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HARDNESS OF AMINO ACIDS

by

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ABSTRACT

Definitions of molecular, atomic and functional group hardness are applied to amino acids. It is concluded that amino acids are soft substances, arginine and lysine being the softest ones. The zwitterionic forms exhibit hardness values within a limited range. The hardness of amino acids is related to structural characteristics.

Key-words: Hardness; Softness; Amino acids.

1. Introduction

The concept of hardness in connection with chemical reactivity has been introduced a long time ago [1], giving rise to ample qualitative work on the subject.

In the last few years, plenty of effort has been devoted to provide a quantitative scale for the hardness/softness concept, be it for isolated atoms, atoms in molecules or molecules [2-6].

Most definitions are based on the finite difference approximation relating hardness to valence state ionization potentials and electron affinities [2]. Another approach distinguishes between chemical and differential hardness [7]. We ourselves proposed to relate the softness of an atom within a molecule to its self-charge [8]. From this approach we deduced a prescription for the softness of an atomic functional group [9]. Finally, we offered an operational definition of molecular hardness which depends on whether N, the number of electrons in the molecule, is even or odd. For odd N, the usual (I-A) expression is found to be appropriate. For even N, hardness is instead determined by the interaction between electrons in the HOMO and the LUMO [10].

We apply here the above definitions to the twenty essential amino acids. The hard-soft-acid-base (HSAB) principle [1] does not seem to have been applied to biological molecules. Among these, amino acids form a particularly challenging family of compounds, for the acid and basic character are found in the

same molecule. This peculiarity, together with the existence of a specific side chain for each amino acid, to which many properties are ascribed, motivates a study of amino acids hardness. As a first approach to this problem, we shall calculate molecular hardness and the hardness of different atomic groups in order to explore their chemical significance.

2. Hardness definitions

The chemical potential μ (or electronegativity χ) of an N-electron species is accepted to be [11]

$$\mu = \partial E/\partial N = -\chi \qquad (1)$$

E being the electronic energy. The absolute hardness has been defined as [2]

$$\eta = \partial^2 E/\partial N^2 \qquad (2)$$

The finite difference approximation [2] leads to

$$\chi = (I+A)/2 \quad ; \quad \eta = I-A \qquad (3)$$

where I and A are respectively the ionization potential and the electron affinity. In Ref. [8] we obtained

$$\partial q_A/\partial \mu_A \Big|_{\mu_A=\mu} = -\beta I_{AA}/2 \qquad (4)$$

In Eq. (4), q_A is the electronic charge of atom A in the N-electron system, I_{AA} its self-charge (duly unburdened of core and lone pair electrons) and $\beta = 1/kT$. We also obtained

$$s_A = 1/\eta_A = -\beta I_{AA}/2 \qquad (5)$$

It is most natural to extend this expression to a functional group G within a molecule [9]

$$s_G = -\beta \sum_{A,A' \in G}' (I_{AA}+I_{AA'})/2 \qquad (6)$$

including the internal active charge I_{AA}, within the self-charge of the group and taking into account that [12]

$$q_A = (I_{AA} + \sum_{A \neq B}' I_{AB})/2 \qquad (7)$$

The electronic charge in Eq.(7) is the same as Mulliken's; the partition however is quite different.

The variation in the number of electrons Δq_A which appears in Eq.(4) must be understood as a statistical average [8]. For the molecule as an entity, ΔN has a different statistical meaning, for a molecule actually looses or acquires electrons under reaction. Thus, it is not straightforward to relate the hardness of a molecule to the hardness of the atoms within it.

For the molecular hardness (N even), formula (2) must take into account the fact that N changes because the occupation numbers of HOMO and LUMO are changing [10]. Thus

$$\eta = \partial^2 E/\partial n_i \partial n_{i+1} \qquad (8)$$

where n_i (n_{i+1}) is the HOMO(LUMO) occupation number.

In the Roothaan equation for a closed-shell system, the energy may be written as [13]

$$E = \sum_i' n_n H_{ii} + (1/4) \sum_{i \neq j}' n_i n_j (J_{ij}-K_{ij}) \qquad (9)$$

where H_{ii} is the expectation value of the one-electron core Hamiltonian corresponding to the i-th MO, J_{ij} and K_{ij} the Coulomb and exchange integrals respectively. Eq.(8) becomes then

$$\eta = (2J_{i,i+1} - K_{i,i+1})/2 \qquad (10)$$

that is, hardness of a closed-shell species is determined by the interaction of electrons in the HOMO and in the LUMO [10].

3. Results and discussion

The initial geometry has been taken from Ref.[14], optimized through an MNDO program [15]. The results shown in the tables follow from a CNDO calculation, accurate enough for a first semi-quantitative study.

Table 1 shows the η values for the twenty essential amino acids, calculated with Eq.(10). Amino acids appear mostly in the dipolar ionic form (zwitterions); the corresponding results are hence the most significant in this work. Nevertheless, we have shown also η for the neutral form, for the sake of comparison. We have attempted to link our results to some structural and reactivity features of amino acids.

Most of the zwitterions have similar hardness (around 4 eV), although they differ in shape, size, polarity, solubility, etc. Now, the hardness/softness concept has been introduced precisely to acknowledge that Lewis acids/bases tend to react with other ones of similar hardness/softness [1]. The known hardness/soft-

ness scales have not been able, up to now, to span the same range of values for acids and bases [2,5,9,16]. It seems therefore stimulating that amino acids appear to be soft species (see below), both acid and basic in character, their ease in combining with each other being related to their similar softness.

We do not find any direct correspondence between the η values and the classical groups according to the side chain; only the basic group shows considerably smaller values of η.

In Eq.(10), for zwitterions, hardness is due only to the Coulomb contribution except for (Met, Phe, Asn, Glu, Tyr) where the exchange integral has a very low absolute value of about 0.01 eV. The neutral form shows a quite different behaviour. Ser, Glu, His and Arg have a $|K|$ value less than 0.01 eV, while for the other amino acids it is higher, reaching 0.30 eV for Met and 0.15 eV for Cys; both Met and Cys contain a sulphur atom.

The pairs (Val, Leu), (Asn,Gln), (Asp, Glu) differ only in one methylene (CH_2). However, while the first two zwitterionic pairs have very close hardnesses, the acidic pair shows appreciably different values.

The only structural difference between Cys and Ser is that Cys has a sulphur atom where Ser has an oxygen; $\eta(\text{Cys}) < \eta(\text{Ser})$. We had already found in much simpler compounds (XH_n-type) that replacing a first-row with a second-row atom of the same group

in the periodic table softens the molecule [10].

Four amino acids have aromatic rings in the side chain. Of them, the benzene ring in Phe and Tyr does not seem to affect η, while the indole ring in Trp and imidazole in His could have an influence in lowering hardness. It is known that the imidazole groups in His are active sites in many enzymes [17].

Arg and Lys are the softest amino acids. The softness of Arg may be related to the fact that it appears among the main types of energy-rich substances; through phosphorylation of its guanidino group, it functions as an energy pool [18]. Also, a highly specific cleavage in the carboxyl side of Arg and Lys of polypeptide chains is obtained through trypsin.

The neutral form sweeps a much wider hardness range (2.1-11.4 eV), offering a greater discrimination among amino acids. The change to zwitterionic form decreases systematically the value of η, that is zwitterions are softer than the neutral form; under ionization amino acids become more labile.

The values of η obtained through Eq.(10) yield an absolute scale for hardness for molecules calculated with the same method. We have calculated elsewhere [10] the hardness of a number of small molecules, where the range of η was 4.53-19.72 eV. Amino acids under zwitterionic form are softer than any of them, in particular softer than H_2O (η=17.46 eV) and NH_3 (η= 16.11 eV). The neutral form shows a certain overlap with the mentioned range.

The functional group softness s_G calculated with Eq.(6) depends in an obvious way upon the number of atoms in the group. Both the α-amino and the α-carboxyl groups have practically constant values of s_G for all amino acids, being respectively 13.84-13.92 and 16.52-16.68 in β units. In Pro, the amino group is secondary rather than primary, thus showing a different s_G of 11.40.

We have shown [9] that the alcohol, aldehyde and acid functional groups fulfil the transferibility requirement in derivatives of methane, ethane, propane and butane. Table 2 shows s_G for some side chain groups. Transferibility seems to be obeyed by β(γ)carboxyl, isopropyl and by $-NH_2$ (note that in arginine NH_2 does not belong to an amide group), while -OH has three distinct values; in Thr there is a branched hydrocarbonated chain, the -OH in Tyr belongs to a phenolic group.

The atoms in the main chain have, as expected, characteristic atomic softnesses along the amino acid series. The carboxylic group carbon is harder than the other carbon, and O^- harder than O.

Both HOMO and LUMO of the amino acids fall within three diverse energy "bands", depending on whether the zwitterion is neutral (polar or nonpolar), acidic or basic; they are reported in Table 3.

One could wonder if the HOMO and LUMO wavefunctions Ψ are

ascribed to the main chain or to the side chain, or if they are delocalized along the whole molecule. An inspection of the frontier orbitals leads ~~to the~~ striking result

$$\Psi_{HOMO} \simeq 0.6\ p(O_1) \pm 0.8\ p(O_2) \qquad (11)$$

with the apparent exception of Pro and Asp. Actually, even in these the main contributions to the HOMO arise from the carboxylic oxygens, O_2 denoting O^-, with an increased mixture of 2p orbitals instead of the pure 2p of expression (11).

Although the LUMO is not equally constant, in most amino acids the largest contributions may be similarly ascribed to the main chain amino group atoms:

$$\Psi_{LUMO} \simeq 0.6(N) \pm 0.4(H+H'+H") \qquad (12)$$

Trp, His and Arg disagree with this behaviour, their LUMO being due to lateral chain contributions; in Pro and Cys, the LUMO mixes main chain and side chain contributions.

In short, it may be said that the hardness of amino acids is chiefly due to the main chain interaction between the carboxylic oxygens (HOMO) and the amino group atoms (LUMO).

Expressions (11) and (12) give a hint in another direction. Amino acids, as we have seen, are all more or less equally soft, despite the variety in their chemical formulae. They look hence, in the sense of Refs. [19,20], as similar molecules. Furthermore, from (11) and (12) their frontier orbitals are also very similar.

Similarity criteria furnish a quite peculiar information which seems worthwhile investigating further. Softness/hardness could be matching parameters.

4. Conclusions

- The twenty common amino acids are harder in the neutral form than in the zwitterionic form; most of them are predicted to have, in the last form, a hardness of about 4 eV.

- Amino acids are soft substances; in particular, much softer than water. Arginine and lysine are the softest.

- The α-amino and α-carboxyl groups have characteristic softness values for all the series.

- The hardness of amino acids is due, in most of them, to the main chain interaction between the carboxylic oxygens (HOMO) and the amino group atoms (LUMO).

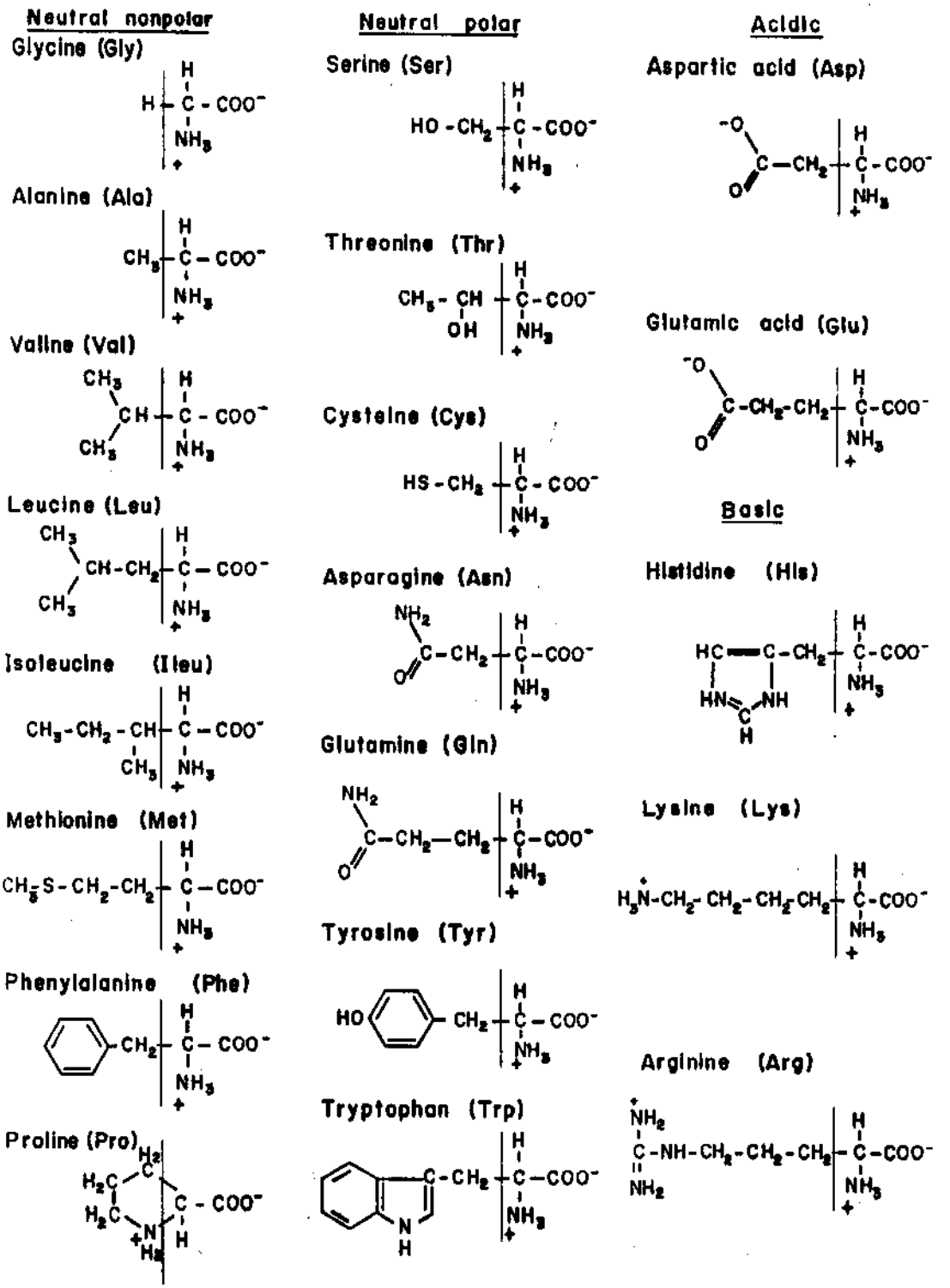

FIG.1

Structure of the 20 common amino acids.

Amino acid	η (eV)		Side chain
	Zwitterion	Neutral	
Gly	4.13	9.62	
Ala	4.15	9.44	
Val	4.14	8.97	
Leu	4.15	8.80	Neutral
Ilu	4.14	8.72	nonpolar
Met	4.15	10.44	
Phe	4.43	5.31	
Pro	4.14	7.63	
Ser	4.24	9.19	
Thr	4.24	8.94	
Cys	3.74	11.37	Neutral
Asn	4.36	6.91	polar
Gln	4.47	5.44	
Tyr	4.41	6.72	
Trp	2.39	6.94	
Asp	4.00	7.72	Acidic
Glu	2.77	4.29	
His	2.26	3.54	
Lys	1.74	8.13	Basic
Arg	1.70	2.07	

Table 1 : Hardness η for amino acids, calculated from eq.(10)

Table 2 : Softness (s_G) for some side chains groups, calculated from eq.(6).

Amino acid	Side chain group			
	-OH	$-NH_2$	Isopropyl	β(γ) Carboxyl
Val			18.39	
Leu			18.46	
Ser	8.72			
Thr	7.23			
Asn		9.66		
Gln		9.66		
Tyr	6.57			
Asp				17.39
Glu				17.34
Arg		9.22		

Table 3 : HOMO and LUMO range (in eV) for amino acids.

Amino acid group	HOMO	LUMO
Neutral polar and nonpolar	-0.34; -0.32	0.08; 0.11
Acidic	-0.17; -0.15	0.21; 0.24
Basic	-0.46; -0.41	-0.15;-0.06

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