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REGULAR ARTICLE

Exploring the potential energy surfaces of association of NO with aminoacids and related organic functional groups: the role of entropy of association

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Abstract Association between NO and each of the 20 amino acids and their related organic functional groups was studied by exploring the configuration space of the potential energy of association surface by using the multiple minima hypersurface procedure. AM1 semiempirical Hamiltonian was used in order to explore such complex hypersurfaces of biological molecular interactions at finite computational times. An appropriate test with a set of NO and small molecule complexes obtained at the MP2/6-311++g(2d,2p) level of theory was also carried out. Stabilization energies of larger models were also evaluated at the conventional PBE1PBE/6-31g(d,p) DFT level. NO–aminoacid hypersurface explorations yielded that interactions of NO with NH group together with the C=O belonging to the backbone appeared predominant in all cases. Models of polar aminoacids and NO also show stable interactions with the lateral chains. Interactions with charged amino acids were found as the most stable and Lys was, undoubtedly, the preferred association. The study of these kinds of interactions must take into account the deepest and other minima because the entropy of association plays an important role.

Keywords Aminoacids · Nitric oxide interactions · MMH

Contribution to the Serafin Fraja Memorial Issue.

Dedicated to Prof. Serafín Fraga, an unforgettable friend.

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1 Introduction

The chemistry of certain diatomic molecules is very important to understand key biological processes. Especially diatomics such as $^3O_2$, CO and NO regulate cellular respiration and many other functions. The sites for molecular association of small molecules like these are, necessarily, less specific than those that involve drugs, vitamins, and other life active species. Proteins or oligosaccharides fixing active molecules show, generally, a docking site that is specific to complement the spatial structure and charge distribution of the arriving molecule. A simple chemical reasoning brings the conclusion that interactions of simple molecules with proteins must be represented by several different structures with different and significant populations. Therefore, the very-frequently-found-in-literature calculation of a single most stable structure, i.e., dismissing entropic effects, is often a partial modeling of a real object, and not necessarily conducting to good and useful knowledge about the molecular process under study.

In the last decade, enormous efforts have been done in the field of NO research, because of the discovery of its role in many physiological functions such as neurotransmission, platelet aggregation, regulation of blood pressure, heart contractility, host defense, and others. A family of enzymes known as the NO synthases converts L-arginine into L-citrulline and NO [1–3].

There are also other proteins such as nitrophorines that transport NO in serum under certain conditions. In such cases, the biological functions of NO are related with its interaction with proteins; in particular those that contain *heme*, iron–sulfur, zinc–sulfur and copper clusters [4] Nevertheless, in these processes the role of amino acids belonging to the protein chains is very important, determining the ligand diffusion to the active sites, the specific function, and its

modulation. Many proteins with the same active site have different functions, eg., the case of *heme* proteins [5]. The amino acids that appear directly linked to the active site (proximal residue) and those in the cavity (distal pocket) change the electron density of the active site and can establish direct interactions with ligands. In the case of myoglobins, some cavities which allow for small molecules have been explored [6,7]. At the same time, external aminoacids could serve as scavengers for further fixation of NO in a *heme* site.

This work approaches NO–amino acid pair interactions independent of the different kind of cavities where they could be located in a given protein. It takes into account only the electron exchange in a single site which is the ultimate cause of such interactions. Therefore, in order to obtain an understanding of the interactions between each one of the 20 most common aminoacids in proteins with NO, the multiple minima hypersurface (MMH) approach was used. MMH combines quantum chemical Hamiltonians (for the calculation of the internal energy of molecular clusters) with statistical weighting after the generation of an ensemble of the most significant molecular pairs by means of formulae for the calculation of thermodynamic association functions. In our case, the AM1 Hamiltonian and PBE1PBE hybrid functional were used. In addition, and because of intrinsic inaccuracy of semiempirical calculations, the reliability was tested for a set of intermolecular complexes between NO and small molecular models.

One of the pioneers in the computational prediction of the structure of proteins was the late and never forgotten Prof. Serafin Fraga [8]. This work has been inspired by his teaching and know-how, generously and largely imported to one of us (LAMC) several years ago. Now, the obtained results of this paper are intended to be employed for molecular interpretation and understanding of the huge biological data related with several NO functions and its relationship with protein structural building blocks.

2 Models and methods

The treatment of molecular interactions remains as a challenging field for theoretical modeling. The use of accurate methods based on the SCF approach is limited to systems with a reduced number of atoms. Besides, the problem of basis set superposition error (BSSE) appears as a spurious stabilization for interacting systems both at HF and popular Kohn–Sham's DFT levels [9].

Molecular mechanical methods are not always appropriate because of the specific quantum features of certain molecular interactions that are not taken into account in general parameterizations of classical potentials. Independent of the well-known shortcomings, semiempirical methods remain competitive and useful in the elucidation of molecular interactions, considering that they take into account quantum effects [10]. These methods seem not affected by the BSSE probably due to the orthogonality of the atomic orbital basis set. Correlation effects are also implicitly considered during the parameterization procedures with respect to experimental values. However, the most important fact to decide exploration of hypersurfaces with parametric, i.e., semiempirical, Hamiltonians is that they allow a huge number of calculations with a lower computational cost than ab initio methods. Their principal disadvantages are the occarional erratic accuracy and the lack of ab initio theoretical rigor [11,12]. Our previous paper shows that this problem appears to have overcome in the case of water clusters, apparently because the cancellation of errors in statistical weighting and the calculation of relative association energies [12].

The size of NO molecule, in comparison with any of the 20 naturally selected amino acids, is quite small. Therefore, a key point of validity for models is that several stable configurations of one NO molecule may coexist around each amino acid, all of them being at a sufficiently low energy for contributing to the ensemble properties. Consequently, a large set of these atomic arrangements should be explored with a subsequent increment of computational cost, which should be significantly large if more accurate Hamiltonians were employed. For this reason, the use of semiempirical methods should be the choice in order to explore the energy hypersurfaces of the complexes, although they must be tested for reliability.

In our work the AM1 semiempirical Hamiltonian was selected for this purpose. Its performance for this kind of systems was tested with respect to accurate ab initio MP2/6-311++g(2d,2p) calculations in cases of selected small models of molecular interactions. This level of theory shows a good performance for the CH_4–NO complexes, as it was shown in a previous work [13]. Therefore, all small complexes were optimized at both ab initio and semiempirical levels. The stabilization energies of the complexes obtained with the ab initio procedures were corrected from BSSE using the counterpoise procedure (CP) of Boys and Bernardi [14]. The models were so simple that the lateral chains were imitated with the following molecules: CH_4, NH_3, NH_4^+, H_2S, H_2O and C_6H_6. For the peptidic bond, the $HCONH_2$ molecule was used. A group of symmetric complexes was selected (Figs. 1 and 2) These calculations were performed using Gaussian 03 program [15]. All geometrical optimizations were carried out employing a tight convergence criterion.

Then, in order to simulate the electronic density of amino acids in proteins, a model system was used (Fig. 3), R being the lateral chain that imposes the difference between amino acids. All 20 naturally occurring amino acids were studied; starting from the most stable conformation obtained from "in vacuo" calculation. These conformers are similar to those leading to the β sheet form (the NH and CO groups being

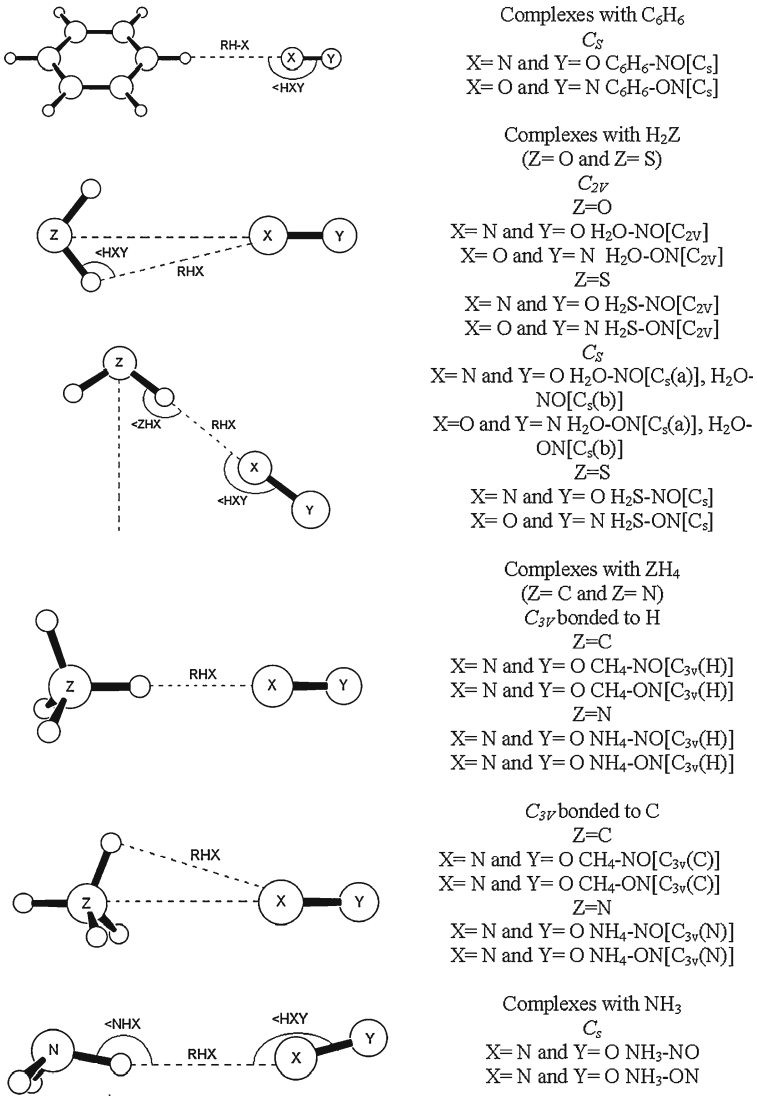

Fig. 1 Conformations calculated for the selected complexes for modeling the lateral chains

almost parallel). In this model, peptide bonds are simulated with dimethylamine and acetyl groups. $N(CH_3)_2$ was used instead of $NHCH_3$ because the latter group shows a great affinity to NO and introduces subsequent fictitious interactions that are not related with a particular amino acid, as we accurately tested. In any case, all interactions with these blocking groups were carefully eliminated from our aminoacid–NO molecular sets in order to avoid artifact entropic values.

In order to analyze the results, amino acids were classified in four groups according to the polarity of the R moiety: nonpolar (aliphatics and aromatics), polar without charge, polar with negative charge (acids) and polar with positive charge (bases).

The MMH procedure [11,12,16–19] is used to explore the energy hypersurface and subsequently to find minima structures that have a significant contribution to thermodynamical properties. MMH has been successfully employed in the study of several systems [16,20–22]. It combines the use of a quantum Hamiltonian for the calculation of atomic arrangement energies of one or several molecules around another molecule and statistical thermodynamics for the calculation of collective properties by means of a Boltzmann distribution. Isolated molecules (in this case, the amino acid and NO) were optimized independently first using a semiempirical method (AM1 in this work). After that, a group of random arrangements of NO around each amino acid were generated using a program called GRANADA, where randomness has been carefully tested [11]. Then, a gradient driven path for optimizing each initial random structure is followed until the desired threshold. In some cases, different arrangements converged to the same minimum, and then the Tanimoto similarity index was used in order to eliminate redundant structures. All minima related with interactions of NO with our artificial blocking groups were also eliminated. Then, the partition function for the system is calculated assuming a canonic ensemble with the isolated

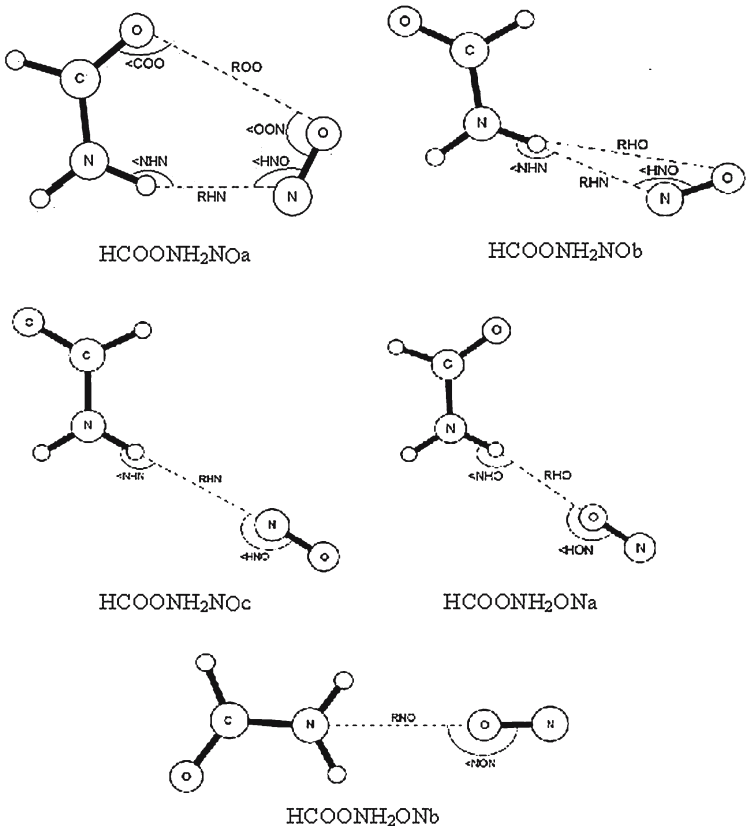

Fig. 2 Complexes between nitric oxide and formamide

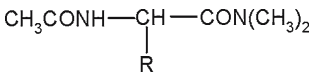

Fig. 3 Aminoacid model

molecules taken as reference. Thermodynamic properties, such as association energy, entropy, and Helmontz free energy are then calculated by this procedure [11,12], according to the following formulae:

$$\Delta E^{\text{assoc}} = E - E^{\text{ref}} = RT^2 \frac{q^{*\prime}}{q^*} \tag{1}$$

$$S^{\text{assoc}} = S - S^{\text{ref}} = R \ln q^* + \frac{E^{\text{assoc}}}{T} \tag{2}$$

$$\Delta F^{\text{assoc}} = F - F^{\text{ref}} = -RT \ln q^* \tag{3}$$

where the partition function is:

$$q^* = \sum_i g_i \mathrm{e}^{-\Delta\varepsilon_i/\mathrm{RT}} = q\mathrm{e}^{\varepsilon^{\text{ref}}/RT} \tag{4}$$

and each cell energy with respect to the reference scale, $\Delta\varepsilon_i$, is:

$$\Delta\varepsilon_i = \varepsilon_i - \varepsilon^{\text{ref}} \tag{5}$$

where

$$\varepsilon^{\text{ref}} = \varepsilon_{\text{tot(aa)}} + \varepsilon_{\text{tot(NO)}} \tag{6}$$

where $\varepsilon_{\text{tot(aa)}}$ and $\varepsilon_{\text{tot(NO)}}$ are the total energies of isolated amino acids and NO, respectively.

As mentioned, optimizations are performed using the AM1 Hamiltonian with the MOPAC v. 6 program [23]. The *eigenvector following* (EF) routine for searching minima was used in all cases. The association energies were also evaluated at PBE1PBE/6-31g(d,p) level employing the AM1 geometries. All convergence criteria were increased 100 times with respect to defaults. Semiempirical approaches do not reproduce correctly the rotational barrier of peptidic bonds: here a molecular mechanic term that corrects this systematic error was employed. All figures shown here were made with ORTEP v. 3.0 [24].

3 Results

3.1 Calculations on small models for testing reliability of semiempirical Hamiltonians

3.1.1 Lateral chain models

The selected small molecules represent all aliphatic, aromatic, polar hydroxyl, and thiol groups that are present as residues of the 20 naturally occurring amino acids.

Table 1 Tests of AM1 Hamiltonian toward MP2 calculations[a]

Complexes	ΔE_{AM1}	$\Delta E_{\mathrm{MP2/6-311++G(2d,2p)}}$	$\Delta E^{\mathrm{CP}}_{MP2/6-311++G(2d,2p)}$[a]	$\Delta E_{\mathrm{PBE1PBE/6-31G(d,p)//MP2/6-311++G(2d,2p)}}$
C_6H_6–NO[C_s]	−0.2	−3.2	−1.5	−1.9
C_6H_6–ON[C_s]	−1.3	−3.3	−1.0	−1.5
H_2O–NO[C_s(a)]	–	−6.4	−4.8	−7.6
H_2O–NO[C_s(b)]	−0.5	−5.9	−4.3	−7.1
H_2O–ON[C_s(a)]	−3.6	−3.5	−1.8	−6.8
H_2O–ON[C_s(b)]	−3.4	−3.5	−1.8	−2.8
H_2O–NO[C_{2V}]	−2.7	−4.4	−2.9	−5.1
H_2O–ON[C_{2V}]	−6.4	−2.7	−1.0	−5.2
H_2S–NO[C_s]	−2.0	−4.1	−1.2	−2.1
H_2S–ON[C_s]	−1.1	−3.2	−2.5	−4.3
H_2S–NO[C_{2V}]	−1.2	−3.7	−2.4	−3.2
H_2S–ON[C_{2V}]	−1.2	−2.9	−1.2	−2.3
CH_4–NO[C_{3v}(H)]	0.0	−1.5	−0.8	−1.4
CH_4–ON[C_{3v}(H)]	−1.2	−1.9	−0.6	−1.2
CH_4–NO[C_{3v}(C)]	0.0	−1.4	−0.6	−0.8
CH_4–ON[C_{3v}(C)]	0.0	−2.2	−0.8	−0.8
NH_4–NO[C_{3v}(N)]*n*	−17.0	−11.0	−8.7	−17.0
NH_4–ON[C_{3v}(N)]	−16.3	−18.7	−17.2	−14.5
NH_4–NO[C_{3v}(H)]	−11.1	−24.2	−21.5	−23.4
NH_4–ON[C_{3v}(H)]	−14.1	−13.7	−10.5	−18.8
NH_3–NO	−1.0	−3.4	−1.5	−2.6
NH_3–ON	−8.5	−2.4	−1.2	−2.6

Interaction energies in kJ mol were calculated as the difference between the complex and the isolated molecule energies (ΔE=E(molecule $\cdots$ NO)-E(molecule)-E(NO))

[a] $\Delta E^{\mathrm{CP}}_{\mathrm{MP2/6-311++G(2d,2p)}} = \Delta E_{\mathrm{MP2/6-311++G(2d,2p)}} + \mathrm{BSSE}$

Table 1 shows the stabilization energies obtained at all AM1, MP2/6-311++G(2d,2p) (BSSE uncorrected and corrected) and PBE1PBE/6-31G(d,p) levels. The late hybrid functional had demonstrated a relative good performance to treat weak interactions [25]. Calculations with PBE1PBE/6-31G(d,p) were done employing the MP2 geometries and the stabilization energies show a mean absolute error of 2.2 kJ/mol with respect to those at the MP2/6-311++G(2d,2p) level results with BSSE correction, which is supposed to be the best model. The equivalent error of AM1 is 2.5 kJ/mol. If we exclude the case NH4–NO[C_{3v} (H)], AM1 mean absolute error reduces to 1.8 kJ/mol and DFT to 2.0 kJ/mol. The order of stabilities is well reproduced and for this reason this method was selected for the calculation in larger models of aminoacids. It must be pointed out that, in spite of the important effect of BSSE in absolute energies, the order of stabilities of different conformations does not significantly change and therefore, uncorrected energies can be used from the qualitative point of view.

The selected models were found useful to evaluate the ability of AM1 Hamiltonian for the study of complexes with hydrogen joined to aromatic molecules and others. For the benzene–NO system, a couple of planar configurations were tested: C_6H_6–NO[C_s] and C_6H_6–ON[C_s] (Fig. 1), as the structures related to interaction with one hydrogen atom and NO oriented by O or N atoms. The AM1 stabilization energy for the C_6H_6–ON[C_s] is in the order of that obtained at BSSE corrected by MP2/6-311g++(2d,2p) level (Table 1). For the N oriented complex, the obtained stabilization energy is poor and the obtained complex is only slightly attractive. Geometries of all these complexes with all methods basically maintain the conformation, and the distances to hydrogen atom are overestimated around 0.3 Å for N orientation at AM1 with respect to the distances calculated at MP2/6-311++g(2d,2p), while the same distance to O atom in the equivalent complex is underestimated in the same amount (Table 2).

A set of complexes where the interaction is with molecules of H_2Z stoichiometry were studied. The Z atom could be sulphur or oxygen, representing the weak complexes of NO with H_2O and H_2S. These kinds of interactions could appear in amino acids such as cysteine and methionine. The study of complexes with water is interesting. First, water is the most important solvent and is really ubiquitous in biological conditions. Second, the water molecule could serve as

Table 2 Geometrical parameters for lateral chain models[a]

Complex	RHX (Å)		<HXY (degrees)		<ZHX (degrees)	
	AM1	MP2/ 6-311++ G (2d,2p)	AM1	MP2/ 6-311++G (2d,2p)	AM1	MP2/ 6-311++G (2d,2p)
C_6H_6–NO[C_s]	3.05	2.77	180	180	–	–
C_6H_6–ON[C_s]	2.47	2.68	180	180	–	–
H_2O–NO[C_{2v}]	2.83	2.84	112.8	112.8	–	–
H_2O–ON[C_{2v}]	2.34	2.89	109.4	112.8	–	–
H_2O–NO[C_s(a)]	–	2.32	–	118.3	–	169.1
H_2O–NO[C_s(b)]	3.65	2.33	161.1	131.5	180.0	170.6
H_2O–ON[C_s(a)]	2.30	2.37	141.3	129.0	163.3	165.5
H_2O–ON[C_s(b)]	2.30	2.38	177.5	133.6	179.6	163.4
H_2S–NO[C_{2v}]	3.12	3.02	114.0	115.6	–	–
H_2S–ON[C_{2v}]	2.74	2.98	111.6	115.2	–	–
H_2S–NO[C_s]	2.95	2.60	163.1	179.5	179.9	172.9
H_2S–ON[C_s]	2.45	2.61	160.8	144.2	179.2	167.1
CH_4–NO[C_{3v}(H)]	4.86	2.97	–	–	–	–
CH_4–ON[C_{3v}(H)]	2.47	2.88	–	–	–	–
CH_4–NO[C_{3v}(C)]	19.9	3.36	–	–	–	–
CH_4–ON[C_{3v}(C)]	10.3	3.19	–	–	–	–
NH_4–NO[C_{3v}(H)]	2.56	2.03	–	–	–	–
NH_4–ON[C_{3v}(H)]	2.13	2.03	–	–	–	–
NH_4–NO[C_{3v}(N)]	2.82	2.82	–	–	–	–
NH_4–ON[C_{3v}(N)]	2.81	2.71	–	–	–	–
NH_3–NO	2.90	2.58	149.2	166.4	169.5	166.7
NH_3–ON	2.70	2.61	103.9	178.8	91.9	167.9

[a] See Fig. 1

a model for amino acids that have hydroxyl groups such as Thr, Ser, and Tyr. Two previous theoretical studies regarding the van der Waals complexes between NO and water have been found [26,27].

Infrared spectra was also recorded and analyzed, showing that a very weak complex appeared [28]. For H_2O–NO complexes, four configurations of C_s symmetry (H_2O–NO[C_s(a)], H_2O–NO[C_s(b)], H_2O–ON[C_s(a)] and H_2O–ON[$C]_s$(b)]) and two configurations of C_{2V} symmetry (H_2O–NO[C_{2V}], H_2O–ON[C_{2V}]) were calculated (Fig. 1 and Table 2). According to our ab initio results the N oriented complexes are more stable than the O oriented complexes for the same symmetry. These results are in line with those obtained in previous theoretical works [26]. At the same time, stabilization energies obtained at a BSSE corrected level are in good agreement with previous works. Stabilization energies reported for the H_2O-NO[C_s(a)], H_2O–NO[C_s(b)] at the CCSD(T)/aug-cc-pVTZ level are −4.55 and −4.28 kJ mol while our MP2/6-311++g(2d,2p) are −4.75 and −4.25, showing that the employed level of theory is a good choice for this kind of complexes. For the O oriented complexes, the reported stabilization energies at CCSD(T)/aug-cc-pVTZ (−3.24 and −3.00 kJ mol are larger than that obtained at this level. Nevertheless, the value reported by Ball [27] for the H_2O-NO[C_s(b)] complex at G2 level is −1.96 kJ mol and appears near to our value of −1.79 kJ mol. All conformers obtained at ab initio level are also obtained at AM1 level, except H_2O–NO[C_s(a)] (Table 3). On the other hand, the corresponding N oriented complex H_2O–NO[C_s(b)] is obtained with a larger intermolecular distance. Stabilization energies obtained with the AM1 method for O oriented complexes are overestimated with respect to the BSSE corrected energies. Complexes with H_2S are relatively well treated by the AM1 Hamiltonian. Stabilization energies are in good agreement with the MP2/6-311++G(2d,2p) values. Although, the stability order is not exactly reproduced by this method, the obtained values are satisfactory and in the dependability range for the employed methods. Geometries obtained by AM1 method show again that the intermolecular distance of the N oriented complexes are larger than the ab initio ones while the intermolecular distances of the O oriented complexes are smaller than the ab initio.

A couple of atomic configurations were calculated for complexes with ZH_4 formula, where Z could either be C or N (in this case the complexes are charged). As expected,

Table 3 Geometric parameters for peptidic bond models

Complex	Method						
		RHN	ROO	<NHN	<HNO	<COO	<OON
$HCOONH_2$–NO(a)	AM1	2.82	3.12	161.2	112.2	109.8	92.9
	MP2 / 6-311++G (2d,2p)	2.39	3.48	132.2	136.2	134.5	74.4
		RHN	RHO	<NHN	<HNO		
$HCOONH_2$–NO(b)	AM1	2.86	3.31	177.2	135.3		
	MP2 / 6-311++G (2d,2p)	2.40	3.97	178.9	176.8		
		RHN	<NHN	<HNO			
$HCOONH_2$–NO(c)	AM1	2.86	158.0	176.8			
	MP2 / 6-311++G (2d,2p)	2.42	179.4	139.6			
		RHO	<NHO	<HON			
$HCOONH_2$–ON (a)	AM1	2.43	170.3	178.1			
	MP2 / 6-311++G (2d,2p)	2.27	163.9	146.9			
		RNO	<NON				
$HCOONH_2$–ON (b)	AM1	2.94	178.8				
	MP2 / 6-311++G (2d,2p)	3.18	179.0				

complexes with CH_4 are less stable because of the low polarizability of this molecule. Complexes where NO is interacting with one H are better calculated at this level than complexes where the interaction is with the CH_3 group, although the latest complexes are the most stable for this molecule and the AM1 Hamiltonian does not yield a bounded structure (Table 1).

Semiempirical geometries corresponding to NH_4^+ and NO associations are in good agreement with the ab initio structures. The largest deviation is obtained for R_{NH} distance in the NH_4–NO[C_{3v}(H)] complexes, that is deviated around 0.5 Å. These complexes are the most stable and their stabilization energies calculated at MP2/6-311++G(2d,2p) level are more than 8 kJ mol stable than the isolated molecules. The most stable complex is that obtained with one hydrogen bonded to N interacting (NH_4–NO[C_{3v}(H)]) with the NO oriented by N atom, having an interaction energy of −21.48 kJ mol. Again, relative stabilities of AM1 structures with respect to ab initio are coincident with the general trend, although there are some changes of order.

Calculated complexes between NH_3 and NO were the NH_3–NO and NH_3–ON (Table 1). The AM1 method overestimated the stability of the second complex and changes their geometry decreasing the value of the <NHX angle. We can see that group protonation increases the stability of the complex with NO molecule and the complexes obtained with NH_4^+ are considerably more stable than that obtained with NH_3.

3.1.2 Peptidic bond model

When NO associates with a protein, one of the most frequent interacting molecular fragments should be the peptidic bonds, because they appear in every amino acid. Calculated geometries of five planar complexes with NO were obtained (Fig. 2, Table 3). All conformations of the ab initio minima were maintained in the semiempirical calculations, although some variations on angles were detected. AM1 method gives intermolecular distances for N oriented complexes ($HCOONH_2$NOa, $HCOONH_2$NOb, $HCOONH_2$NOc) larger than those calculated at the ab initio method. The main interaction in these kinds of complexes involved the NH_2 group. According to the ab initio results, N oriented are more stable than O oriented complexes and this order is not correctly reproduced by the AM1 method.

3.1.3 About the performance of AM1 method with respect to small model-NO complexes

Our analysis was done comparing the energies and geometries obtained at MP2/6-311++G(2d,2p) and AM1. Generally, the calculated complexes at the AM1 level reproduce

the conformation obtained with the ab initio procedure. Critical cases are the complexes between CH_4 and NO, but it is not a rare result taking into account the weakness of the corresponding complexes.

There are some systematic errors found in the AM1 treatment of complexes with NO. First, intermolecular distances for N oriented complexes are overestimated with respect to there calculated at MP2/6-311++G(2d,2p) level. Excluding the methane–NO complexes, the average overestimation is around 0.4 Å. Second, for the O oriented complexes obtained with small models that have no nitrogen atoms, and NO oriented by O, the same intermolecular distances are underestimated with respect to the ab initio calculations. The average for the intermolecular distance underestimation is 0.2 Å. Intermolecular distances in O oriented complexes containing N are very well treated by AM1 (complexes with NH_4^+ and NH_3). The average overestimation of these distances is only 0.09 Å. In general, AM1 intermolecular distances of O oriented complexes are in better agreement with ab initio calculations than the intermolecular distances of the N oriented complexes.

For the obtained complexes between $HCONH_2$ and NO, we can see that intermolecular distances in N oriented complexes are again overestimated around 0.4 Å. For O oriented complexes these are not a regular behavior; for one of the calculated complexes the distance is overestimated and for the other is underestimated, in both cases around 0.2 Å.

Analyzing the stabilization energies of different complexes at AM1, we can see that the O oriented complexes are generally predicted as more stable than the N oriented complexes for the same model. Ab initio and DFT calculations give the opposite tendency. This fact could be related with the general underestimation of intermolecular distances in this kind of complexes by the AM1 method. The AM1 method is not able to obtain the ab initio order of the conformer stabilities. Nevertheless, it can reproduce interactions with the charged NH_4^+ as the most stable. It conducts to a cautious but positive view to AM1 results when interactions are found with the O side of NO, because distance deviations in these cases are not large.

According to this test, a set of minima conformations can be obtained at the AM1 Hamiltonian level, although the detected systematic errors must taken into account. The previous general consideration on this testing allows us to use the AM1 hypersurface exploration to detect and compare minima energies, mostly among similar interactions given the fact, above mentioned, that we are dealing with systematic absolute errors of the order of 2.5 kJ mol (0.6 kcal mol or 0.03 eV). Our strategy consists in the use of MMH methodology to obtain a set of significant minima of the amino acid–NO systems. As stabilization energies were also evaluated at the PBE1PBE/6-31G(d,p) level, it has been found that it behaves similar to AM1 with respect to MP2/6-311++G(2d,2p) calculations, according to our test. Very weak interactions are susceptible to errors, just as it could occur with other accurate methods as well. It must also be taken into account that comparisons among energy values also cancel systematic errors, and it increases the reliability of statistical averages.

3.1.4 Several significant association structures for the smallest amino acid: glycine

Our model of the isolated glycine has two hydrogens bonded to the α carbon and the blocked chain positions according to Fig. 3. These last blocking groups are obviously excluded from our considerations although they are included in the calculations.

Glycine is considered among the nonpolar amino acids group in some textbooks. On the other hand, it would also appear as the noncharged polar amino acid group. Therefore, in our work the interaction with Gly is analyzed in a separate group and the predominance of the peptidic bond gives one of the most stable interactions with NO. Figure 4 shows four significant minima obtained around glycine, which are mostly related with the interaction with the peptidic backbone and the α hydrogens. Table 4 reports the AM1 and PBE1PBE/6-31g(d,p) stabilization energies of each of these four structures and their relative populations, as total and calculated values according to Boltzmann distribution. At the AM1 semiempirical level, it can be seen that the interaction energies of the complexes where the O atom is nearer to each contact site are around 3 kJ mol more stable than that obtained for the corresponding N orientation complex. It will be shown that this behavior is concurrent with all the considered models and it must be related with the previously detected systematic error of the AM1 Hamiltonian. PBE1PBE/6-31g(d,p) results are similar to these obtained with small models and stabilities of N and O oriented complexes are with similar or the former is more stable than the second. For the sake of reliability, in the case of the remaining amino acids, we will only show the stabilization energies as obtained at the DFT level. Therefore, AM1 results of these kinds of calculations must be carefully taken and valid conclusions can only be of qualitative character in individual cases, because there are no method capable of providing exact proportions when energy differences among the several molecular arrangements are of the order of 0.6–0.9 kJ mol.

The case of glycine is significant because it is a very simple system, without much conformational opportunities for molecular interactions. If populations are calculated according to Boltzmann distribution considering only the four lowest energy complexes we can obtain, at PBE1PBE level that the 1*a*, 1*b*, 1*c* and 1*d* configurations contribute roughly 31, 14, 30 and 25 % (Table 4), respectively, to the significant population. These proportions are a good indication of a very

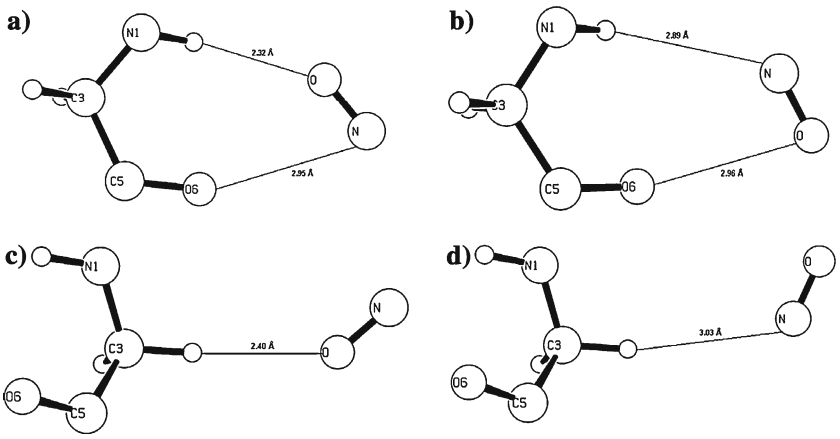

Fig. 4 Four significant minima obtained for Glycine–NO system

Table 4 Interaction energies of some selected atom orientations of NO around glycine

	ΔE(kJ mol^{-1})[a]		Atomic interactions (amino acid/NO)	Populations (percent)[b] AM1	PBE1PBE/ 6-31g(d,p)
	AM1	PBE1PBE/ 6-31g(d,p)			
1a)	−10.2	−5.5	NH/O, CO/N	47	30
1b)	−9.3	−2.6	H α/O	32	14
1c)	−6.7	−5.3	NH/N, CO/O	11	29
1d)	−6.5	−4.6	Hα/N	10	25

[a] The interaction energy was calculated as the difference between the complex and the isolated molecule energies employing the AM1 geometries ($\Delta E = E(aa\cdots NO) - E(aa) - E(NO)$, where aa is glycine, and the aa. . .NO is the corresponding Gly· · ·NO complex)
[b] Populations calculated according to Boltzmann distribution at 298 K

remarkable fact: *a molecular modeling study of these kinds of associations to understand biological processes must take into account more structures than that of the sole "deepest" minimum because the entropy of association plays a significant role in the appearance of the related phenomena in life at the scale of living systems and experiments, that is not the molecular but the human world scale*.

3.1.5 Aliphatic non-polar amino acids

This group presents amino acids with aliphatic chains, which are to be located at protein cores [Alanine (Ala), Isoleucine (Ile), Leucine (Leu), Proline (Pro), Valine (Val) and Methionine (Met)]. Pro is unique, since this amino acid has a lateral chain cycled to the backbone. This feature plays an important role in protein folding.

Analyzing the minima structures obtained, we can classify them according to their association energies and the site of the interaction with NO. The most stable interactions for nonpolar amino acids (excluding Pro) are those where association occurs with the NH and C=O belonging to the amino acid backbone (Fig. 5). Amino acid conformations similar to β sheet favor this kind of simultaneous interaction. Stabilization energies for this pattern of interaction ranged widely from −4 to −8 kJ mol. It means that this group is not preferred by NO over other aminoacids. Neither of them involves the lateral chain and the results are similar to those previously shown for Gly (Fig. 4). In the case of proline no peptidic hydrogen is present, thus, the most stable interaction is obtained with the hydrogen bonded to the α carbon (Fig. 6).

A second group of atomic arrangements of NO around amino acids is related to the interaction between the H bonded to α carbon and the O side of the NO molecule. These structures showed stabilization energies of around 1 kJ mol below the previous interaction with peptide group.

A third group can be considered when NO interacts with aliphatic hydrogens. These interactions are very weak and contribute with around 1 and 2 kJ mol. Interactions with–SCH_3 have a stabilization energy of around 1.5 kJ mol. Due to their low polarity, such interactions with NO molecule must be fundamentally determined by dispersive forces. These kinds of interactions are a challenge for computational chemists because a proper treatment requires accurate methods that include a large calculation of electron correlation. In a recent work [13], very accurate calculations dealing with H_3C–H · · · [NO] interactions showed that stabilization energies of this interaction calculated at the MP2 level and using several extended basis sets are around 1 kJ mol. It is congruent with the obtained semiempirical results. Accordingly,

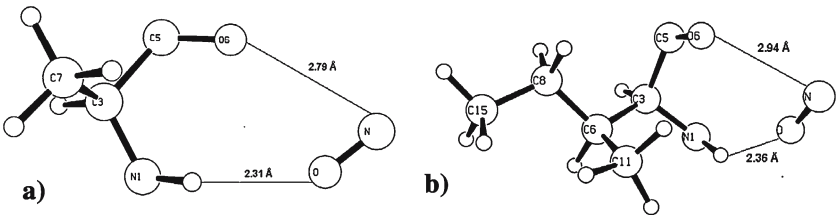

Fig. 5 Most stable minima obtained for alanine (**a**) and isoleucine (**b**) amino acids interacting simultaneously with NO by amide and carbonyl groups. In all figure hydrogens are the *empty balls* and blocking groups are omitted for simplicity

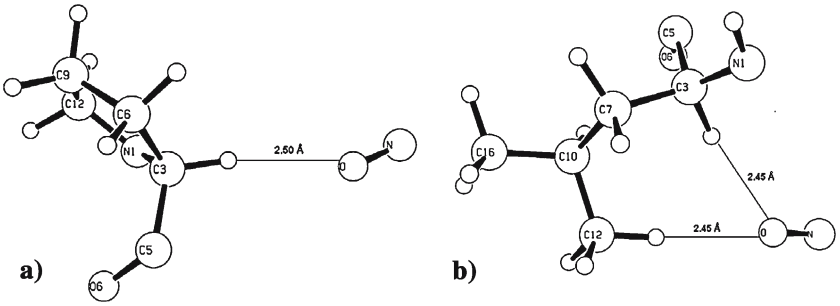

Fig. 6 Interactions with hydrogen bonded to α carbon (C3). Minima obtained for proline (**a**) and leucine (**b**) amino acids. In the case of Leu one of the hydrogen's methyl is also interacting with NO

in the case of these amino acids, the stabilization energy is slightly larger due to the increase of polarity of amino acids with respect to the simple hydrocarbons. In spite of the–SCH_3 substitution in Met, this amino acid has a very similar behavior to that obtained for the other amino acids of this group.

3.1.6 Aromatic nonpolar amino acids

Phe, Trp and Tyr belong to the group of amino acids with aromatic lateral chains. The most stable interactions found were also related to the peptidic backbone (similar to Figs. 4a, 4b, 5). Interactions with H bonded to either α carbons, the aromatic and the aliphatic groups also appear in some minima.

In Figs. 7 and 8 three other interesting interactions with the lateral aromatic chains appear. In the shown minimum of Trp, that has no significant population, the NO molecule is almost perpendicular to the aromatic ring (Fig. 7). In this case, the N atom is close to the N–H belonging to the aromatic ring. A similar minimum where the NO is oriented by O is almost as stable as the interaction where the NO is oriented by N.

These kinds of interactions involve the interaction between π clouds of both molecules, and also stacking interactions (Fig. 8b). In one of the minima obtained for Phe amino acid, the O atom of NO, simultaneously interacts with *ortho* and *meta* (with respect to backbone substitution) hydrogens (Fig. 8a). This pattern is slightly affected by the hydroxyl substitution in Tyr. The OH substitution increases the electron density because of the higher ability of *meta* hydrogen (*ortho* with respect to OH) to interact with NO. The intermolecular distances to hydrogens decrease with respect to that obtained for Phe (Fig. 7).

3.1.7 Polar amino acids without charges: Alcohols (Ser, Thr), thiol (Cys) and amides (Asn, Gln)

The group of amino acids with polar lateral chains is analyzed here. Several minima relating with the interaction between the polar groups ($CONH_2$ in amides of glutamic and aspactic acids: Asn, Gln and OH in Ser and Thr) appeared. In these cases the interaction with lateral chains are equally or even more stable than the interactions with the protein backbone

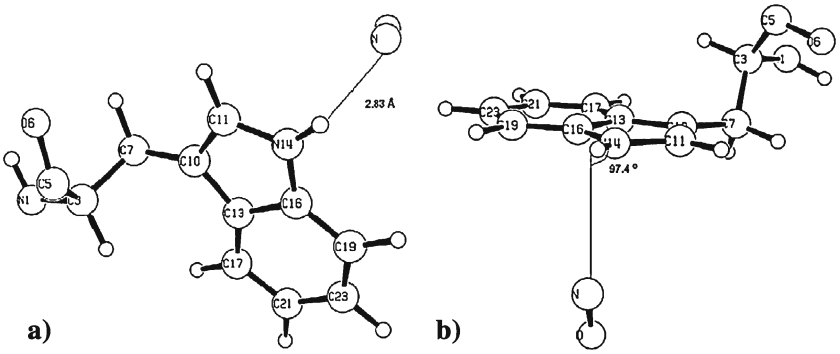

Fig. 7 Two views of one Triptofane nitric oxide interaction with NH

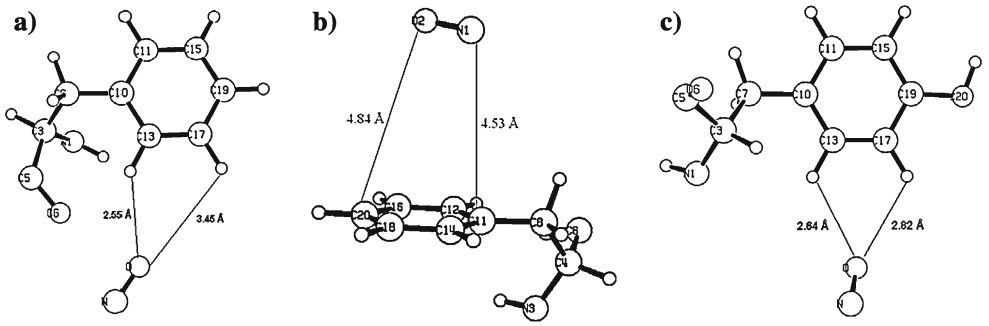

Fig. 8 Interaction with aromatic chains of Phe and Tyr

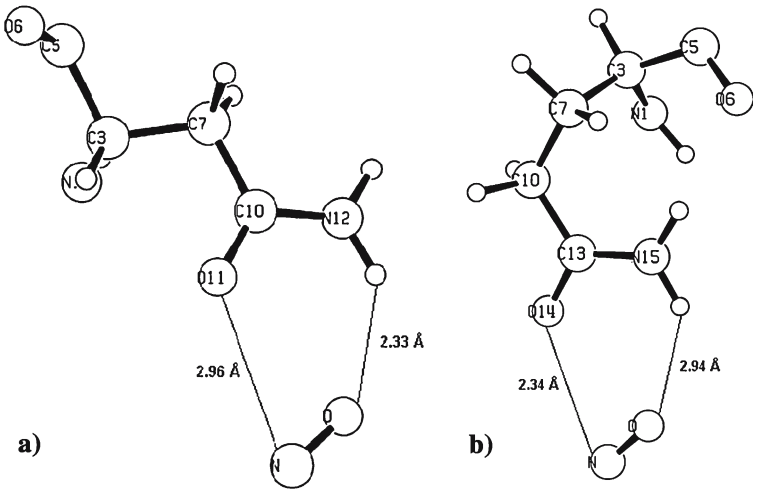

Fig. 9 Minima for Asn and Gln amino acids where NO interacts with the amide groups. This figure shows a geometrical pattern similar to Fig. 2

or with H bonded to α carbons. Nevertheless, minima that involve backbone interactions also appear.

Figure 9 represents two minima for Asn and Gln. These are the most stable minima obtained for these amino acids. As it can be seen, each minimum shows a geometrical pattern similar to that obtained for the interaction with peptidic NH, although the involved groups belong to the lateral chains in these cases. The calculated distance to NH_2 is of around 2.3 Å and the distance to C=O is of 2.9 Å. There are other minima where the main interactions are with NH_2 without the participation of C=O groups. These arrangements are in correspondence with the previously calculated at ab initio level for the peptidic bond model. These minima are similar to those obtained for the amino acids in a conformation such as α helix, where C=O and N–H are not in the same direction.

Figure 10 shows two minima where Thr and Ser interact with the OH groups. In both cases, the interaction is augmented by a secondary group.

Cysteine has a thiol group in its lateral chain although this group is not as polar as OH group in Tyr and Ser. The great propensity for nitroso–thiol formation represents a modulation mechanism for the action of macromolecules containing NO-reactive Cys residues at their active or allosteric sites [29]. In this case, the NH peptidic interaction also appears as the most stable, but there is a atomic arrangement where NO molecule is interacting with the S–H group, related with that calculated for the smallest model (H_2S).

3.1.8 Polar amino acids with charges

These amino acids are predominantly charged at physiological pH and, consequently, they were calculated in their charged form. Therefore, positive charges for His, Lys, and Arg and a negative charge for Asp and Glu were considered. The interactions with charged lateral chains are especially strong when they are compared with the other groups.

3.1.9 Negative charge at physiological pH: Asp and Glu

The most important interaction for these amino acids is obtained by the carboxylic group. This is an interesting association where the NO is almost perpendicular to the COO– group (Fig. 11), where N and O atoms of NO are located at 2.9 Å of carboxylic oxygens. Association energies for these interactions are around −6 kJ mol, which are more stable than the interaction with NH belonging to peptidic bonds. Other minima are related to the interaction with the CH_2 directly bonded to COO^-. These CH_2 groups are influenced by the

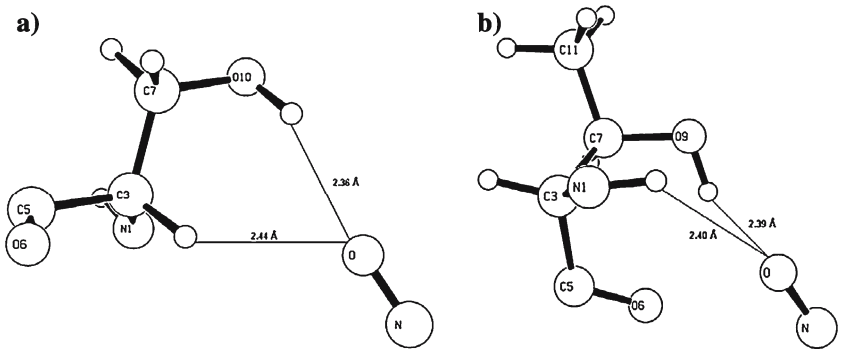

Fig. 10 Minima for Ser and Thr amino acids

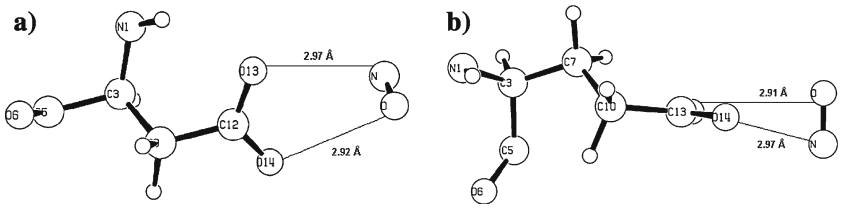

Fig. 11 Global minima obtained for aspartic and glutamic acids

negatively, charged carboxylic group, and the stabilization energy obtained for these interactions is around −7 kJ mol. In these complexes N atoms are located near C=O of peptidic bonds.

3.1.10 Positive charge at physiological pH: His, Lys, Arg

Many structures for this group appears with similar energies that contribute to the ensemble properties. The interaction with Lys amino acid is the strongest. The most stable obtained minimum (Fig. 12b) has a stabilization energy of −32 kJ/mol and is related to the interaction with the NH_3^+ group when the NO is oriented by N atom. These kinds of interactions were also obtained as most stable in the set of small complexes. The atomic arrangement represented in Fig. 12a is other minimum. The main interaction (according to the distance) is obtained between two hydrogens belonging to NH_3^+ and the oxygen atom of NO molecule. A third interaction, between the C=O and N atom (this kind of interaction is aided by a carbonyl oxygen) appears as very probable for most of the amino acids.

There are other structures with similar stabilization energies but different orientations of the NO molecule with respect to the NH_3^+ group. The structure shown in Fig. 12a, stabilizes the system in −12 kJ mol. In this case, the NO is facing the N atom. There are H atoms bonded to α carbon and C=O located at distances smaller than 3 Å.

For Arg many minima associated with the interaction with guanidino group also appears. Figure 13 shows two of these minima. In Fig. 13a the most stable of all arrangements explored where the NO is pointing toward the guanidino carbon appears (C16 in Fig. 13). It has intermolecular distances to hydrogen atoms of 2.26 Å. In Fig. 9b, a second minimum is shown. It has an interaction with both hydrogens of one guanidino NH_2 (N18 in Fig. 13) group. Other minima, which implicate the interaction with hydrogens belonging to N14 and N17, are also obtained. The energy stabilizations with respect to the isolated molecules of both atomic arrangements are −9 and −8 kJ mol, respectively.

The interaction with Histidine is really important. There are many proteins, which have histidine groups in their distal pocket, which include many hemoproteins such as myoglobin and hemoglobin. Again, the interaction with the lateral charged group predominates, in this case with the imidazole group. The most stable interactions are obtained with the hydrogens bonded to N17 and C15 (in Fig. 14b), the smallest being r(N–H) bond length of 2.69 Å, while the H bonded to C15 is located at 2.85 Å of the N of NO. This interaction stabilizes the system at −14 kJ mol. The second (shown) interaction (Fig. 14b) involves the H bonded to N11 atom and the C=O oxygen, their stabilization energy is −11 kJ/mol.

3.2 Thermodynamic Properties

According to the comparison between the ab initio and AM1 calculations we concluded that the direct energetic at AM1 level must be used with care. Nevertheless, we think that one of the values of MMH methodology is the calculation of thermodynamic association properties. Our experience indicates that the statistics usually produce reasonable results due to systematic error cancellations [12].

Table 5 shows the calculated thermodynamic properties of the canonical ensembles at temperature of 298 K and the stabilization energy of the corresponding most stable mini-

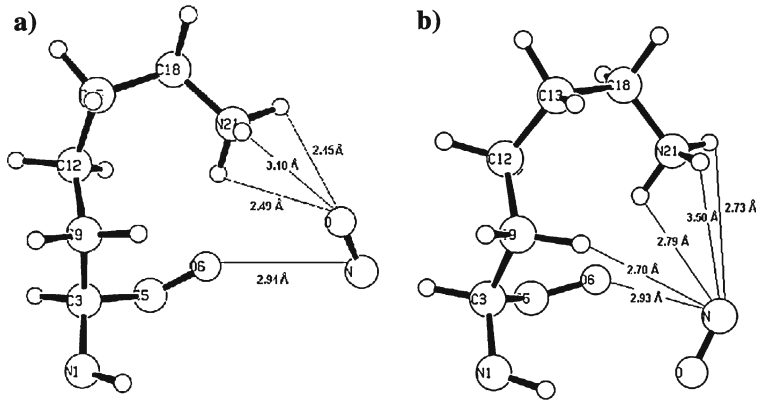

Fig. 12 Two molecular minima arrangements of NO around Lys amino acid

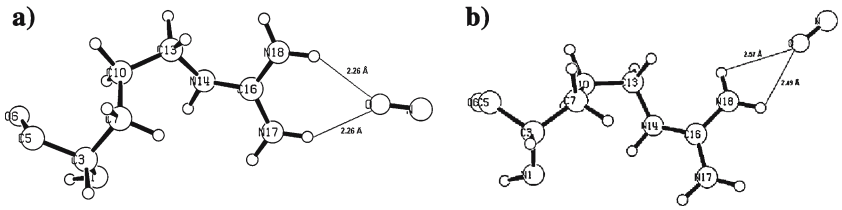

Fig. 13 Minima obtained for the interaction of NO with arginine amino acid

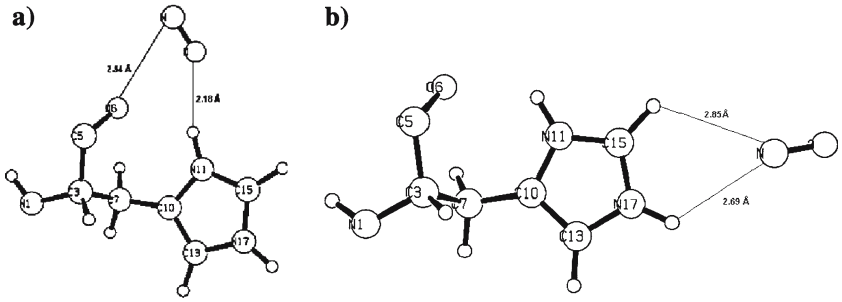

Fig. 14 Minima obtained for the interaction of NO with histidine amino acid

mum for each amino acid–NO system at the AM1 level. As can be seen, the stabilization energies for each of the most stable obtained minima ranged between −8 and −16 kJ mol. These limits are similar at the PBE1PBE/6-31G(d,p) level, although one of the minima of Lys deviated with an stabilization energy of −32 kJ/mol). These energies are in the order of very weak hydrogen bonds (4–16 kJ mol) which are mostly determined by dispersion energy components that are implicitly treated in the semiempirical methods by means of the Hamiltonian parameterizations. Association energies are lower than the global minimum energies because the calculated thermodynamic properties are affected by the presence of all atomic arrangements taken into consideration.

According to the energy of the minima, the most stable interaction is with Lys (Fig. 12). In general, interactions with charged amino acids (both acid and basic) are the strongest. These results follow the same tendencies with those obtained at ab initio and DFT levels for the model complexes. For these charged amino acids, the most stable minimum is more stable than or as stable as the interaction with backbone atoms. Taking into account the high polarity of N–H and C=O groups belonging to the protein backbone, the interaction of ligands with NH group is really favorable. In fact, a search in PDB entries of complexes between ligands and proteins done by Moreno and León [30] showed that one of the most probable interactions is obtained with NH of the peptidic group. According to our calculations, in the case of lysine–NO interaction, the complex where the interaction occurs with NH_3^+ is especially stable; similar to the cases of Asp and Glu. In these cases, a stable interaction with COO^- groups is obtained. On the other hand, lateral chains of Arg and His have many active hydrogen atoms with relatively large association energies. These amino acids show a high tendency to interact with NO.

The entropies of association for the calculated ensembles are positive in all cases. In spite of the possible inaccuracies, this calculation on entropies has an undeniable qualitative importance due to the fact that several cluster geometries are

Table 5 Thermodynamic properties for canonical ensembles of amino acid–NO weak complexes[a] calculated at AM1

Amino acids	ΔE^{assoc} (kJ mol)	S^{assoc} (J K mol)	ΔF^{assoc} (kJ mol)	E_{min} (kJ mol)	Interaction features of global minima[a]
Lys	−15.4	18.8	−21.0	−16.4	Class II
Arg	−10.8	18.4	−16.2	−12.9	Class II
His	−10.2	19.9	−16.1	−12.3	Class II
Trp	−9.3	17.4	−14.5	−11.3	Class I
Asn	−8.7	20.7	−14.9	−10.4	Class I
Gly	−8.4	20.4	−14.5	−10.2	Class I
Asp	−8.4	23.8	−15.5	−9.8	Class II
Ala	−8.3	22.5	−15.0	−9.9	Class I
Gln	−8.0	17.0	−13.1	−9.6	Class II
Phe	−8.0	20.9	−14.2	−9.5	Class II
Met	−7.7	21.4	−14.1	−9.8	Class I
Ser	−7.6	20.5	−13.7	−10.1	Class II
Val	−7.5	21.2	−13.8	−9.3	Class I
Thr	−7.4	20.7	−13.5	−8.5	Class II, Class I
Glu	−7.3	23.3	−14.3	−9.1	Class II
Ile	−7.0	22.4	−13.5	−9.2	Class I
Leu	−6.8	21.4	−13.2	−8.6	Class II
Cys	−6.7	21.7	−13.2	−8.6	Class I
Tyr	−6.5	18.8	−12.1	−8.3	Class II
Pro	−6.4	24.1	−13.6	−7.6	Class II

[a] Class I refers to global minima where the interaction is with N–H, the general geometrical pattern is shown is Figs. 4a, 4b and 5. Belonging to Class II are the amino acids where the most stable interaction is obtained with lateral chains

possible near the global minimum and it is a very important fact for modeling of molecular association. This fact was shown for the case of glycine (See Table 4). According to Boltzmann distribution, the four stable minima have similar populations. The negative value of Helmontz's free energy ($\Delta F^{assoc} < 0$) implies that the association processes are even more favorable from the thermodynamical point of view if we consider this undeniable factor in our modeling.

Our results were obtained at gas phase and employing a peptide model, these are models for the real situation of NO in a protein environment. Some of the obtained minima are not accessible in certain specific protein environments due to steric hindrances. If there are free water molecules in the environment, these can also compete with NO. Nevertheless, the NO–water affinity is rather small and the competition is not expected to be very important. NO and water establish very weak interactions, as was obtained by us and in previous investigations. A recent molecular dynamic study of NO hydration shows a small amount of water molecules around the NO molecule [31]. At the same line, amino acids should be hydrated (especially the charged ones) and the formation of amino acid–NO complexes should also be determined by the hydration of the environment. Nevertheless, the presence of a large amount of water molecules around a buried amino acid is not frequent in protein and an anhydrous surrounding is the most common situation.

4 Conclusions

The interactions between NO and the 20 protein contributing amino acids were studied employing a molecular model that considers electron densities by different Hamiltonians. The MMH methodology allows us to obtain a group of significant association minima for each interacting system. It must be remarked that several conformations or molecular arrangements of NO around each amino acid have similar populations and they contribute significantly to the ensemble properties, as was shown for the case of glycine amino acid. It introduces the qualitative understanding that entropies of association take a major role in these kinds of processes and that modeling single and deepest minima of such hypersurfaces could bring wrong results when "macroscopic" physiological processes are approached.

The optimization processes were carried out at AM1 semiempirical level. A set of molecular complexes between NO and small molecules were calculated and the results were compared with ab initio calculations at MP2/6-311++G (2d,2p) and DFT calculations at PBE1PBE/6-31g(d,p) levels. These calculations show that the AM1 Hamiltonian produces complexes with the right conformations, although some intermolecular geometrical parameters could deviate.

According to our results, interactions between NH and CO belonging to backbone and NO appear in all amino acids.

Interaction with H bonded to α carbon is also important. According the chemical features of the lateral chain residue, these interactions can be more stable than the related with the backbone atoms. For charged amino acids the most stable complexes with NO occur.

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