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Studies of Solvent Effects

III. Solvent Effect on the Conformation of Acetylcholine

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The effect of water on the conformational preferences of acetylcholine has been studied within the "discrete", the "continuum" and the combined "discrete-continuum" models described in parts I and II of this series. All the models lead to the conclusion that the *trans-gauche* form which is, following refined quantum-mechanical computations, the intrinsically preferred one and the one observed in the crystal of acetylcholine and of a number of analogues should remain also the preferred conformation in water. This result agrees with NMR studies. The results of the empirical discrete model used here compare favorably to those obtained by an *ab initio* "super-molecule" treatment. The continuum model utilized here represents a net improvement above such models utilized in other works.

Key words: Solvent effect on the conformation of acetylcholine – Acetylcholine, solvent effect on the conformation of ~

1. Introduction

In papers I and II of this series [1, 2] we have presented, respectively, three possible approximate methodologies for the study of solute-solvent interactions and have checked the accuracy of these representations on simple systems. In the present paper we apply these models to the study of a relatively large molecule in connection with the effect of the solvent on its conformational properties.

The molecule investigated is acetylcholine (AcCh) and this choice was dictated both by the great importance of this substance, which is the principal natural intercellular effector in nervous transmission systems and by the large amount of experimental and theoretical works devoted to the exploration of its conformational properties (for general reviews see e.g. Refs. [3–6]).

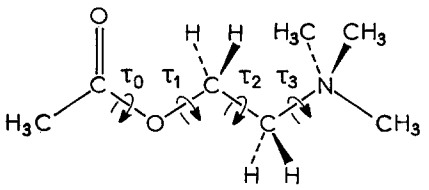

Fig. 1. Torsion angles in acetylcholine

As seen in Fig. 1, acetylcholine is a flexible molecule with four main torsion angles, τ_0–τ_3. In fact, the two essential of these angles upon which all the studies center are τ_1 and τ_2 [3–6].

X-ray crystal structure studies were the first to provide information about the values of τ_1 and τ_2. They were obtained for two such crystals, the chloride [7] and the bromide [8, 9]. The geometries of the molecule differ somewhat in the two crystals and so do also the values in particular of one of the torsion angles, namely τ_1. Thus $\tau_1 = -167°$ and $\tau_2 = 85°$ in the crystal of the chloride and $\tau_1 = 85°$ and $\tau_2 = 78°$ in the crystal of the bromide. We shall designate the structure as *trans-gauche* (*tg*) in the first case and as *gauche-gauche* (*gg*) in the second.

A large number of theoretical computations have been carried out with a view to determining the conformational possibilities and preferences of acetylcholine, both by the empirical and quantum-mechanical methods [6]. The most refined of them, such as e.g. those carried out by the self-consistent field *ab initio* procedure [10, 11], indicate strongly that the intrinsically preferred conformation of acetylcholine is *trans-gauche*, close to the one present in the crystal of the chloride. This preference results from the electrostatic interactions between the cationic head and the ester oxygen. The *gauche-gauche* form observed in the crystal of the bromide seems to be due to the crystal packing forces and does not represent an important form for AcCh biochemistry. These studies have also shown that the *trans-trans* conformation ($\tau_1 = \tau_2 = 180°$) considered as significant in some proposals related to the structure and activity of acetylcholine and in some computations (see [6]) and deeply implied in the discussion concerning the conformation of AcCh in solution (*vide infra*), is 3–4 kcal/mole less stable than the *trans-gauche* one.

Parallelly, the solution conformation of acetylcholine was also studied extensively both experimentally and theoretically. The experimental investigations rely primarily on the NMR techniques. As a general conclusion of these studies, the *trans-gauche* conformation with respect to τ_1 and τ_2, characteristic of the AcCh chloride crystal and corresponding to the global energy minimum on the *ab initio* conformational energy map, is essentially preserved in water. The most recent and elaborate evaluation [12] indicates the presence of 91 % of this *trans-gauche* form at room temperature. It also estimates at 65°–69° the value of τ_1 and at no more than 1 kcal/mole the value of $\Delta G°$ between the two forms at room temperature. A number of other authors indicate the proportion of the *gauche* form to be practically equal to 100 % [13, 14]. The conformation of AcCh in solution seems to be independent of the counter-ion, Cl^- or Br^- [14].

Noticeable differences seem on the other hand to characterize the results of the theoretical evaluations of the solvent effect on the conformational properties of acetylcholine. Three such studies are available and we shall examine them in some detail.

1.1.

An *ab initio* SCF investigation by the discrete "super-molecule" approach (for a general presentation of this method see [15]) has indicated that the stabilization of the *trans-trans* conformer of AcCh, due to the interaction with its first hydration shell, is about 2 kcal/mole greater than the stabilization of the *trans-gauche* form, the first hydration shell being formed in both cases of five water molecules [16]. Taking into account the *ab initio* results on free AcCh [10, 11] which indicate that the *trans-gauche* conformer is intrinsically more stable than the *trans-trans* one by about 3 kcal/mole, these studies lead to the conclusions that: a) the *trans-gauche* form is still the predominant one in solution; b) the solvent effect decreases its energy difference with respect to the *trans-trans* form.

1.2.

A simplified "continuum" model for the solvent effect was used by Beveridge *et al.* [17, 18]. Parallelly, these authors used for the study of the free molecule the INDO method which predicts a particular global energy minimum at $\tau_1 = 90°$, $\tau_2 = 50°$. With respect to this starting point the solvent favors, following these authors, an extension of τ_1 and τ_2, leading to a global minimum in solution at $\tau_1 = 160°$, $\tau_2 = 100°$ which they consider representing a *trans-gauche* conformer. A large energy difference ($\simeq 7$ kcal/mole) separates this conformer from the *trans-trans* one.

A detailed examination of the procedure utilized by this group of authors, in particular as concerns the electrostatic part of the hydration energy, raises, however, a few critical objections. Thus in this model, the solute molecule is treated as a sphere of radius a (thus the exact shape of the molecule is not taken into account) with a point charge Q and a total dipole moment $\boldsymbol{m}$ positioned at its center. The solute-solvent electrostatic interaction is then evaluated as $E_{es} = Q\Phi_R - \boldsymbol{m} \cdot \boldsymbol{\mathscr{E}}_R$ where Φ_R and $\boldsymbol{\mathscr{E}}_R$ are, respectively, the reaction potential and the reaction field at the center of the sphere (in principle, Φ_R and $\boldsymbol{\mathscr{E}}_R$ are total reaction quantities, namely they are induced by the total field created by the charge and the dipole; but in the special case where both Q and $\boldsymbol{m}$ lie at the center of a sphere, the reaction potential Φ_R is due to the charge only, while the reaction field $\boldsymbol{\mathscr{E}}_R$ is due to the dipole only).

There is, however, an essential difficulty in determining the value of the dipole to be used in such a simplified model. Indeed, when the total charge is non-zero, the dipole moment depends on the origin chosen for the multipole expansion, and, for example, it would vanish if the origin was chosen to be the center of *total charge*. Now, the choice of such an origin is in no way unreasonable, but if we also continue to take this origin as the center of the sphere which simulates the molecule, E_{es} would then reduce to $Q\Phi_R = (1/D - 1)Q^2/a$, where a denotes the radius of the sphere and D the dielectric constant of the solvent. By contrast, Beveridge *et al.* evaluate the dipole

moment at the center of *positive charge*, and then find non-zero values, very different for the two conformations under investigation: 1.5*D* for the *trans-gauche* form and 9.8*D* for the *trans-trans* one. Hence, the dipole part of the energy $-\boldsymbol{m}\cdot\boldsymbol{\mathscr{E}}_R$ (which varies like m^2/a^3) now gives widely different values. On the other hand, it could also be argued that, in the real molecule, the charge is closer to its boundary than to its center (however reasonably defined), and that the charge should therefore be placed at some point different from the center in the simulating sphere, this position being altogether different according to which conformation of acetylcholine we wish to simulate. This discussion shows that it is very difficult to avoid considerable arbitrariness in the numerical results obtained by a too rough simulation of complex molecules or ions and that, consequently, it seems necessary to have at one's disposal a method which takes properly into account both the real shape of the molecule and its charge distribution in space, as our own continuum model [1] does. As we shall see later in this paper, it turns out that realistic values for the electrostatic solute-solvent energy are quite close for the two conformations of acetylcholine. Finally, Beveridge *et al.* evaluated the cavitation energy in terms of the surface tension, modified for taking into account the microscopic size of the molecular cavity, while, as explained in part I of this series [1], recent Monte-Carlo results incite to use a rather different procedure derived from Pierotti's formula.

1.3.

Hopfinger [19] used the concept of hydration shell of Moelwyn-Huges to calculate the change in free energy as a function of the conformation of acetylcholine in aqueous solution. In this model all solvent molecules distribute themselves about an atom or group so that they may be encapsulated in a hydration shell of radius R_v. If Δf is the change in free energy associated with the removal of one solvent molecule from the hydration shell for the solute in conformation k, then it is possible to calculate the total free energy $F(k)$ associated with the hydration shell following formulas presented by this author. With this model Hopfinger finds a preference of AcCh for the *trans-trans* form in solution, this form being 2.3 kcal/mole more stable than the *trans-gauche* one. As this result disagrees with experiment, Weintraub and Hopfinger [21] propose to refine the previous model by taking into account "dynamic interactions" between the water molecules and solvation groups. Then, they found that the *trans-gauche* form of solvated acetylcholine becomes the predominant one.

However, the theoretical foundation of the original (unsuccessful) model as well as of its modification is not clear and it seems thus difficult to assess the extent of their validity (see [6]). In fact, it appears that the failure of the primitive model of Hopfinger is primarily due to the much too strong hydration, in these authors' method, of the ester oxygen of AcCh and of the hydrogen atoms bound to two of the methyl carbons at the cationic head [22].

In view of the divergences between the results of the different theoretical procedures and of the manifest insufficiencies of the empirical models we thought it useful to apply to this important system the three methods presented in part I of this series:

the discrete model, the continuum model and the discrete-continuum model. The utilization of our discrete model should enable the comparison of its results with those of quantum-mechanical *ab initio* computations. The effect of its extension beyond the number of water molecules introduced in the latter is also worthwhile exploring. The utilization of our continuum model which takes into account the shape of the molecule, the charge distribution, the reorganization of the solvent molecules in the presence of the solute and the correction of the free energy variation calculated with a dielectric constant, factors neglected in previous such treatments, should provide more reliable information about the significance of a continuum model. Finally the utilization of the combined continuum-discrete model should delimit the extent to which the discrete model accounts or fails to account for the overall solvent effect.

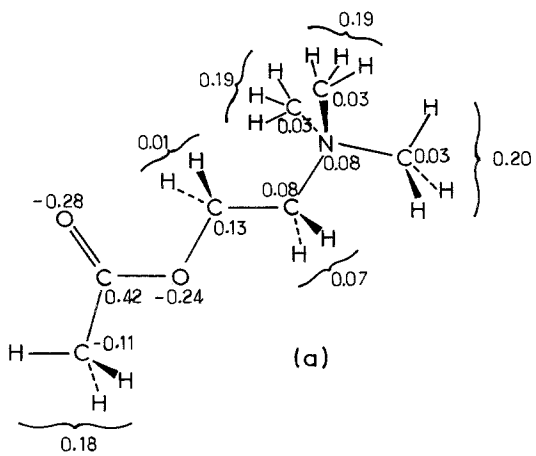

Fig. 2a. Net charges corresponding to electronic populations (CNDO method)

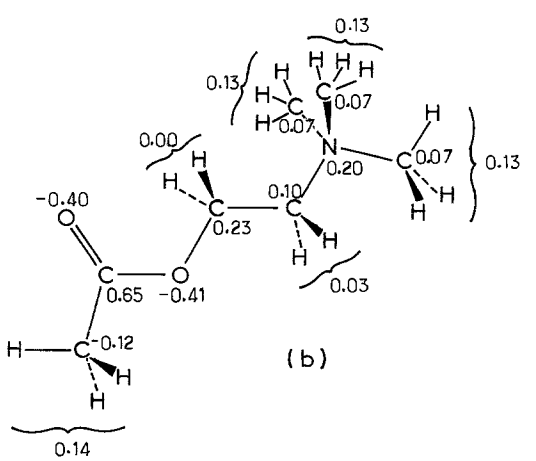

Fig. 2b. Effective net charges (corresponding to the total effective electronic charges)

2. Technical Details

The geometry of AcCh used in the computations is that derived from the crystal of its chloride [7]. The distribution of the electronic charges was computed using the CNDO/2 method [23]. The atomic net charges are displayed in Fig. 2a, b for the *trans-trans* form: Fig. 2a corresponds to the usual electronic populations, while Fig. 2b gives the net charges used for the calculation of the intermolecular interactions. It must indeed be emphasized that these two kinds of charges do not coincide, since the complete electrostatic charge distribution of the molecule involves, in the CNDO framework, atomic dipole moments (so-called hybridization moments) besides the usual electronic populations [24–27] (in an *ab initio* framework, since the zero differential overlap approximation is no more used, dipole moments associated with the overlap between different atoms would also appear besides the atomic dipole moments and the usual Mulliken electronic populations [24–27]). It would be possible to use directly the atomic dipole moments in the evaluation of the electrostatic interaction, but for computational simplicity we preferred to replace them by suitable "effective" charges. For that purpose we followed the procedure described in [27]: each atomic dipole moment is split into components directed along the bonds, associated with the atom considered, and each of these components is replaced by two effective "hybridization" charges put on the atoms corresponding to the bond; the total electronic charges used (corresponding to the net charges of Fig. 2b) are the sums of the electronic populations (corresponding to the net charges of Fig. 2a) and of these "hybridization" charges.

As concerns the charges of the *trans-gauche* form, they are identical with those of the *trans-trans* form within the first decimal.

It is worth stressing that the electronic populations of Fig. 2a are quite close to the Mulliken electronic populations given by the SCF *ab initio* method (see Ref. [11]), namely the positive charge is mainly distributed on the hydrogen atoms surrounding the nitrogen. Therefore the positive effective charge appearing on the nitrogen in Fig. 2b is almost entirely due to the hybridization charges which mimic the atomic hybridization dipole moments.

3. Results and Discussion

3.1. The Discrete Model

In the first step of utilization of this model we wished to compare the results of these empirical computations with those available from the *ab initio* treatment of Pullman and Port [11]. As shown in Fig. 3a and 3b our own investigation is in very close agreement with theirs: with five water molecules we find similar preferred binding sites as obtained by these authors and also that the interaction of the water molecules with the two conformers of AcCh favors the *trans-trans* conformation over the *trans-gauche* one by about 2 kcal/mole (Table 1). It brings thus the energies of these two conformers more closely together leaving nevertheless the *trans-gauche* conformation as the preferred one.

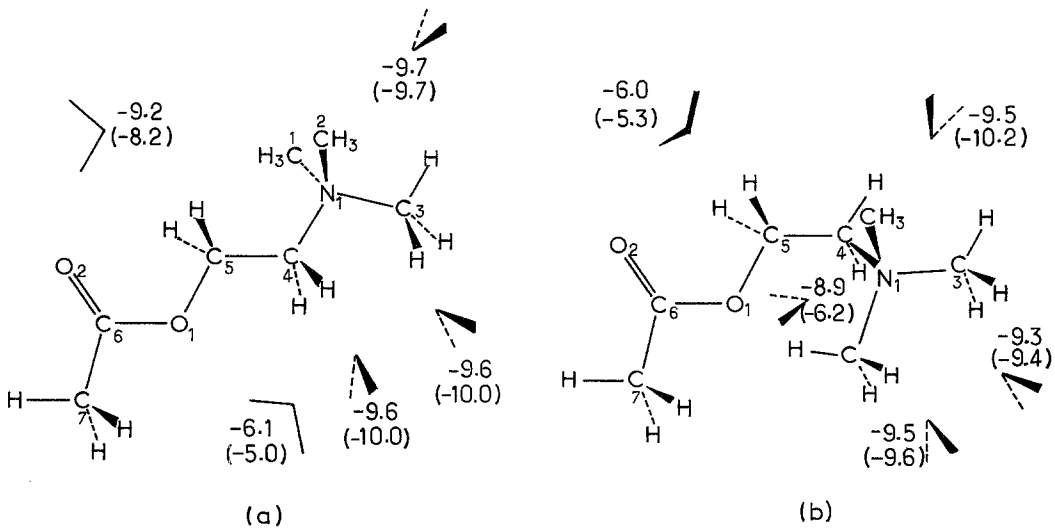

Fig. 3. Principal hydration sites of acetylcholine (*trans-trans* (3a) and *trans-gauche* (3b)) as given in Ref. [11]

Table 1. Solvation energies of acetylcholine calculated by the discrete model (kcal/mole) (after minimization)

Number of water molecules	*trans-trans* form	*trans-gauche* form
5	−22.5[a]	−23.6[b]
23	−38.9	−41.0
25	−31.0	−35.0
41	−40.0	−39.0

[a] −16.4 before minimization.
[b] −14.4 before minimization.

In a second step we have carried out a minimization of the total energy of the complex, with respect to simultaneous rotations and translations of the five water molecules. As seen from the comparison of Figs. 3 and 4, this results for both conformations in a displacement of two water molecules, which come closer together and form a hydrogen bond. In this modified situation it is the *trans-gauche* form which becomes slightly more stabilized by the solvent than the *trans-trans* one, which leads altogether to an enhancement of the overall stability of the former with respect to the latter.

In the next steps of the procedure we have increased the number of water molecules put around the AcCh molecule. Calculations have been performed, according to the procedure described in Ref. [1], with 23, 25 and 41 water molecules. The main results may be summed up as follows.

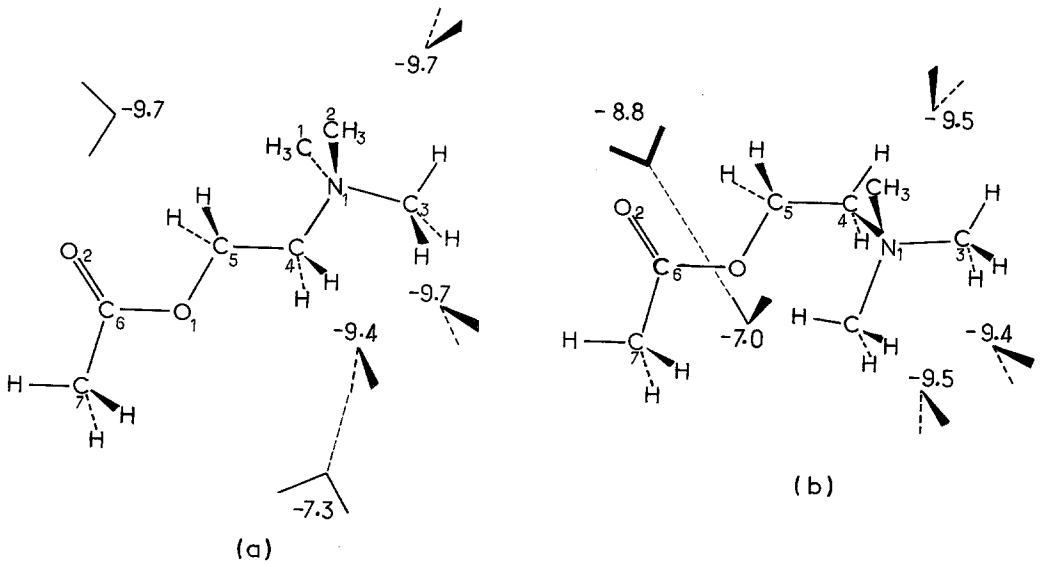

Fig. 4. Principal hydration sites of acetylcholine *trans-trans* (4a) or *trans-gauche* (4b) after minimization. (Present discrete model)

Computations with 23 or 25 water molecules confirm the existence of five preferred interaction sites with the AcCh skeleton, located altogether very closely to the sites indicated by Ref. [11], the remaining water molecules organizing themselves around this lattice. After minimization these hydration schemes favor the *trans-gauche* form by about 2–4 kcal/mole and produce thus an enhancement of its stability over that of the *trans-trans* form with respect to the relative intrinsic stabilities of these two forms. In the last computation, involving 41 water molecules, we added 36 water molecules around the five water molecules put in the hydration scheme of Ref. [11]. General minimization did not produce any important displacement of these water molecules from their primitive sites but the overall result (last line of Table 1) favors the *trans-trans* form by 1 kcal/mole over the *trans-gauche* one. Taking into account the intrinsic preferences of the free conformers, the result signifies the persistence of a preference for the *trans-gauche* form but with a diminished difference with respect to the *trans-trans* one. It is therefore similar to the result of the "super-molecule" treatment of Ref. [11].

Thus, in spite of a certain instability of the results with respect to the differential effect of the solvent on the stabilities of the two conformers of AcCh, the discrete treatment leads consistently to the conclusion that the *trans-gauche* form should be the predominant one in solution.

3.2. The Continuum Model

The solvation energy and its partitioning into its different components obtained by this model are indicated in Table 2. The main result, indicated in the last column,

Table 2. Solvation energies of acetylcholine calculated by the "continuum model"

Conformer	$E_{cavitation}$	$E_{dispersion}$	$E_{repulsion}$	$E_{electrostatic}$	E_{total}
trans-trans	−0.9	−27.0	+3.75	−45.4	−69.55
trans-gauche	−0.9	−26.4	+3.7	−46.3	−69.9

shows that the total solvation energy is practically identical for the two conformers and thus leaves the intrinsic preference for the *trans-gauche* one practically unperturbed. It is worth stressing that, in contrast to the calculations of Refs. [17] and [18], the electrostatic component of the solvation energy, which is the principal term, differs by less than 1 kcal/mole between the *trans-trans* and the *trans-gauche* forms.

3.3. The Combined Discrete-Continuum Model

In this model the effect of the continuum was superposed upon the system acetylcholine + five water molecules of the first hydration shell, fixed as in Ref. [11]. As described in paper I of the series [1] and symbolized in Fig. 5 the procedure involves the consideration of four states of the system (+2 intermediate ones) and of three steps in the hydration scheme. In the first step we take five water molecules out of the continuum, a process associated with a loss of energy. In the next one we proceed with the hydration of acetylcholine by these five water molecules. In the last

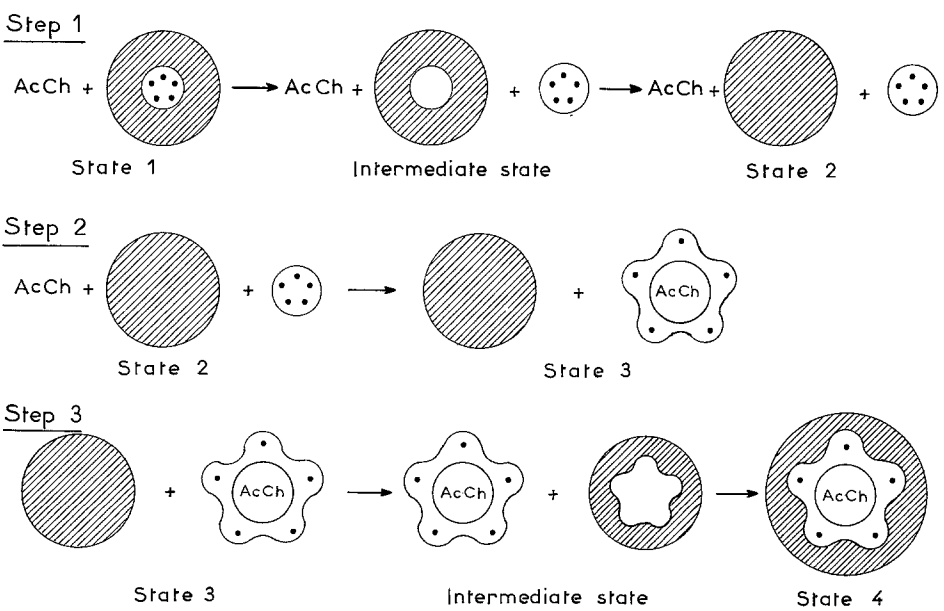

Fig. 5. The solvation process in the combined discrete-continuum model

Table 3. Solvation energy of acetylcholine[a] obtained by the combined discrete-continuum model (kcal/mole)

State[b]	$E_{cavitation}$		$E_{dispersion}$		$E_{repulsion}$		$E_{electrostatic}$		E_{total}[c]	
1										
Intermediate	−0.7		−23.3		+6.1		−4.0		−21.9	
2			−15.0		+20.9		−37.1		−31.2	
	trans	*gauche*	*trans*	*gauche*	*trans*	*gauche*	*trans*	*gauche*	*trans*	*gauche*
3			−24.4	−21.0	+25.8	+21.0	−48.9	−45.5	−47.5	−45.5
4	−1.5	−1.5	−39.5	−40.0	+5.4	+5.6	−33.0	−33.9	−68.6	−69.8
Total	−0.8	−0.8	−25.6	−22.7	+4.2	−0.4	−40.8	−38.3	−63.0	−62.2

[a] *trans* stands for *trans-trans* and *gauche* for *trans-gauche*.

[b] See Fig. 3.

[c] $E_{total} = E_{state\ 4} + E_{state\ 3} - E_{state\ 2} - E_{state\ 1}$.

step a continuum of water is made to interact with this system. It must be mentioned that, in the present state of the method, the computation time for the continuum model is still rather important, so that we have not attempted to perform minimizations with respect to the configuration of the discrete water molecules of the hydration shell. The results of the relevant computations are summarized in Table 3. The overall result indicates a small preferential stabilization of the *trans-trans* conformation (by about 0.8 kcal/mole) by the general solvation effect, which means that it decreases only very little the intrinsic preference of the free molecule for the *trans-gauche* form (which is of the order of 3 kcal/mole). From the results of the intermediate steps we may notice that the favoring of the *trans-trans* form by the formation of the discrete first hydration shell, which is of the order of 2 kcal/mole, is partially counteracted by the effect of the added continuum which favors the *trans-gauche* form by about 1.2 kcal/mole.

4. Conclusion

The essential conclusions which may be drawn from this work are twofold, referring to the comparison of the different methods on the one hand and to the problem of the solvent effect upon the conformation of AcCh on the other.

Concerning the first point the most significant result seems to relate to the new aspect of the effect of the continuum as obtained in this work in comparison with previous ones, which shows the importance of a careful definition of this method. Concerning the second point the main conclusion seems to be that the solvent-effect is altogether small in the example studied here and does not perturb to a large extent the intrinsic preference of AcCh for the *trans-gauche* conformation. This essential result persists in the three types of approach described here and agrees satisfactorily with the conclusion reached by experimentalists [6]. The different approaches differ somewhat in their evaluation of the differential solvent effect on the relative energies of the two conformers under investigation but the differences are in this case so small that it would not be reasonable to decide on that basis which of them is better. It seems probable that the combination of a discrete treatment for the structured first hydration shell [5] with a refined continuum treatment for the effect of the surrounding bulk water may represent the most satisfactory procedure. We are exploring now along similar lines the effect of hydration on other important biological molecules and systems with the hope that such an extended study will enable to build a coherent and satisfactory theoretical representation of the solvent effect.

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