

Determination of the standard Gibbs energies of transfer of cations and anions of amino acids and small peptides across the water nitrobenzene interface

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Summary. The standard Gibbs energies of transfer of anions and cations of amino acids and small peptides across the water nitrobenzene interface were determined with the help of a novel electrochemical technique using three-phase electrodes. This is the first time that reliable data are reported for the anions of amino acids. The main result is that the standard Gibbs energies of transfer of the anion and cation of an amino acid are almost the same.

Keywords: Amino acids – Voltammetry – Ion transfer – Lipophilicity

Introduction

Here we report a new approach for the determination of the standard Gibbs energies of transfer of cations and anions of amino acids and small peptides by voltammetry at three-phase electrodes to assess their lipophilicity. The lipophilicity of compounds is regarded as a key for understanding their biological activity, since the passive transcellular transport through membranes involves the transfer from an aqueous to a rather lipophilic environment, and vice versa. The standard method to assess the lipophilicity of compounds is to determine the octanol-water partition coefficients and partition coefficients for other two-phase liquid systems (Karplus, 1997), or related data by chromatography using reversed phases (Plass, 2000). For neutral compounds this can be certainly done in a relatively simple way, although a lot of rou-

tine analytical work is usually involved. For ionic compounds chemical methods can provide information for the salt as a whole, and the lipophilicity of particular ionic species is accessible only on the base of an extrathermodynamic assumption (Grunwald et al., 1960). In the latter case, electrochemical measurements lend themselves for the determination of the Gibbs energies of transfer because the transfer of ions from one phase to the other is an electrochemical process, and because an electrochemical method will yield these values for a single kind of ions and not for the entire salt. However, the only electrochemical method that was known until very recently was the voltammetry at the interface of two immiscible electrolyte solutions (ITIES) with the help of a four-electrode potentiostat (Girault and Schiffrin, 1989). In these experiments, the accessible potential range is very limited (300 to 500 mV) that can be extended either by a microelectrode approach or by coupling chemical equilibria on both sides of the interface, however, all these approaches have only a limited practicability. Recently we have introduced a new experimental arrangement to determine the standard Gibbs energies of ion transfer (Scholz et al., 2000; Komorsky-Lovrić et al., 2001a,b) that is based on coupling an electron transfer and an ion transfer when three phases, the aqueous solution containing the ions to be transferred, an organic solution of an electroactive compound (e.g. decamethylferrocene dissolved in nitrobenzene), and a solid electrode (e.g. graphite) (Donten et al., 2002) are in contact. This

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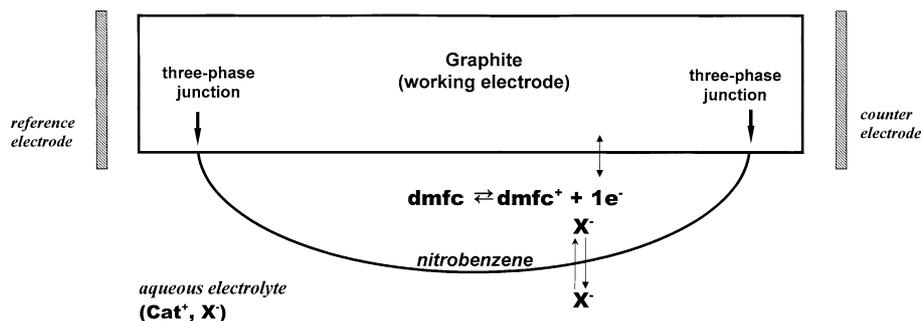


Fig. 1. Schematic representation of the three-phase electrode consisting of a nitrobenzene droplet containing decamethylferrocene (*dmfc*), which is attached to a graphite electrode and immersed in an aqueous salt solution

simple experimental methodology has been already successfully applied to measure the lipophilicity of series of inorganic and organic ions in *n*-octanol, (Gulaboski et al., 2002) and nitrobenzene (Lovrić, 2002). The proposed experimental design is highly attractive since it retains many benefits of conventional aqueous three-electrode voltammetric cells and overcomes some disadvantages of standard ITIES configurations, related mainly to the narrow potential window and a limited number of polarizable organic water interfaces.

Materials and methods

For the determination of the Gibbs energy of transfer of amino acid anions a 0.1 mol dm⁻³ solution of decamethylferrocene (*dmfc*) was prepared by dissolving it in water saturated nitrobenzene. 2 μL of *dmfc* solution were attached as a droplet to the surface of a paraffin impregnated graphite electrode (PIGE) by help of an Eppendorf-type pipette. The approximate radius of the hemispherical droplet was 0.15 cm. The PIGE was a graphite rod with 0.5 cm diameter and an exposed surface area of 0.2 cm², which fabrication is described by Scholz and Meyer (1998). The electrode with the attached droplet was immersed into an aqueous electrolyte solution, containing ions which energy of transfer between water and nitrobenzene has to be measured. For determination of the Gibbs energy of transfer of amino acid anions the aqueous phase contained the sodium salt of the amino acid in a concentration of 1 mol dm⁻³.

All aqueous solutions were saturated with nitrobenzene. All chemicals used were of analytical grade. Decamethylferrocene and amino acids were products of Acrös Organics and Bachem (Heidelberg-Germany), respectively, and were used without further purification. The water used was Millipore Q. All experiments were carried out at 25°C.

For determination of Gibbs energy of transfer of amino acid cations a 0.1 mol dm⁻³ solution of iodine in water saturated nitrobenzene has been prepared. The aqueous phase contained 0.25 mol dm⁻³ hydrochloride salt of amino acids and 0.05 mol dm⁻³ HCl.

The electrochemical oxidation of *dmfc* and reduction of iodine in the three-phase arrangement was studied utilizing a three electrode potentiostat. Square-wave (SW) voltammetry was used as a working voltammetric method (Lovrić, 2002). It is a fast technique providing a precise measurement of the peak potential, i.e. the formal potential of the system. Due to the short measurement time, the nitroben-

zene droplet was stable and neither a significant distortion nor a change of the color of the droplet was observed. SW voltammograms were recorded using the commercial electrochemical measuring system μAUTOLAB (Eco-Chemie, Utrecht, Netherlands). An Ag | AgCl | NaCl (saturated solution) reference electrode ($E = 0.200$ V vs. SHE) was used and a platinum wire served as auxiliary electrode.

Results and discussion

Figure 1 shows schematically the situation when a droplet of a decamethylferrocene (*dmfc*) solution in nitrobenzene (NB) is attached to the surface of a graphite electrode and immersed in an aqueous solution (W) of a salt. When the *dmfc* is oxidised to *dmfc*⁺, two possibilities arise to maintain electroneutrality: either the *dmfc*⁺ is transferred to the aqueous phase where its charge is compensated by anions, or anions are transferred to the nitrobenzene phase to compensate the positive charge of the *dmfc*⁺ ions remaining in this phase (of course, in any case, cations, e.g. H⁺, will be removed on the counter electrode by reduction). What will happen depends only on the Gibbs energies of transfer of both ions. Since *dmfc*⁺ cations are very hydrophobic, it is easy to transfer most of the monovalent inorganic and organic anions from water to nitrobenzene. We have also shown that this new method can be used to determine the standard Gibbs energies of transfer of anions from water to *n*-octanol (Gulaboski et al., 2002). Utilising a neutral compound which can be reduced in the organic phase, e.g. reduction of iodine to iodide, it is even possible to determine the Gibbs energies of transfer of cations (Mirčeski et al., 2002). Here we can show that these two approaches are applicable for measurements of the transfer of rather fragile anions and cations of amino acids and small peptides.

Figures 2 and 3 show SW voltammograms recorded at the three-phase electrodes for the transfer of amino

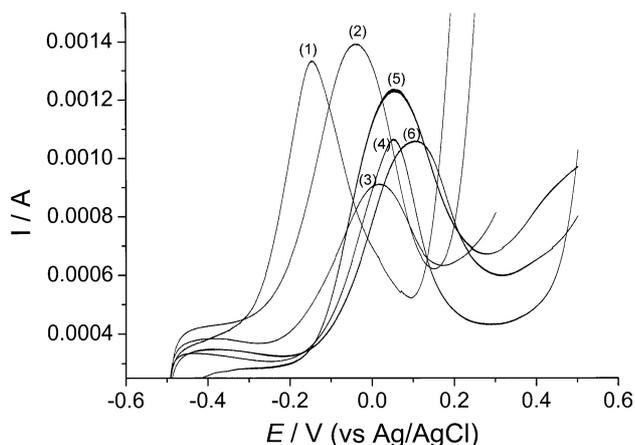


Fig. 2. Square-wave voltammetric responses of a nitrobenzene droplet containing 0.1 mol/L solution of dmfc, which is attached to a graphite electrode and immersed in aqueous solutions containing 1 mol/L NaOH and 1 mol/L sodium salt of the following amino acids: tryptophane (1), tyrosine (2), methionine (3), histidine (4), lysine (5), and proline (6). The other conditions were: SW frequency $f = 100$ Hz, SW amplitude $E_{sw} = 50$ mV, and potential increment $dE = 0.15$ mV

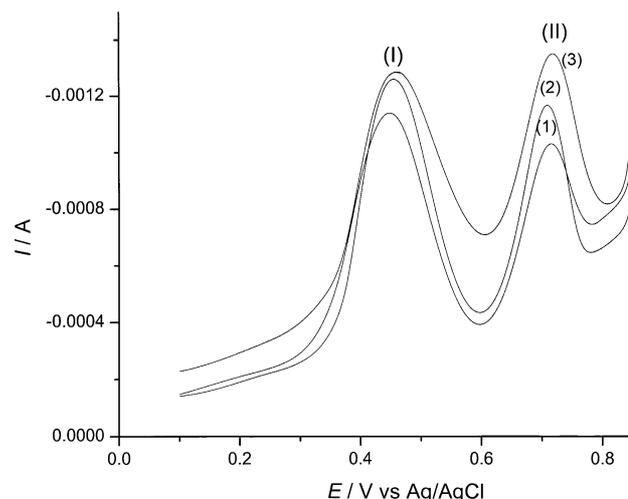


Fig. 3. Square-wave voltammetric responses of a nitrobenzene droplet containing 0.1 mol/L iodine solution, which is attached to a graphite electrode and immersed in aqueous solutions containing 0.05 mol/L HCl and 0.25 mol/L hydrochloride salt of alanine (I), leucine (II), and phenylalanine (3). The other conditions were: SW frequency $f = 50$ Hz, SW amplitude $E_{sw} = 50$ mV, and potential increment $dE = 0.15$ mV

acid anions and cations, respectively. The transfer of anions and cations from water to nitrobenzene was electrochemically driven by the oxidation of dmfc and reduction of iodine in the organic phase, respectively. The Gibbs energies of anion transfer $\Delta G_{X^-}^{\theta}(W \rightarrow NB)$ can be determined because the formal potential of the voltammetric systems E_c^{θ} is related to the standard potential of the ion transfer $\Delta_W^{NB} \varphi_{X^-}^{\theta}$ as follows (Scholz, 2000):

$$E_c^{\theta} = E_{dmfc^+(NB)|dmfc(NB)}^{\theta} + \Delta_W^{NB} \varphi_{X^-}^{\theta} - \frac{RT}{F} \ln(a_{X^-(NB)}) + \frac{RT}{F} \ln\left(\frac{a_{dmfc(NB)}^*}{2}\right) \quad (1)$$

$$\Delta G_{X^-(W \rightarrow NB)}^{\theta} = -\Delta_W^{NB} \varphi_{X^-}^{\theta} zF \quad (2)$$

Here $a_{dmfc(NB)}^*$ is the activity of dmfc in the bulk of the organic phase, X^- is the symbol for transferable anions, z is the charge of the ions, and other symbols have their usual meanings. The formal potentials can be derived from cyclic voltammograms as the mid-peak potentials (Scholz, 2002), or from square-wave voltammograms as the peak potentials (Lovrić, 2002). The standard potential of the $dmfc^+ | dmfc$ redox couple $E_{dmfc^+(NB)|dmfc(NB)}^{\theta}$ in nitrobenzene is -0.184 V vs Ag/AgCl, that has been determined by calibrating the system with ions with known standard Gibbs energies of transfer, which is depicted in Fig. 4.

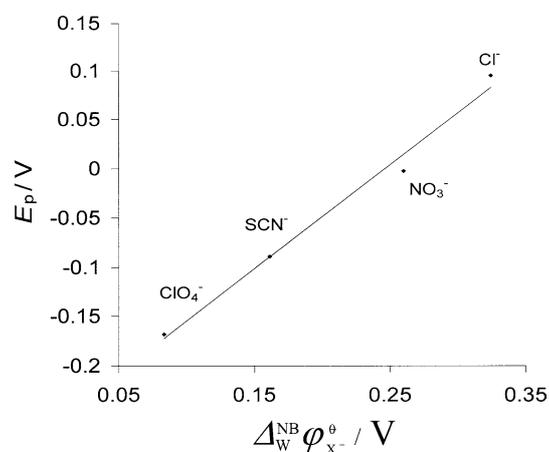
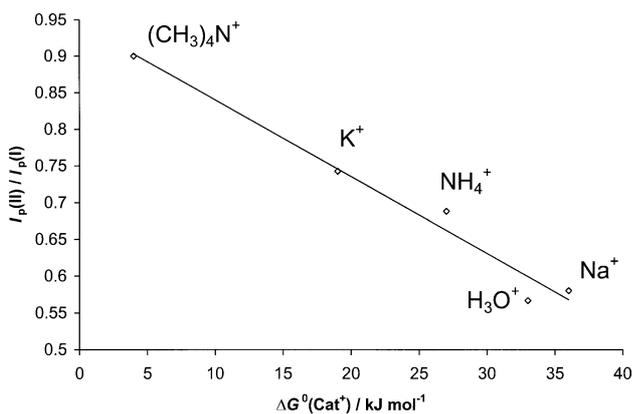


Fig. 4. The dependence of the peak potential of the SW voltammetric response of a nitrobenzene droplet, containing 0.1 mol dm^{-3} dmfc solution recorded in different aqueous electrolyte solutions, on the standard potential of transfer across water | nitrobenzene interface of some anions. All other conditions were the same as in Fig. 2

In the case of cation transfer, the evaluation is more complex, as the following mechanism is operative: When the organic phase containing iodine is in contact with the aqueous solution containing chloride ions and certain cations, a spontaneous chemical partition of the chloride salt, facilitated by the formation of I_2Cl^- , occurs between the organic and aqueous phase. As a consequence, a significant amount of chloride ions and co-partitioned cations are introduced in the organic

Table 1. Gibbs energies of transfer of amino acid anions and cations and peptide anions across the water | nitrobenzene interface

Amino acids and peptides	Formulae	Anion $\Delta G_{X^-}^{\theta}(W \rightarrow NB) / \text{kJ mol}^{-1}$	Cation $\Delta G_{C^+}^{\theta}(W \rightarrow NB) / \text{kJ mol}^{-1}$
Glycine	$C_2H_5NO_2$	26.60	/
Alanine	$C_3H_7NO_2$	27.53	27.03
Valine	$C_5H_{11}NO_2$	26.79	26.60
Leucine	$C_6H_{13}NO_2$	23.90	28.90
Phenylalanine	$C_9H_9NO_2$	21.00	19.00
Tyrosine	$C_9H_9NO_3$	21.18	20.04
Methionine	$C_5H_{11}NO_2S$	24.55	/
Tryptophane	$C_{11}H_{12}N_2O_2$	10.80	/
Lysine	$C_6H_{14}N_2O_2$	27.30	28.71
Proline	$C_5H_9NO_2$	29.52	/
Histidine	$C_6H_9N_3O_2$	27.71	29.09
2-Glycine	$C_4H_8N_2O_3$	26.97	/
3-Glycine	$C_6H_{11}N_3O_4$	26.05	/
2-Alanine	$C_6H_{12}N_2O_3$	27.78	/

**Fig. 5.** The correlation of the peak current ratio with the standard Gibbs energy of cation transfer from water to nitrobenzene for different cations present in the chloride aqueous phase with a concentration of 0.25 mol/L ($R^2 = 0.9742$). Other conditions were the same as in Fig. 3

phase. Upon reduction of iodine to iodide, chloride ions are transferred from nitrobenzene to the aqueous phase. The mechanism is such that the rate of the overall reaction, and thus the height of the voltammetric response (SW peak II in Fig. 3), is directly proportional to the standard Gibbs energy of cation transfer, because the cations are co-partitioned in the preceding partition. At more negative potentials a second process appears (SW peak I) which is attributed to the reduction of iodine to iodide, connected with an expulsion of iodide from nitrobenzene to water. It has been demonstrated that the measurement of the ratio of these two peaks depends linearly on the standard Gibbs energies of transfer of the cations, and a calibra-

tion can be easily obtained by using some ions with known transfer data, as depicted in Fig. 5 (for details of the mechanism see ref. Mirčeski et al., 2002).

Table 1 summarises the experimentally determined data of some amino acid and small peptide cations and anions. For all anions a plot of the formal potentials versus anion concentration gave slopes between -50 and -70 mV, what is in agreement with equation (1). As can be seen from Table 1, the data for some cations are missing. These amino acids are either chemically reactive with iodine, affecting significantly the reduction of iodine at the three-phase electrode, or their energies of transfer as cations are higher than the energy of transfer of protons present in the aqueous solutions that can be concurrently transferred across the W | NB interface.

Analysing the data presented in Table 1 it is important to note that there is only a small difference between the energy of transfer of the anion and cation of a respective amino acid. A possible explanation can be provided by the simple Born theory. According to the Born electrostatic theory, ions are considered as hard spheres and their energy of transfer between two immiscible liquids depends only on their ionic radii and charges, and the dielectric constants of both solvents. Thus, on the base of this theory, it is expected that both the amino acid cation and anion have a similar energy of transfer, since their ionic radii are almost equal. However it is well-known that the Born theory is rather approximate, since it does not take into account the short range interactions between solute and the solvent. The energy of ion transfer is affected by

the formation of cavities in the organic phase, co-transfer of water molecules, and charge transfer interactions between the ions and the solvent, (Osakai and Ebina, 2001). All these effects depend on both the radii and charges of the ions, as well as their chemical properties. Due to appreciable similarities in the chemical structure of the cationic and anionic forms of an amino acid, it is reasonable to suppose that all mentioned phenomena play a similar role in the transfer of both amino acid ions. It is furthermore worth noting, that the ion transfer process can be also influenced by some steric constraints. For instance, the energy of transfer of simple inorganic ions from water to some polar organic solvent depends significantly on the accessibility of the opposite charge in the polar group of the organic solvent (Fernandes et al., 2002). If the molecular structure of the organic solvent permits electrostatic interactions with the transferred ions without any steric constraints, an easy transfer has to be expected. In the present system, considering the charge distribution of the nitrobenzene molecule, it is known that it is a weak dipole, with its negative part on the side of the nitro-group, and the positive part on the side of the aromatic ring. Both parts of the nitrobenzene molecules are equally accessible for both cations and anions, which further supports the experimental results. It should be finally pointed out that the energies of transfer of the cationic forms of phenylalanine, alanine, and valine are in good agreement with the data obtained by Takeda et al. (1995) applying chemical methods. In the latter study it has been found that the difference between the energy of transfer of the cationic and neutral form of an amino acid is a constant value of about 5 kJ mol^{-1} . This is an important fact which implies that the lipophilicity of the neutral form of the amino acid can be assessed by measuring the energy of transfer of its cationic form.

Table 1 also gives the data of some very simple peptide anions. Obviously, the Gibbs energies of transfer of the anions of glycine and triglycine are very similar to that of glycine, and the same holds true for di-alanine and alanine. A continuation of this study will allow to build up an increment system that allows to calculate the lipophilicity of larger peptides, with the aim to get reliable data for modelling hydrophobic interactions and folding of proteins.

This study shows that the new method allows the determination of Gibbs energies of ion transfer of rather fragile ions of biologically important compounds.

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