Solvent Constraints on the Property Space of Acetylcholine. I. Isotropic Solvents

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The objective of this study was, first, to examine the property space of a test molecule and, second, to assess solvent constraints. Acetylcholine was chosen as the object of study given its interesting molecular structure and major biological significance. Molecular dynamics simulations of long duration (30 ns) were carried out with acetylcholine in a vacuum or in a box of solvent (chloroform, water, water plus one chloride counterion). For each of the 6000 conformers stored during each run, various geometric and physicochemical properties were calculated, namely, N^+-C8 distance, solvent-accessible surface area (SAS), polar surface area (PSA), dipole moment, and lipophilicity (virtual log *P*). The variations of these properties as a function of the dihedral angles τ_2 and τ_3 were unexpectedly broad for such a small molecule. Dipole moment and virtual log *P* were well correlated, and they varied in a complex manner with the dihedral angles. For example, each of the seven conformational clusters was able to access much of the lipophilicity space of acetylcholine. Solvent constraints on the property space clearly indicate that a polar medium tends to favor polar conformers, whereas the opposite is true for a solvent of low polarity.

Introduction

One of the fundamental quests in chemistry is to constantly deepen our understanding of molecular structure taken in its broadest sense, namely, its geometric features and physicochemical properties. A poorly explored aspect in this research agenda is the interdependence of geometric and physicochemical properties. Some of the geometric features of a given compound are invariant (i.e., its connectivity and configuration), whereas others can vary within a given range (e.g., its conformation[1,2]). The conformational behavior of molecules as assessed experimentally or computationally can be expressed in conformational hypersurfaces, and the ensemble of all conformers of a given compound is often taken as defining a conformational space.

Physicochemical properties of great pharmacological and biological relevance include hydrophobicity, hydrogen-bond donor and acceptor capacity, and lipophilicity, in other words, properties expressed as recognition forces in chemical and biochemical processes.[3−5]

A powerful method to study the recognition forces of biomolecules is the computation of molecular fields, for example, MEPs (molecular electrostatic potentials), which encode electrostatic forces,[6] MLPs (molecular lipophilicity potentials), which encode hydrophobicity, H-bonding capacity and polarizability,[5,7] and the recent MHBPs (molecular hydrogen-bonding potentials[8]). MLPs are of particular relevance in the context of this study, since they allow one to back-calculate a partition coefficient of a given molecule.[5,7] However, a complicating factor is that MLPs, like all 3D-molecular fields, are strongly dependent on the 3D-geometry of the investigated molecules, in other words, on their conformational state. This dependence has led researchers to calculate a "virtual" log *P* for conformers,[7] a computational achievement that has received experimental validation. Indeed, there is experimental evidence that "rigidified" conformers (i.e., mainly diastereomers) differ in their log *P* values.[9] Recent kinetic studies[10] have afforded a direct proof that conformers differ in their octanol/water partition coefficient.

Since a virtual log *P* can be computed for each conformer in the conformational space of a molecule, a lipophilicity space must correspond to the conformational space. The first objective of this study is to establish the postulated existence of a lipophilicity space and its one-to-one correspondence with the conformational space.

A second objective of the study is to investigate solvent effects on the lipophilicity space, given the well-known constraints exerted by the medium on the conformational behavior (and hence the conformational space) of molecules. But while solvent-dependent conformational equilibria have been extensively investigated, medium-dependent constraints on the hypothetical lipophilicity space remain unexplored. Yet such constraints should not be without influence on the behavior of compounds in biological systems, for example, when they permeate membranes or bind to biological targets such as enzymes and receptors.

Acetylcholine was chosen as the object of study given its major biological significance and the many data (experimental and computational) accumulated on its conformational behavior.[11−23] Acetylcholine, despite its small MW, also has an interesting molecular structure characterized by a great flexibility and the presence of

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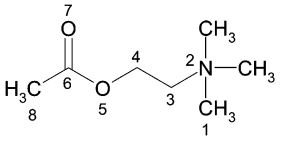

τ_1 = C1-N2-C3-C4

τ_2 = N2-C3-C4-O5

τ_3 = C3-C4-O5-C6

τ_4 = C4-O5-C6-O7

monitored distance = N2-C8

Figure 1. Dihedral angles in acetylcholine. Their values in this study are defined according to Klyne and Prelog.[2]

polar and nonpolar groups. In our study, the small size of acetylcholine was both an advantage (since molecular dynamics simulations were very time-effective) and a limitation (since it was obvious at study design that the property space of such a small molecule must be quite limited).

Molecular dynamics (MD) simulations were used to explore the conformational space of acetylcholine in a vacuum and in two solvents of very different polarity, that is, water and chloroform. A number of geometric and physicochemical properties were calculated for each conformer. The geometric properties were the dihedral angles τ_2 and τ_3 (Figure 1) and the distance between N^+ and C8. The physicochemical properties included lipophilicity (log *P*), dipole moment, polar surface area (PSA), and solvent-accessible surface (SAS), which is a mixed geometric and physicochemical property. The results reveal the interesting shape of the lipophilicity space of acetylcholine in the τ_2, τ_3, and log *P* system of Cartesian coordinates. Solvent constraints on the lipophilicity space have also been evidenced.

Methods

Conformational Properties of Acetylcholine. The initial geometry of acetylcholine was constructed and energy-minimized using the Quanta/CHARMm package (MSI, Burlington, MA). The computation of partial atomic charges and the final geometry optimization were carried out at the semiempirical level with the MOPAC6.0 program (keywords = AM1, PRECISE, GEO-OK).

Acetylcholine has four dihedral angles defined in Figure 1. A previous study [24] undertaken under computational conditions similar to the present ones had confirmed literature data and indicated that τ_1 and τ_4 vary in a narrow range and independently of the conditions ($\tau_1 = 60° \pm 20°$; and $\tau_4 = 0° \pm 20°$), due to the symmetry of the triple rotor (τ_1) and to the rigidity of the ester group (τ_4). This study revealed 9 minimum-energy conformations and 15 transition conformations (see Table 1 for clusters and definitions). The previous study had also highlighted that +**g**+**a** and +**gt** conformers form a unique +**gt** cluster, while the −**gt** and −**g**−**a** conformers form the −**gt** cluster. In other words, 7 low-energy conformational clusters were seen (see Figure 2A). The other possibilities in Table 1 correspond to highly improbable conformations and are left blank. The present work does not focus on relative conformational energies; hence, all conformers having τ_2, τ_3, or both in an anticlinal conformation are considered as transition forms.

Molecular Dynamics Simulations. All calculations were carried out in a dual Athlon PC. The package Namd2.51[25] with the force-field CHARMm v22 was used. The initial structure of acetylcholine for all simulations was the +**g**+**g** geometry. The previous study[24] had shown that the nature of the starting conformer had no influence on the results. This structure was submitted to molecular dynamics simulations. The simulations were carried out for 30 ns in a vacuum, in chloroform, and in water. No constraint was imposed on any of the dihedral angles, but only τ_2 and τ_3 were monitored.

For the simulations in chloroform and water, the starting structure of acetylcholine was placed using VEGA[26] in a cluster of 10 Å radius containing 161 water molecules described by the model TIP3S, or 88 chloroform molecules described in VEGA templates. Simulations were also carried out in the same cluster of water to which one chloride anion had been added. In simulations with solvents, a spherical boundary condition (radius = 18 Å) was applied to stabilize solvent clusters, which were then optimized for the relative position of the solvent molecules to eliminate any high-energy interaction.

All simulations had the following characteristics: minimizations with the conjugate gradients algorithm; convergence limit (RMS) = 0.01; maximal number of iterations = 5000; molecular dynamics with constant temperature in the range 300 ± 25 K; integration of Newton's equation each 1 fs according to Verlet's algorithm; frame stored each 5000 iterations (5.0 ps), yielding 6000 frames per trajectory. The molecular dynamics were carried out in three phases: initial period of heating from 0 to 300 K over 3000 iterations (3 ps, i.e., 1 K/10 iterations), equilibration period of 3 ns, and the monitored phase of simulation of 30 ns. Only the frames memorized during this third phase were considered.

Computation of Geometric and Physicochemical Properties of Conformers. The results of the MD simulations were analyzed with VEGA. The geometric parameters include the dihedral angles τ_2 and τ_3 as defined in Figure 1, and the distance between the N^+ atom and the methyl C8 atom (see Figure 1), because it is intuitively suitable to indicate the folding degree of acetylcholine. The virtual log P was calculated according to the MLP approach.[7] The SAS was calculated with a solvent molecule of radius equal to 1.4 Å. The PSA was calculated by subtracting from the SAS the contributions of carbon and nonpolar hydrogen atoms.[27]

Results

Conformational Behavior of Acetylcholine in a Vacuum, in Chloroform, and in Water. The conformational space of acetylcholine was simulated during 30 ns in a vacuum, in chloroform, in water, and in water in the presence of a chloride anion. The 6000 conformers of acetylcholine recorded in a vacuum, chloroform, and water are displayed in Figure 2, parts B, C, and D, respectively. The conformational distributions (also including the simulation in water plus one Cl^-) are presented in Table 2. In a vacuum, τ_2 was mainly in a gauche conformation, as was τ_3. In chloroform, τ_2 was mostly gauche, whereas τ_3 showed no clear preference for gauche or trans conformations. In water (in the absence or presence of a Cl^- anion), τ_2 again was mainly gauche, and τ_3 showed no clear preference in the range 60°−300°. When analyzing the relative abundance of each conformational cluster, one observes that all solvents significantly reduced the relative abundance of the **gt** cluster without markedly affecting the relative abundance of the **gg** cluster. In contrast, the relative abundance of the more extended geometries was influenced by the polarity of the solvent, since water induced a significant increase in the population of **tt** conformers, whereas in chloroform an increase in **tg** conformers is noticeable. In all solvents, about 25% of conformers were classified as transition forms (τ_2 or τ_2 anticlinal), while in vacuo the corresponding relative abundance was 12.8%.

These results are in good agreement with those obtained by Segall et al. using ab initio methods based

Table 1. The Various Conformers of Acetylcholine Designated as Combinations of τ_2 and τ_3[24] [a]

	τ_2 (range in deg)					
τ_3 (range in deg)	**0** (330−30)	**+g** (30−90)	**+a** (90−150)	**t** (150−210)	**−a** (210−270)	**−g** (270−330)
0 (330−30)				**(t0)**		
+g (30−90)		**+g+g**	**(+a+g)**	**t+g**	**(−a+g)**	
+a (90−150)		**+g+a**	**(+a+a)**	**(t+a)**	**(−a+a)**	**(−g+a)**
t (150−210)		**+gt**	**(+at)**	**tt**	**(−at)**	**−gt**
−a (210−270)		**(+g−a)**	**(+a−a)**	**(t−a)**	**(−a−a)**	**−g−a**
−g (270−330)			**(+a−g)**	**t−g**	**(−a−g)**	**−g−g**

[a] Combinations *not* in parentheses designate the most probable conformers (energy minima), combinations in parentheses designate conformers of slightly higher energy (transitional conformers), whereas empty boxes correspond to highly improbable conformers (**g** = gauche, **t** = trans, **a** = anticlinal, **0** = synperiplanar).

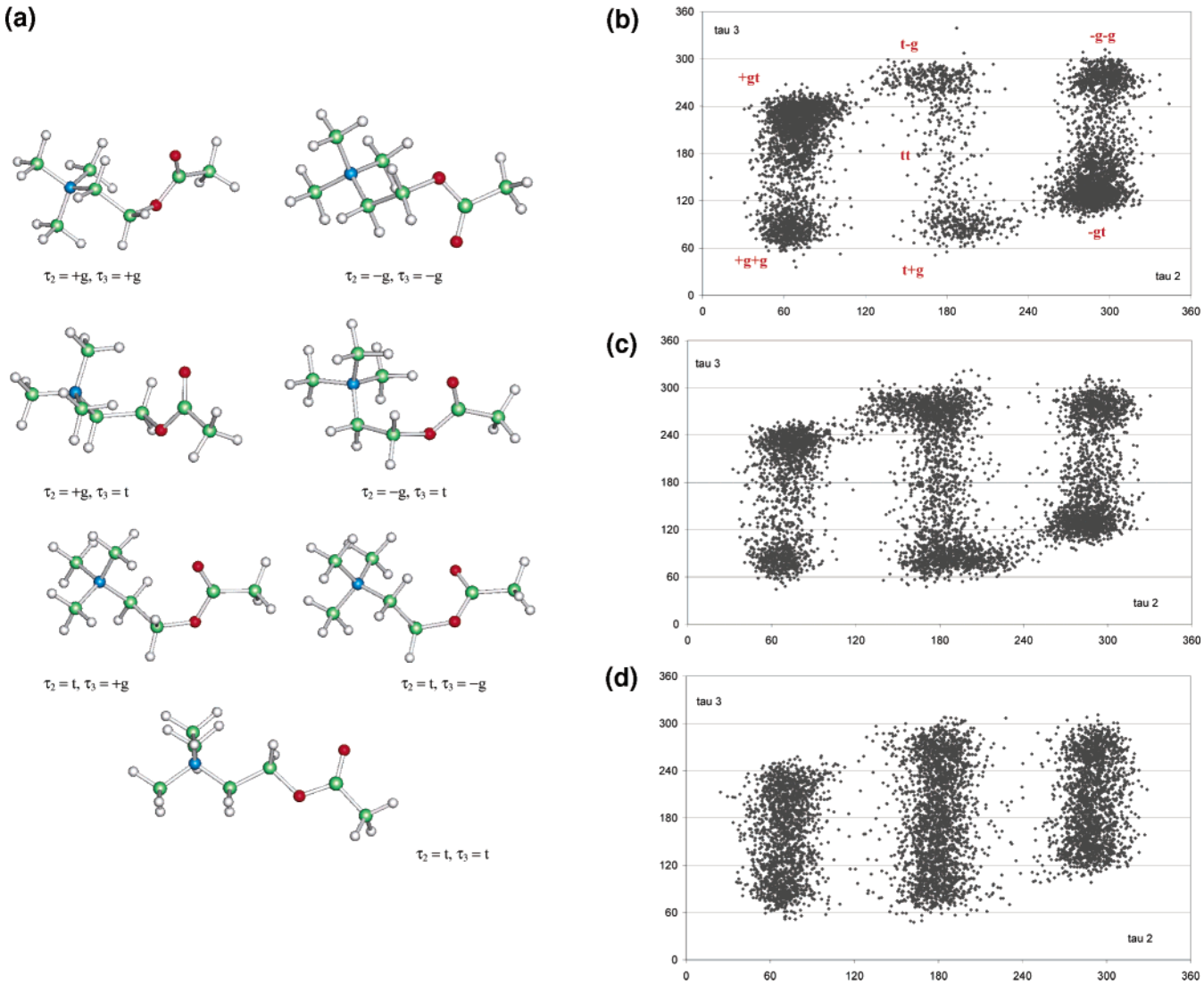

Figure 2. The conformational behavior of acetylcholine (τ_2 vs τ_3 plot) as simulated for 30 ns at 300 K: (a) the seven low-energy conformers of acetylcholine; (b) conformational behavior in a vacuum (the conformational classes are indicated in red); (c) conformational behavior in chloroform; (d) conformational behavior in water.

on density functional theory.[21] Indeed, their study revealed that the angles τ_2 and τ_3 can exist in the gauche and trans ranges with an energy minimum for the **−gt** and **tt** conformers existing only in relaxed molecular structures. The present results are also in excellent agreement with our previous results in a vacuum, methanol, and water using the same methodology.[24]

Solvent effects on the conformational profile of acetylcholine (Table 2) show that the solvents act as filters and favor conformers with properties most similar to theirs. The solvent effects can be summarized as follows:

(a) Polarity. The solute assumes conformers best able to mimic the properties of the solvent. This can be seen with the significant increase of fully extended (**tt**) conformers in water compared to a vacuum (the percentage varies from 1.1% in vacuo to 9.3% in water). This effect is 2-fold, namely, (a) intrinsic polarity and (b) a solute−solvent interaction that selects the conformers able to maximize interactions with the solvent. For example, the **tt** conformers are simultaneously the most polar ones and the best suited to form H-bonds with water molecules.

Table 2. Number of Conformers (and %) in Each Class as Accumulated during 30 ns Simulations

	conditions			
conformers	vacuum	chloroform	water	water plus one chloride
+g+g	731 (12.2%)	558 (9.3%)	741 (12.35%)	726 (12.1%)
−g−g	723 (12.1%)	768 (12.8%)	801 (13.35%)	828 (13.8%)
+gt	1777 (29.9%)	936 (15.6%)	928 (15.5%)	876 (14.6%)
−gt	1620 (27.0%)	1068 (17.8%)	964 (16.1%)	1086 (18.1%)
t+g	153 (2.5%)	492 (8.2%)	217 (3.6%)	216 (3.6%)
t−g	161 (2.5%)	444 (7.4%)	230 (3.8%)	228 (3.8%)
tt	63 (1.1%)	246 (4.1%)	557 (9.3%)	498 (8.3%)
anticlinal forms (**ga**, **ta**, **ag**, **at**, **aa**)	772 (12.8%)	1488 (24.8%)	1562 (26.0%)	1542 (25.7%)
total gg and **gt**	4851 (80.8%)	3330 (55.5%)	3434 (57.3%)	3516 (58.6%)
total tg and **tt**	377 (6.4%)	1182 (19.7%)	1004 (16.7%)	942 (15.7%)

Table 3. Ranges, Mean Values ± SD, and Mean ± 99.9% Confidence Limits for the Computed Molecular Properties of 6000 Acetylcholine Conformers Generated during 30 ns MD Simulations

	medium[a]			
property	vacuum ($\epsilon = 1$)	chloroform	water	water plus one chloride
distance[b]	4.37 to 6.39	4.35 to 6.37	4.37 to 6.36	4.29 to 6.41
	5.25 ± 0.22	5.40 ± 0.40	5.43 ± 0.45	5.42 ± 0.45
	5.25 ± 0.009	5.40 ± 0.018	5.43 ± 0.019	5.42 ± 0.020
SAS[c]	343 to 377	336 to 376	341 to 378	337 to 374
	358 ± 5	356 ± 6	361 ± 7	359 ± 7
	358 ± 0.2	356 ± 0.3	361 ± 0.3	359 ± 0.3
PSA[d]	24.2 to 44.0	28.5 to 50.4	24.4 to 44.8	30.0 to 51.4
	35.0 ± 2.8	40.1 ± 3.7	37.8 ± 2.6	42.4 ± 3.2
	35.0 ± 0.12	40.1 ± 0.16	37.8 ± 0.11	42.4 ± 0.15
log P_{oct}[e]	−2.53 to −2.15	−2.53 to −2.19	−2.55 to −2.20	−2.53 to −2.23
	−2.34 ± 0.06	−2.36 ± 0.06	−2.42 ± 0.06	−2.41 ± 0.05
	−2.34 ± 0.003	−2.36 ± 0.003	−2.42 ± 0.003	−2.41 ± 0.002
dipole moment	5.51 to 10.1	7.43 to 9.54	7.80 to 9.71	6.14 to 10.68
	7.78 ± 0.82	8.40 ± 0.37	8.88 ± 0.33	8.85 ± 0.75
	7.78 ± 0.035	8.40 ± 0.017	8.88 ± 0.014	8.85 ± 0.033

[a] In each group, the first line shows the range, the second the mean ± SD, and the third the mean ± 99.9% confidence limits (= SD × 3.29/$\sqrt{6000}$ = SD × 0.0425). [b] Distance in Å between N^+ and C8. [c] Solvent-accessible surface area in Å^2. [d] Polar surface area in Å^2. [e] "Virtual" log *P* calculated by the molecular lipophilicity potential.

(b) Friction. The solvent slows down all molecular movements irrespective of its physicochemical properties, an effect mainly proportional to solvent molecular weight, as seen in a previous work (with methanol and octanol)[24] and in the present study. As a result, the percentage of anticlinal structures increases dramatically from a vacuum (12.8%) to water (26.0%), since the former can be considered as transitional conformations of which the percentage increases in relation to the slowing of the molecular movements. In summary, this preliminary exploration has identified preferred conformers and preferred conformational transitions of acetylcholine.

The goal here was purely indicative, namely, to ensure a broad exploration of the accessible conformational space, and served to lay the ground for the real objective of this study, which was to explore the space of physicochemical properties of acetylcholine and its solvent dependence.

An Overview of the Property Space of Acetylcholine in a Vacuum, in Chloroform, and in Water. For each of the 6000 conformers stored during the 30 ns simulations in a vacuum, in chloroform, in water, and in water in the presence of a chloride anion, a number of geometric and physicochemical properties were calculated, as compiled in Table 3. The distance between N^+ and C8 is seen to span a broad range of about 40%. The variation in the solvent-accessible surface area (SAS), another geometric property, is limited to about 10%. Physicochemical properties related to polarity and lipophilicity also span markedly broad ranges, for example, the polar surface area (PSA) about 50% and the dipole moment about 35%. Lipophilicity itself (i.e., log *P*) spans about 0.35 unit, a rather large value considering the smallness of the molecule.

Table 3 highlights that in chloroform acetylcholine shows average values of log *P*, distance, dipole moment, and polar area that are intermediate between those in water and in a vacuum. All solvents, chloroform included, interact with acetylcholine and compete with its intramolecular interactions, thus increasing its polarity (log $P = -2.36$ to -2.41). In a vacuum, intramolecular interactions are not hampered and result in a slightly higher average log *P* value.

In contrast, the lowest average in the solvent-accessible surface area of acetylcholine is seen in chloroform, confirming the significant role of friction in solvent effects. It is intriguing to note how the solute can modulate its physicochemical properties and even minimize its steric hindrance.

A comparison between the MD results in water with and without a chloride ion suggests that acetylcholine is quite insensitive to the presence of the counterion. The sole value that shows a remarkable difference is the average PSA, which is larger in the presence of Cl^-. This behavior may mean that chloride attracts more water molecules around the solute and induces it to increase the exposure of its ester group.

Table 4. Correlation Matrix Obtained Considering Pairs of Properties Monitored[a]

property	log P			dipole moment			PSA			SAS		
dipole moment	**0.77**	**0.76**	**0.81**									
PSA	0.47	0.29	0.46	0.58	0.36	0.60						
SAS	0.39	0.42	0.41	0.40	0.43	0.52	0.35	0.41	0.56			
distance	0.13	0.24	0.16	0.08	0.19	0.21	0.14	0.35	0.42	**0.72**	**0.85**	**0.74**

[a] For each matrix cell, the first column reports the regression coefficient in vacuo, the second column that in water, and the third column that in chloroform. Significant correlations are indicated in bold.

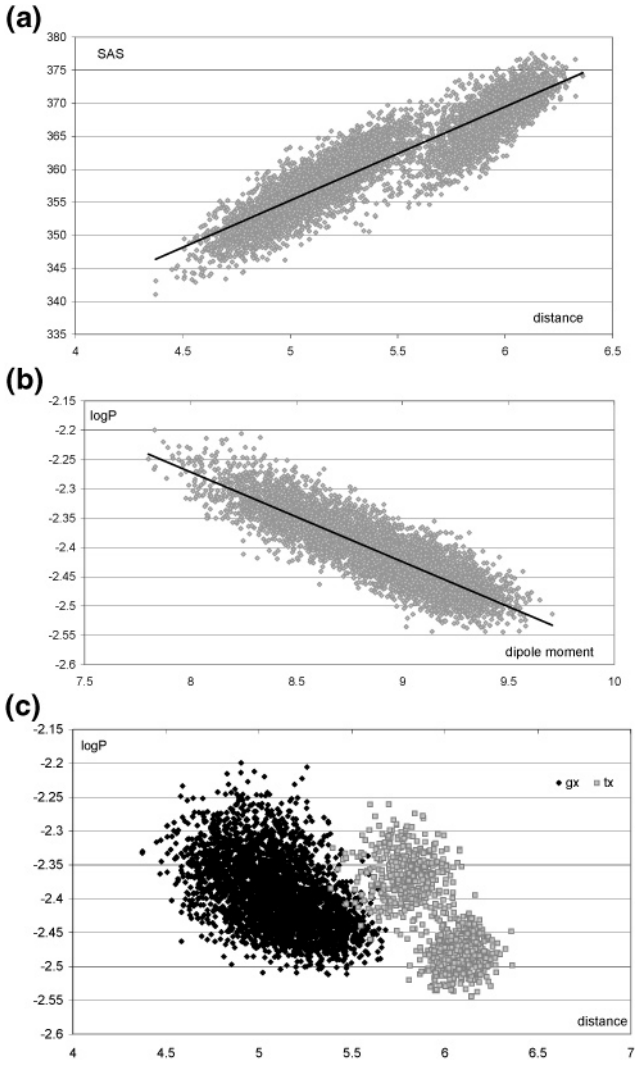

Figure 3. Relations between some of the parameters in Table 2, as calculated for the 6000 conformers obtained during a 30 ns MD simulation of acetylcholine in water: (a) correlation between the solvent-accessible surface area (SAS) and the distance between (N^+) and C8 ($r^2 = 0.85$); (b) correlation between log P (calculated with the molecular lipophilicity potential[7]) and the dipole moment ($r^2 = 0.76$); (c) lipophilicity space vs distance for distinct conformational clusters of acetylcholine. In black is the cluster of the **gg** and **gt** conformers (log P ranging from −2.51 to −2.20, no detectable subclustering); in gray are the cluster of the **tg** conformers (log P ranging from −2.46 to −2.26) and the cluster of the **tt** conformers (log P ranging from −2.54 to −2.41).

With two exceptions, no pair of parameters in Table 4 shows a strong linear correlation (i.e., most r^2 are <0.6). The first exception is the correlation between two geometric parameters, namely, distance and SAS, with r^2 values in the range 0.72 (in the vacuum) to 0.85 (in water, see Figure 3A). This correlation is expected and understandable, folded conformers having a decreased SAS compared to the extended forms.

The other, and more noteworthy, correlation is between dipole moment and log P, the r^2 values of which are in the range 0.76 (in water, see Figure 3B) to 0.81 (in chloroform). Clearly, a higher dipole moment implies a greater hydrophilicity, and the fact that the two parameters correlate despite their different nature can be seen as a mutual validation of the respective algorithms used to calculate them.

Specific plots offer insights into the lack of correlations. This is particularly the case for log P versus distance, where no linear correlation exists (r^2 in the range 0.13−0.24). A closer examination of the log P vs distance plot (Figure 3C) reveals three clusters, namely, (a) the cluster of the **gg** and **gt** conformers (log P ranging from −2.51 to −2.20, no detectable subclustering), (b) the cluster of the **tg** conformers (log P ranging from −2.46 to −2.26), and (c) the cluster of the **tt** conformers (log P ranging from −2.54 to −2.41). Thus, the influence of the torsion angles τ_2 and τ_3 on lipophilicity is a complex one not easily deducible from a 2D-plot. The same conclusion emerges when examining plots of dipole moment vs distance (not shown).

The r^2 matrix (Table 4) shows that correlations between properties are quite insensitive to solvent effects, meaning that these relations are specific parameters that allow a better comparison between and understanding of the dynamic behavior of flexible molecules.

Influence of the Dihedral Angles τ_2 and τ_3 on the Dipole Moment and Lipophilicity of Acetylcholine. Figure 2 has presented the populations of conformers (τ_2 vs τ_3 plots) of acetylcholine in a vacuum, in chloroform, and in water. Adding the dipole moment or log P as a third Cartesian axis yielded 3D-plots, which proved quite revealing.

Figure 4 show how the dipole moment of acetylcholine varies with τ_2 and τ_3. Comparable results were obtained in all media examined, namely, a vacuum (Figure 4A), chloroform (Figure 4B), water (Figure 4C), and water with one chloride anion (not shown). As seen, the dipole moment was not influenced by variations of τ_2, since the dipole range was covered for each of the three classes of conformers (i.e., with $\tau_2 = -\mathbf{g}$ or $+\mathbf{g}$ or **t**). In contrast, the dipole moment was highly sensitive to variations in τ_3 with the most polar conformers having τ_3 = trans, and the least polar conformers having τ_3 = gauche.

Given the high inverse relation between lipophilicity and dipole moment, it comes as no surprise that 3D-plots of log P vs τ_2 and τ_3 (Figures 5) are mirror images of the 3D-plots of the dipole moment. Here again, comparable results were obtained in all media examined, namely, a vacuum (Figure 5A), chloroform (Figure 5B), water (Figure 5C), and water with one chloride anion (not shown). Furthermore, lipophilicity was not influenced by variations of τ_2, since the same range was covered for each of the three classes of conformers (i.e.,

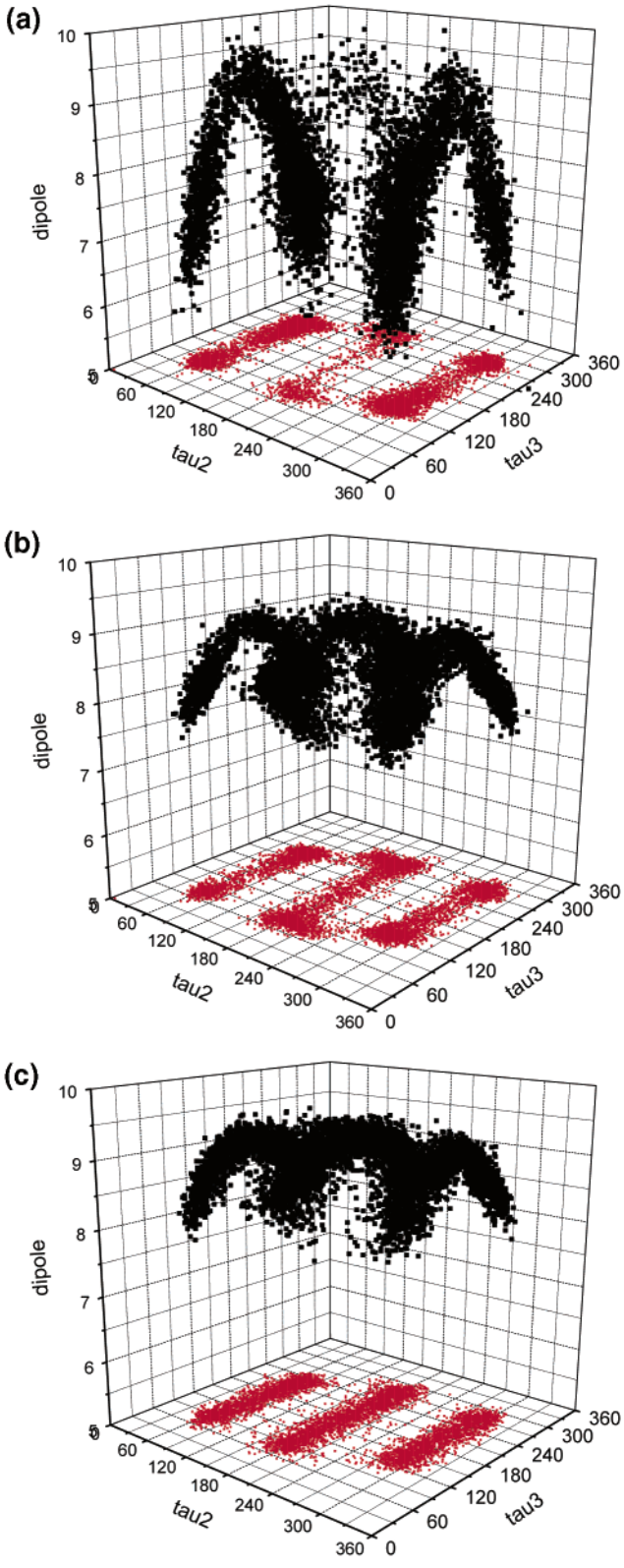

Figure 4. 3D-plots of τ_2 and τ_3 vs dipole moment: (a) in a vacuum; (b) in chloroform; (c) in water.

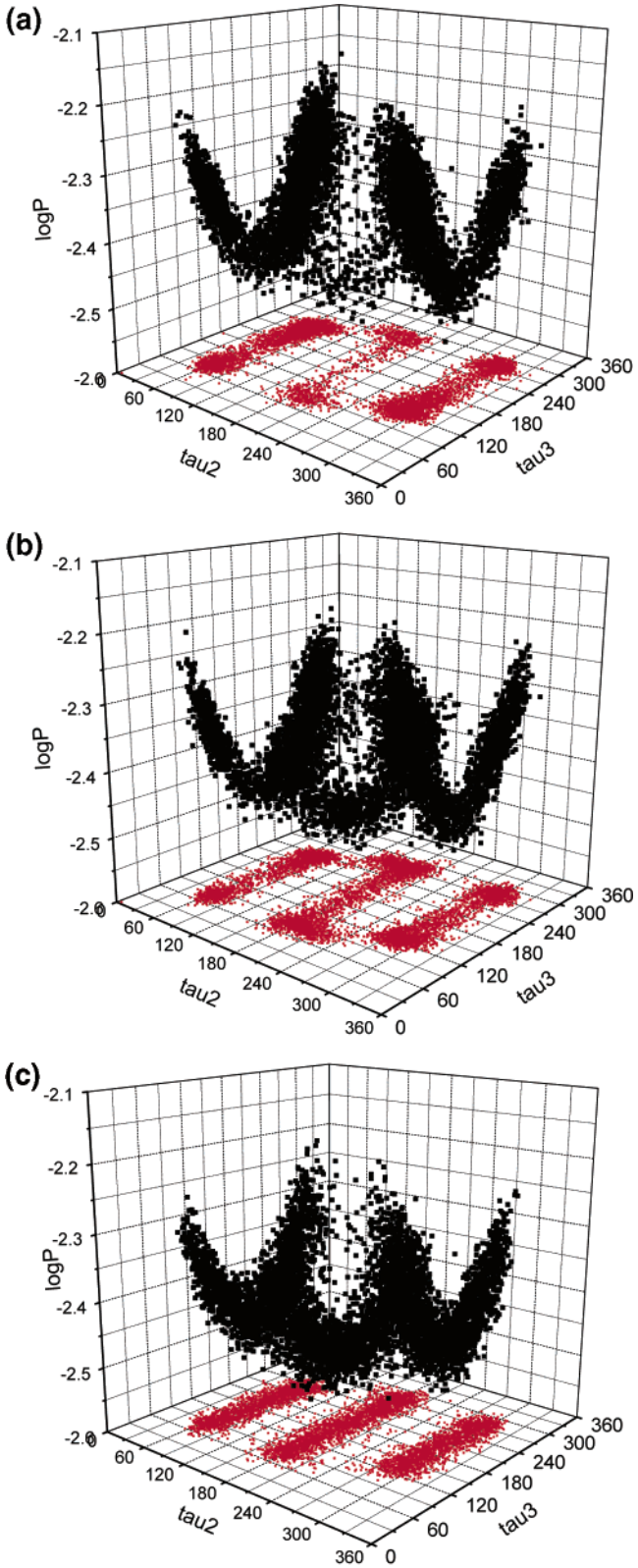

Figure 5. 3D-plots of τ_2 and τ_3 vs virtual log P: (a) in a vacuum; (b) in chloroform; (c) in water.

with $\tau_2 = -\mathbf{g}$ or $+\mathbf{g}$ or $\mathbf{t}$). And again, lipophilicity was highly sensitive to variations in τ_3 with the most lipophilic conformers having τ_3 = gauche and the most hydrophilic ones having τ_3 = trans. This behavior may mean that the ammonium head, which has a strong influence on the hydrophilicity of acetylcholine, is always accessible irrespective of τ_2. In the **tt** conformers, however, the fully exposed ammonium group appears to make a particularly strong contribution to the hydrophilicity of acetylcholine, as seen in the average values per conformational cluster (log P averages ranging from −2.33 for $+\mathbf{g}+\mathbf{g}$ to −2.47 for **tt**). In contrast, the accessibility of the ester group depends mainly on

τ_3 and varies coherently with this dihedral angle, even if the latter has a minor influence on lipophilicity. Thus, the ester group is associated with higher log *P* values when $\tau_3 =$ **g** (i.e., when it is partly masked by the ammonium group) and lower log *P* values when $\tau_3 =$ **t** (i.e., when it is fully exposed).

Solvent Constraints on the Property Space of Acetylcholine. A comparison of data and figures suggests that the various media used here led to some differences in ranges and average values. The statistical significance of the medium-dependent differences in the mean values of calculated parameters can be assessed from the 99.9% confidence limits, as derived from the standard deviations. As seen in Table 3, all differences, even small ones, are highly significant ($p \ll 0.001$). In other words, the medium (vacuum or solvent) does constrain the property space of acetylcholine, although the constraints seem modest based on the criterion of differences between mean values. As a rule, the largest differences (distance, PSA, dipole moment) are seen between the vacuum, on one hand, and the chloroform and water solvents, on the other hand. A difference of particular biophysical significance is seen in lipophilicity when comparing the aqueous medium with chloroform or a vacuum.

A second approach to consider medium-mediated differences is by examining histograms. Thus, Figure 6 compares the distributions of distances, dipole moments, and log *P* values in vacuo, in water, and in chloroform (Figures 6 and 7). Figure 6A clearly shows that the distance values are clustered in a bimodal distribution, the first peak being centered at 5.1 Å and the second at 5.9 Å. The average distances calculated per conformation (not reported) indicate that the first peak is composed of conformers with $\tau_2 =$ gauche, while the second peak includes conformers with $\tau_2 =$ antiperiplanar, highlighting the major influence of τ_2 on the geometry of acetylcholine. This multiple distribution indicates that the conformers obtained can be neatly divided into folded and extended ones with little if any intermediate conformations. This behavior reflects the discontinuous profile of the τ_2 angle, which can never assume anticlinal geometries. Also, the distance values show a bimodal distribution with many more extended conformers and markedly less folded ones in water and chloroform compared to in a vacuum.

The distribution of dipole moments (Figure 6B) is unimodal with a large excess of the more polar conformers in water and chloroform and an excess of the less polar conformers in a vacuum. A similar conclusion emerges from Figure 6C, where the distributions of log *P* values are compared. The difference here is less spectacular than for dipole moments but nevertheless more revealing than a mere comparison of average values (Table 3).

The histograms in Figure 6 confirm that chloroform exerts effects intermediate between those of water and vacuum.

Figure 7 compares the log *P* averages per conformational cluster in vacuo, in water, and in chloroform. It is evident that these averages are all lower in water than in vacuo, while in chloroform they assume intermediate values. This behavior suggests that the higher hydrophilicity of acetylcholine in polar solvents is due

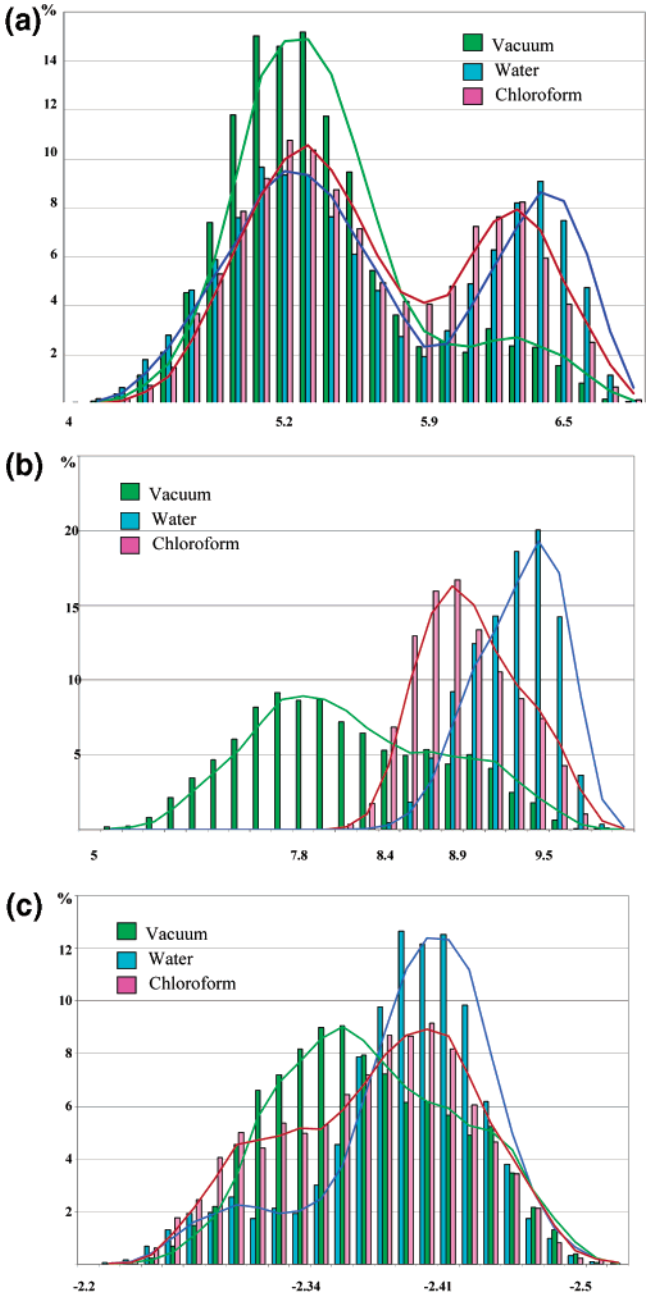

Figure 6. Histograms of some properties of acetylcholine conformers in a vacuum (green), in water (blue), and in chloroform (pink): (a) distribution of N^+ to C8 distances (10 bins/unit); (b) distribution of dipole moments (6 bins/unit); (c) distribution of log *P* values (80 bins/unit).

to both a higher percentage of extended conformers and a lower lipophilicity in all conformational clusters. Thus, acetylcholine can adjust its lipophilicity behavior and assume the most suitable conformers within each conformational cluster, confirming that each of the seven conformational clusters is able to access much of the full lipophilicity space of acetylcholine.

Discussion

The prime objective of this work was to explore the property space of acetylcholine rather than its conformational space. Nevertheless, published data can serve to confirm the validity of the conformational results presented here. Thus, a conformational study of acetyl-

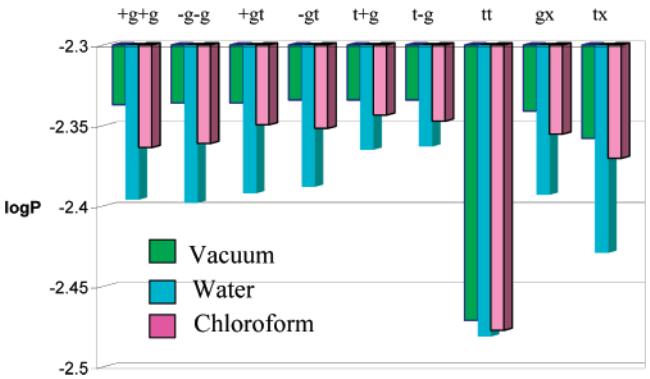

Figure 7. Solvent effects on average log *P* values for each conformational cluster (green = vacuum; blue = water; pink = chloroform).

choline in D_2O showed a predominance of the **tg** form.[12] Recent ab initio studies have refined previous investigations, indicating a preference for the **tg** conformer.[21] The **tt** conformer has been shown to be recognized by acetylcholinesterase[11] and muscarinic receptors, whereas the **tg** conformer interacts with the nicotinic receptors.[19] Also, bidimensional NMR studies have shown that when acetylcholine interacts with the nicotinic receptor, its cationic head undergoes a conversion from an extended **tt** to a folded **tg** conformation.[22]

In our study, there were fewer **tg** but more **tt** conformers in water than in chloroform. There were also more **gg** and almost the same number of **gt** conformers in water compared to chloroform. Compared to a vacuum, any solvent whatever its polarity will tend to favor folded conformers since these will minimize friction with the solvent, as already noted.[24] In other words, the lower the polarity of the medium, the higher the proportion of folded conformers becomes, an effect reinforced by the mere presence of a solvent (compared to a vacuum).

Acetylcholine was chosen as an object of study due to its small size and its great flexibility. The former was expected to yield a limited property space, whereas the latter was expected to have the opposite effect. The outcome of the present simulations points clearly to a noteworthy amplitude of the conformational space of acetylcholine, as shown, for example, by the broad range spanned by the intramolecular distance. And despite the smallness of the molecule, a broad property space is seen to correspond to this flexibility. This is the case for the physicochemical properties being investigated, namely, dipole moment and lipophilicity (i.e., log *P*). The polar surface area, which also varies widely, is both a geometric and a polarity parameter.

An unexpected result is that each cluster of conformers spans most or all of the lipophilicity and dipole spaces of acetylcholine. In other words, this study brings evidence that acetylcholine maintains much of its physicochemical plasticity within each conformational class.

What are the biological implications of this behavior? To discuss this point, we must first examine the influence of the medium on the property space of acetylcholine. As shown in Table 3 and mainly in Figure 3, acetylcholine experiences marked medium-mediated constraints on its property space. As a general trend, apolar media favor the least polar (more lipophilic) conformers, whereas water has the opposite effect. Such a constraint is well documented for preferred conformers, for example, self-coiling and hydrophobic collapse,[28] but the present study goes beyond preferred conformers to explore physicochemical properties within the full conformational space. The results confirm a model of adaptability whereby a medium favors (i.e., selects) those conformers that resemble it most in physicochemical terms.[29,30]

These results add a new dimension to our understanding of the interactions between a compound and its molecular environment.[3,29−31] Furthermore, they invite speculation on the biological and pharmacological relevance of such medium-dependent constraints. Messenger molecules, drugs, and toxins must often penetrate into or cross highly organized biological media (membranes, microtubules, etc.) before reaching their targets (receptors, enzymes, nucleic acids, etc.). The present results demonstrate that a physicochemical adaptability corresponds to the conformational flexibility of biomolecules during their membrane permeation and to their conformational fit upon binding.[32,33] Such a physicochemical adaptability may be relatively modest, but it may afford a mechanism of recognition and selectivity complementary to conformational adaptability. Whatever the ultimate veracity of such a hypothesis, it suggests that the dynamic aspect of the property space of biomolecules may be a fertile ground for future investigations.

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