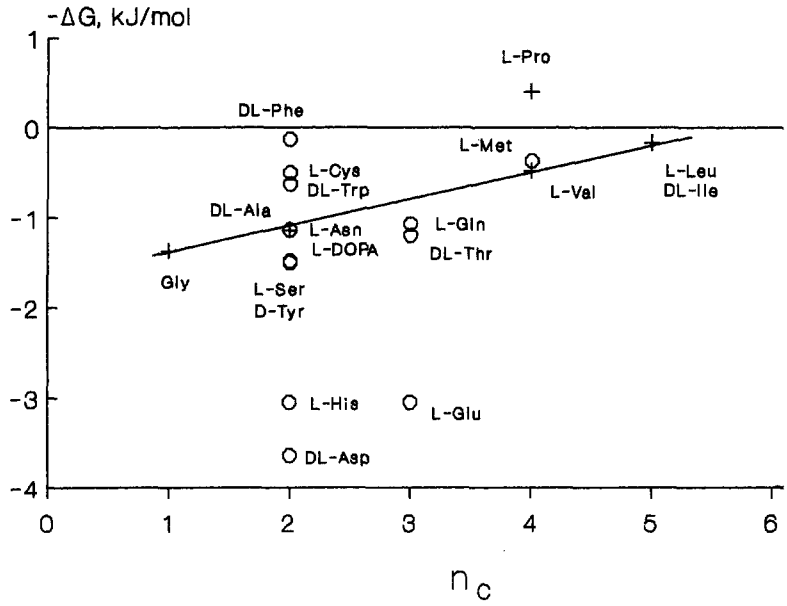

Fig. 1. The plot of free energy ($-\Delta G$) of adsorption on silica from neutral aqueous medium vs. the number of aliphatic carbon atoms (n_c) in amino acid molecules, as estimated from high-performance liquid-chromatographic retention data. (+) Aliphatic bifunctional and (o) other amino acids.

slightly contributes in the case of Met (vs. Val). The presence of a phenyl nucleus results in a $-\Delta G$ 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.

The obtained data can be explained as follows. Amino acid molecules, existing as zwitter-ions in neutral polar media, are surrounded by a hydrate shell. 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 of Gly – glycyl glycine – diglycyl glycine – triglycyl glycine the entropy becomes positive only for triglycyl glycine. For amino acids the entropy factor is generally unfavorable and results in measured negative values of $-\Delta G$. As new polar groups (besides α-amino and α-carboxylic ones) appear in the molecules, the hydrate shell becomes larger and stronger, and the entropy effect is enhanced; and *vice versa*, when the hydrophobic hydrocarbon moiety increases.

Taking into account that amide groups (for Asn and Gln) slightly influences the adsorption characteristics, one may expect that short peptides, derived from hydrophobic amino acids, will have better adsorbability on silica and higher corresponding $-\Delta G$ values. The work on their estimation from liquid-chromatographic retention data is now in progress.

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Received June 18, 1993