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Quantum-Chemical Modeling of Catecholamine Storage Including Continuum Solvent Effects

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Abstract

A model for catecholamine storage in vesicles is analyzed within the SCRF-CNDO/2 approach including continuum solvent effects. The model considers the approach of cationic norepinephrine (NE) to a positively charged guanidinium moiety. Ion-pair formation is found for the whole range of dielectric constants. Even though stable states of H-bonded partners are found for large dielectric constants, this process is ruled out to occur because it involves too high energies. It appears that the medium's polarity is determinant in lowering the energy barrier between the ion-pair complex and the separated partners. Thus, as the medium dielectric constant increases, the equilibrium between the two states is enhanced.

Introduction

Biogenic amines (dopamine, norepinephrine, etc.) are stored in a variety of tissues such as chromaffin granules of the adrenal medulla and catecholamine-storing vesicles in sympathetic nerves [1–4]. The conversion of dopamine to noradrenaline occurs within the chromaffin granules, since the enzyme dopamine β-hydroxylase is found within them.

The storage mechanism has not been elucidated. A first model suggested that catecholamines, ATP, and bivalent cations form an intragranular dynamic storage complex [1,5]. NMR results indicate that ATP and amines interact within the granule because they show less freedom of motion [6]. Other experimental results showed that amine uptake is inhibited by sodium oleate and ethyl and butyl alcohol [2], suggesting that a lipid or a lipoprotein could be an essential component of the storage mechanism.

Recently, a mechanism for the storage of norephinephrine (NE) had been proposed [7]. In this model, catecholamines are bounded to a guanine moiety in an arginine side chain. The bidentate nature of this moiety could permit its interaction with the complementary catechol moiety of NE through a mixture of ion pairing and hydrogen bonding [7](Fig. 1).

In this paper, we report a study of the relative contributions of both H-bonding and ion pairing in the complexation process [7]. This study was carried out within

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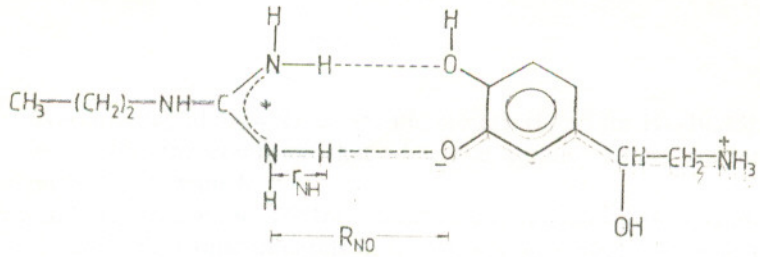

Figure 1. Model for catecholamine and the model site.

the self-consistent reaction field theory at the CNDO/2 level, including continuum solvent effects (SCRF-CNDO/2) [8].

Methods, Models, and Calculations

Within the continuum approach of solvent effects representation, the total free energy of the solute-solvent system is expressed as

$$A(\epsilon) = E(1) + \Delta A_s(\epsilon), \tag{1}$$

where ϵ is the bulk dielectric constant of the solvent, $E(1)$ is the total energy of the isolated solute, and $\Delta A_s(\epsilon)$ is the free energy of solvation. One of the simplest methods for calculating $\Delta A_s(\epsilon)$ consists of building up the free energy of solvation from the atomic contributions of each partially charged atomic center in the molecule plus the corresponding interatomic interaction terms [8–10]. Within the molecular orbital theory at the CNDO/2 level, the solvation energy is written as follows:

$$\Delta A_s(\epsilon) = \frac{1}{2} \sum_A Q_A \, [V_R(\epsilon)]_A, \tag{2}$$

where Q_A is the net charge of atom A and $[V_R(\epsilon)]_A$ is the reaction field potential acting on A.

The desolvation process is probably the main previous step in the dynamics of ion-pair formation [11, 12]. In this work, we employ the desolvation process representation that considers the steric hindrance effects to solvation on each atomic center, created by the vicinal atoms [8, 10]. In this case, a desolvation barrier is expected to appear when the systems come within an overlapping distance. To take into account these effects, the generalized Born formula (GBF) has been modified by incorporating the appropriate term [13–15]. This leads to the following expression for $\Delta A_s(\epsilon)$:

$$\Delta A_s(\epsilon) = -(1/2)(1 - 1/\epsilon) \sum_A \sum_B Q_A Q_B [1 - (f_A + f_B - 2 f_A f_B)] \Gamma_{AB}, \tag{3}$$

where f_A is a function depending only on the neighborhood of A and Γ_{AB} is the solute-solvent interaction integral.

Equation (3) contains a first term corresponding to the standard GBF and the corrective term characterizing the steric hindrance effect upon solvation on each atom produced by the vicinal atoms. Accepting that $f_A \leq 1$ [8], it can be seen that

the corrective term displays an opposite sign to that of the GBF and can be assimilated to a desolvation contribution. f_A has been empirically represented by [10]

$$f_A = \frac{1}{2} \sum_{B \neq A} S_{AB}, \tag{4}$$

where S_{AB} is the overlap integral between 2*s* atomic orbitals (1*s* AO for H) [10, 13]. This representation fulfills the condition that when the atomic centers are far away from each other the *f*'s vanish.

On the other hand, within the CNDO/2 framework, we may identify the Γ_{AB} integrals with the bicentric electronic integrals in such a way that when we neglect neighborhood effects we recuperate the original results [16–18].

We have considered a system in which cationic NE approaches to a binding site represented by a protonated guanidine moiety (i.e., guanidinium) (Fig. 1) [7]. This choice was made by considering that the derivatives of guanidine are the strongest electrically neutral bases known [19] and that catecholamines are present in their cationic form inside the vesicle [1]. We bounded the guanidinium moiety to an alkylic chain to represent the hydrophobic part of the membrane. We have analyzed an approach in which the phenyl ring is coplanar to the guanidinium moiety such that the phenolic oxygens directly interact with the guanidinium's H—N groups. The ethylammonium side chain of NE was placed in a coplanar extended conformation in order to minimize its electrostatic repulsion with the cationic model site.

Even though the two protons can be transferred in an asynchronous way, our calculations were performed for the concerted transfer of both protons from the guanidinium moiety to NE for various N—O intermolecular distances. In this way, our results correspond to an upper bound of the proton transfer barrier height.

To study the relative effects caused by the variation of the medium's dielectric constant, we performed all the calculations in vacuum and in the presence of a continuous polarizable medium (i.e., at various ϵ values). We employed the CNDO/2 approach because it provides a qualitative reliable description of both ion pairing and hydrogen bonding [9, 11, 20–22]. We must stress that, as in the case of our study of proton transfer in water polymers [10], we are looking for possible reaction mechanisms (of storage here) that will lead or not to an acceptance of Portoghese's model. Nevertheless, we must keep in mind that CNDO/2 calculations overestimate proton transfer barriers and that the curve's shape is not the same as the one obtained in ab initio 4-31G calculations. The geometry of the species has been built up with Pople's standard parameters [23].

The system's geometrical arrangement and the calculation method are also supported by a study of the interaction between formiate and guanidium ions performed in the framework of CNDO/2 and ab initio STO-3G methodologies [24]. It was shown that the STO-3G results are similar to the CNDO/2 ones and that the most stable structure is the one in which there are two nearly parallel H bonds between the N atoms of the guanidinium ion and the oxygens of the formiate ion. Therefore, as long as relative energies are compared, the results should be reliable in explaining a given molecular mechanism.

Results and Discussion

To represent the free energy variations as a function of both the intermolecular distance (R_{N-O}) and the position of the protons to be transferred (r_{N-H}), we used the reduced coordinate $Z = r_{N-H} - 1/2R_{N-O}$. $Z = 0$ corresponds to a situation in which the protons are shared by the two partners and $z < 0$ indicates that the protons are closer to the guanidinium N atoms.

Figure 2 shows the proton transfer potential curves (PTPC) in vacuum for several intermolecular N—O distances. The following features are relevant: For 2.2 Å < R_{N-O} < 3.2 Å, we have only single minimum PTPCS. Beyond R_{N-O} = 3.2 Å, double minimum PTPCS appear. In the reaction path passing through the minima of the PTPCS, a stable state appears at $R_{N-O} \approx 2.6$ Å and $r_{N-H} \approx 1.1$ Å. This means that in the case of a low-polarity medium, as expected to be in the vicinity of the membrane, the ion-pair complex appears to be more stable than is the separated species. The presence of this ion pair was confirmed by the population analysis at the equilibrium position, showing a charge of +0.998 au for both partners. The barrier height between the ion-pair complex and the H-bonded system (i.e., $Z > 0$), is of about 14 eV, suggesting that even when CNDO/2 overestimates the barrier heights an H-bonded complex is not formed.

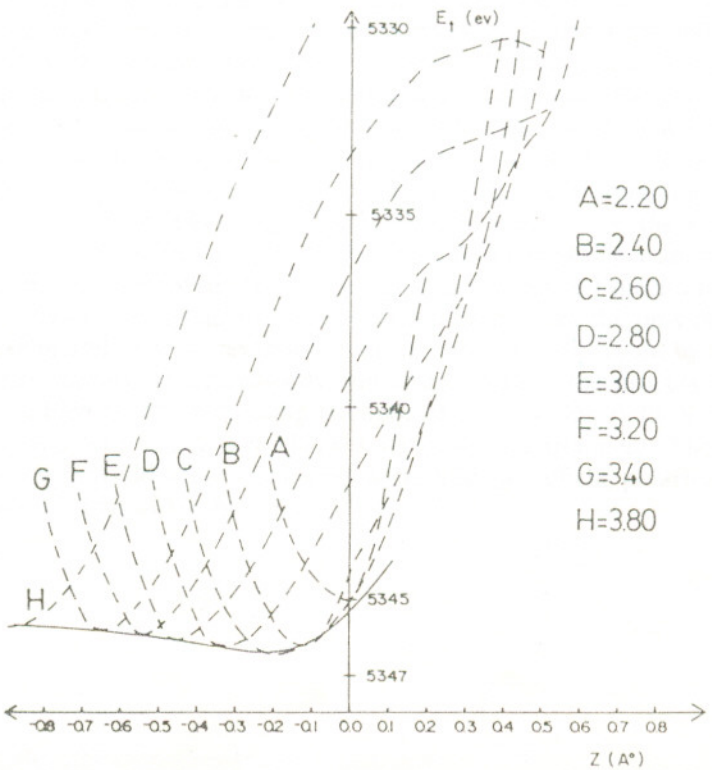

Figure 2. PTPC in vacuum (ϵ = 1). A–H are different N—O distances. A = 2.20; B = 2.40; C = 2.60; D = 2.80; E = 3.00; F = 3.20; G = 3.40; H = 3.80.

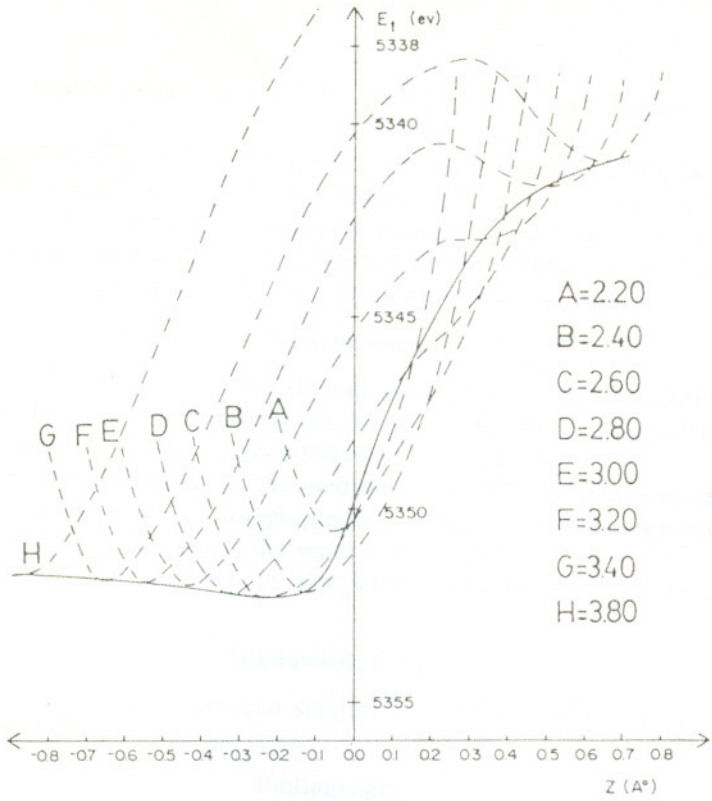

Figure 3. PTPC for $\epsilon = 80$. A–H are different N—O distances. A = 2.20; B = 2.40; C = 2.60; D = 2.80; E = 3.00; F = 3.20; G = 3.40; H = 3.80.

The results for a polar environment ($\epsilon = 80$) are displayed in Figure 3. It may be seen that double-well PTPCs occur for shorter intermolecular distances than in the gas phase. Also, the reaction path shows a flatter minimum, revealing the major role of the electrostatic interactions. The energy difference between the separated and ion-pair bounded species slightly diminishes for $\epsilon = 80$. The latter results also show the stabilization of the ion-pair complex in a wide range of intermolecular distances, but always for $r_{NH} \approx 1.1$ Å. Again, the ion-pair formation was confirmed with Mulliken population analysis, which gave the same results as those obtained for the gas phase. Proton transfer is also not allowed since the energy difference between the ion-pair and the H-bonded species is of about 9 eV. The overall results suggest that ion-paired species are the only ones present in the system and that the reversibility of the process is enhanced by the increasing medium polarity. In other words, the reversibility of the process appears to be determined by the increasing strength of the reaction field of the whole system, including its interaction with the molecular environment. Therefore, the release of NE could occur because of a modification of the polarity of the medium in the vicinity of the binding site.

Another interesting feature of this model is that it may provide an explanation for the inhibition of amine uptake by sodium oleate and ethyl and butyl alcohols. We propose that these molecules compete with NE for the site in the same way.

Finally, our results show that the proposed mechanism for catecholamine storage must be modified in order to exclude an H-bonded complex [7].

Conclusions

1. Norepinephrine interacts with the model site only through the formation of an ion pair. The complexation process occurs in the whole range of dielectric constants. An intermolecular H-bonded complex is not allowed.

2. The electrostatic effects of the medium seem to be determinant factors to allow the reversibility of the storage process, by lowering the energy barrier between the ion-pair complex and the separated species.

3. The mechanism proposed by Portoghese must be modified to rule out H-bonding.

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