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Interaction of Caffeine Molecular Associates with Water: Theory and Experiment

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Results of a Monte Carlo simulation of the association process of caffeine (1,3,7-trimethyl-2,6-dioxipurine) in water are presented. Simulation was performed in a cluster approximation; the system contained 200 water molecules. The nature of the stabilization of caffeine stacking associates in water was considered. Hydrophobic behaviour of methyl groups during association of caffeine molecules in water is shown. The peculiarity of interaction of caffeine associates with water in dependence on the starting geometry of dimer is considered. Results of the simulation on the caffeine association process are compared to experimental data from the study of aqueous solutions of caffeine.

INTRODUCTION

Caffeine is a widely known pharmacological agent. At present it is customary to consider its biological activity to be conditioned by the interaction with biopolymers, including enzymes and nucleic acids. The authors of some investigations, devoted to the effect of caffeine upon the properties of DNA maintain considered that the destruction and change of the DNA hydrated environment in the presence of caffeine is the basis of its biological activity.[1,2]

To elucidate molecular mechanisms of caffeine biological activity, it is necessary to study its physical and chemical properties in detail. Many experimental investigations (see, for example refs. [1—3]) have revealed a high tendency of caffeine to self-association in aqueous solutions. The nature of caffeine stack formation, however, has not been clarified.

METHOD

To investigate the system of caffeine—water, we used a complex approach, including not only theoretical but also a number of experimental methods.

A theoretical study was performed by the Monte Carlo method. The system under investigation included a molecule of caffeine (M) and its stacked dimer (D) in three different configurations, (see Figures 1—3):

D1 — caffeine molecules are arranged above each other in parallel planes, the distance between them amounting to 3.4 Å;

D2 — the same as D1 but an upper molecule in the stack is a mirror reflection of the lower one;

D3 — the same as D1 but an upper molecule is 180° turned around the C_4—C_5 bond in relation to the lower one.

The considered configurations are characterized by a sharply different relative arrangement of methyl groups.

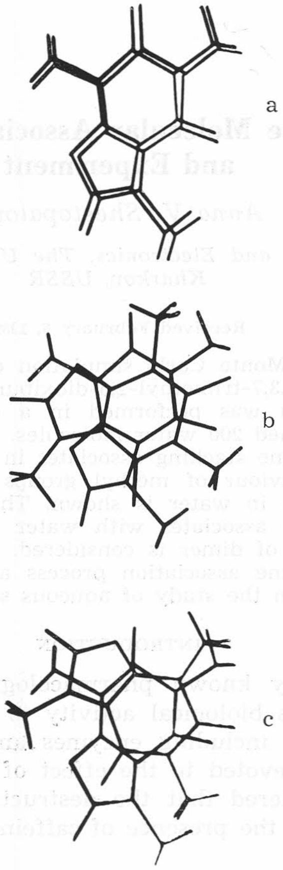

Figure 1. Stacked caffeine dimer D1:

a) starting configuration;
b) energetically most favourable configuration in vacuum (U_{cc} = —11.2 kcal/mol);
c) an equilibrium Monte Carlo configuration in the cluster of 200 water molecules (U_{cc} = —8.9 kcal/mol).

We used a standard Metropolis algorithm. Calculations were performed in a cluster approximation, the investigated system was placed in the centre of a sphere of radius 22 Å. The number of water molecules in each system was 200, $T = 298$ K. The caffeine geometry was taken from crystallographic investigation,[4] charges at the atoms were calculated by means of the Huckel and Del Re methods. Energetic characteristics were calculated using semiempirical atom-atom potential functions.[5] Energetic and structural characteristics of an isolated water cluster were taken from the calculations performed earlier.[6]

During the study of the association of caffeine molecules in water cluster, the vacuum configurations theoretically obtained and energetically most favourable were taken as starting points. In the process of calculations, one of the molecules

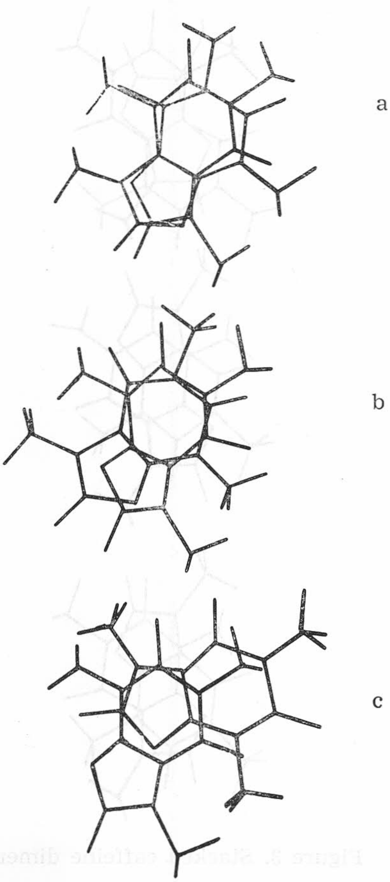

Figure 2. Stacked caffeine dimer D2:

a) starting configuration;

b) energetically most favourable configuration in vacuum (U_{cc} = —10.9 kcal/mol);

c) an equilibrium Monte Carlo configuration in the cluster of 200 water molecules (U_{cc} = —8.5 kcal/mol).

in the stack was moved randomly according to the Metropolis algorithm. The length of the generated Markov chain amounted to 6.0—7.0 $\cdot$ 10^5 elementary steps before reaching the stationary area. In the averaging area, it was equal to (5.1—6.5) $\cdot$ 10^5 steps.

RESULTS AND DISCUSSION

Table I presents the calculation results of the average values of the potential energy U for the system (kcal/mol of water), the water — water interaction energy U_{ww} (kcal/mol of water), the interaction energy of water molecules with a caffeine monomer (dimer) U_{wc} (kcal/mol of the system), the caffeine — caffeine interaction energy U_{cc} (kcal/mol) and the number of hydrogen bonds (H-bonds) by which one water molecule is connected with

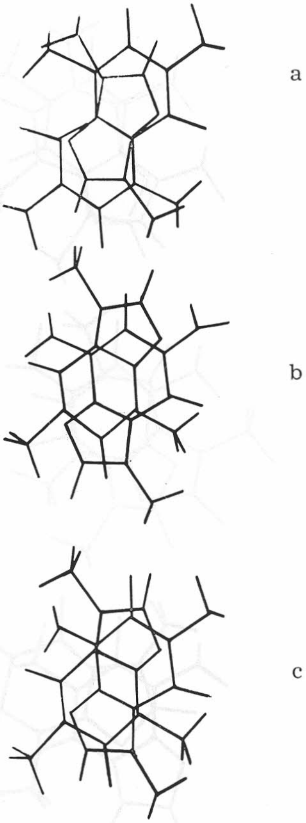

Figure 3. Stacked caffeine dimer D3:

a) starting configuration;
b) energetically most favourable configuration in vacuum ($U_{cc} = —12.6$ kcal/mol);
c) an equilibrium Monte Carlo configuration in the cluster of 200 water molecules ($U_{cc} = —10.4$ kcal/mol).

the rest, n_{Hb} (a water structuring index in the system) for the cluster of 200 water molecules.

The values obtained for the potential energy of the isolated water cluster and the system of M — 200 H_2O allow us to calculate the enthalpy of caffeine hydration. It is equal to —22.0 kcal/mol. This value and the value of caffeine hydration enthalpy calculated on the basis of experimental data, which is equal to —20.5 kcal/mol,[7,8] are in agreement.

Changes of the energy for the association reaction of caffeine molecules during the formation of stacked associate can be calculated using the data in Table I, determined from the folowing relationships:

TABLE I

Energetic and structural characteristic of the hydration of caffeine

System		U^{a} ± (0.02)	$U_{ww}{}^{a}$ ± (0.02)	$U_{wc}{}^{b}$ ± (0.2)	$U_{cc}{}^{b}$ ± (0.2)	n_{ns} ± (0.02)
200	D 1	—8.24	—7.59	—121.6	—8.9	1.80
	D 2	—8.24	—7.61	—118.8	—8.5	1.81
	D 3	—8.12	—7.57	—109.8	—10.4	1.80
	M	—8.00	—7.69	—63.1	—	1.80
	H_2O	—7.89	—7.89	—	—	1.95
70	D 1	—9.30	—7.80	—104.9	—8.9	1.87
	D 2	—8.99	—7.53	—102.7	—8.5	1.83
	D 3	—8.87	—7.42	—101.6	—10.4	1.79
	M	—9.22	—8.48	—51.7	—	2.03
	H_2O	—9.72	—9.72	—	—	2.63

[a] Kcal · mol^{-1} water.
[b] Kcal · mol^{-1} system.

TABLE II

Energetic characteristics of the caffeine association reaction in water (kcal · mol^{-1})

	System	ΔU_{ww}	ΔU_{wc}	ΔU_{cc}	ΔU
200	D 1	—20.0	+4.6	—8.9	—24.3
	D 2	—24.0	+7.4	—8.5	—25.1
	D 3	—16.0	+16.0	—10.4	—10.4
70	D 1	—39.2	—1.5	—8.9	—49.6
	D 2	—20.3	+0.7	—8.5	—28.1
	D 3	—12.6	—1.8	—10.4	—24.6

$$\Delta U_{wc} = U_{wc}(D) - 2U_{wc}(M),$$

$$\Delta U_{ww} = N[(U_{ww}(D) - U(H_2O)) - 2(U_{ww}(M) - U(H_2O))].$$

The results are given in Table II.

The calculated values show that in water the formation of a stacked caffeine dimer is energetically favourable. The main factor stabilizing the stacking is the change in the interaction of water molecules with each other, which is connected with the change in water structure around monomera during their association. The energetic preference for the formation of a stacked dimer, caused by a more favourable structure of water, persists in the subsystem of 70 water molecules taken from the calculations of full cluster (see Table I and II) situated in the centre of the sphere. The surface effects that could distort the picture in the full cluster are considerably smaller in this subsystem.

Analysis of the values of U_{ww} and n_{Hb} for the isolated water cluster and the system of M—200 H_2O shows that the appearance of a caffeine molecule in the system has a distinctive effect on water structure. Analogous results were obtained upon the study of the three configurations of caffeine stacked dimer. The distribution of n_{Hb} values in the spherical layers makes it possible to identify the most structured water layers around a monomer (dimer). These are situated at a distance of 4.3—6.3 Å from the sphere centre ($n_{Hb} = 2.02 — 2.22$). These layers are in the region of the van der Waals contacts of caffeine methyl groups located at 3.8—4.7 Å from the sphere centre. The highest structure of water clusters is found in the layer at 5.3—5.8 Å. In this layer the water — water interaction energy is maximal in absolute value ($U_{ww} = —8.80$ kcal/mol) while for the cluster as a whole $U_{ww} = —7.69$ kcal/mol.

Analogous results were obtained after the analysis of the distribution of the U_{ww} and n_{Hb} values in the spherical layers in the systems, containing the caffeine stacked dimer. The more structured layers of water cluster are situated near the van der Waals contacts of methyl groups with water molecules. For all dimer configurations methyl groups are situated at 3.5 to 6.0 Å from the sphere centre. The layers with a relatively high structuration of water are in the intervals of 5.3—8.3 Å (n_{Hb} changes from 1.88 to 1.98; U_{ww} from —7.80 to —7.90 kcal/mol).

When comparing the given values of n_{Hb}, U_{ww} and the depth of the layers with relatively high structuration of water for the system of M-200 H_2O and the systems of D — water, it is seen that the depth of the indicated layers in the second case is smaller than in the first, and the n_{Hb} and U_{ww} values are somewhat worse.

Thus, against the background of the general destruction of solvent structure the methyl groups oppose the destruction of the adjacent water structure.

This conslusion is confirmed by the comparison of the equilibrium Monte Carlo configurations belonging to the water cluster of caffeine monomer and dimer (see Figure 4). From these figures it is seen that the number of water molecules in the nearest layer around a dimer is smaller than around a monomer. A concentration of water molecules at the boundaries of the chosen clusters is observed for all the considered systems. For comparison, the spheres of radius 6 Å for monomers, and 7 Å for dimers are taken: these spheres correspond to the region of the van der Waals contacts of methyl groups with water molecules.

A larger structuration of water is observed in the layers more distant from the sphere centre around the considered configuration of the dimer (10.3—13.8 Å), as compared to the analogous situation for the system of M-200 H_2O. In these layers the values of n_{Hb} change from 1.88 to 2.04 and the values of U_{ww} are more in the absolute value. In addition, the number of water molecules forming two to four H-bonds with other water molecules is maximal in these layers.

It is necessary to mention that the greatest destruction of the water structure of water cluster in the system of M-200 H_2O is observed in the sphere of radius 4.0—4.5 Å. Such destruction of water clusters in the systems of caffeine dimer — water is observed even in the spheres with radius as large as 9—10 Å.

Thus, a Monte Carlo simulation of the association process of caffeine molecules has shown that its monomer as well as its dimer causes a destruction of water cluster. But the interaction of caffeine monomer and its stacked dimer with water is considerably distinguished.

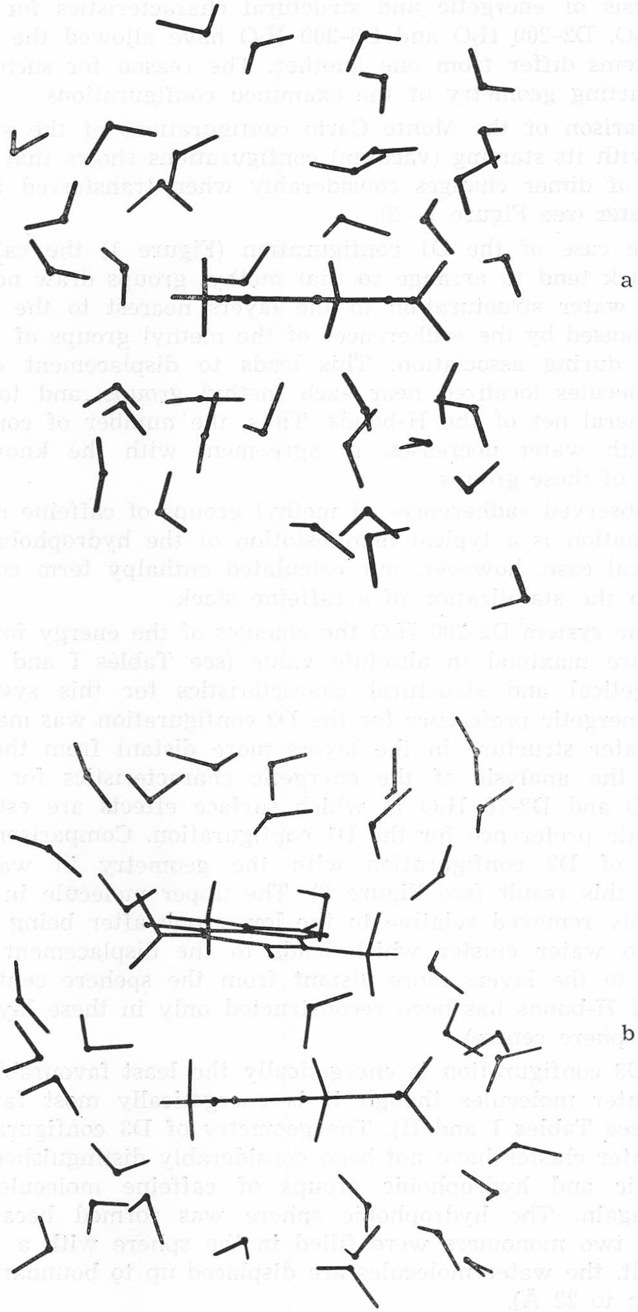

Figure 4. Equilibrium configurations of the nearest water molecules with:

a) caffeine molecules in XZ plane;

b) caffeine stacked dimer D3 in XZ plane.

Analysis of energetic and structural characteristics for the systems of D1-200 H_2O, D2-200 H_2O and D3-200 H_2O have allowed the assumption that these systems differ from one another. The reason for such difference may be the starting geometry of the examined configurations.

Comparison of the Monte Carlo configurations of the stacked dimer of caffeine with its starting (vacuum) configurations shows that the equilibrium geometry of dimer changes considerably when transferred from vacuum to water cluster (see Figure 1—3).

In the case of the D1 configuration (Figure 1) the caffeine molecules in this stack tend to arrange so that methyl groups draw nearer. Therefore, decreased water structuration in the layers nearest to the dimer described above is caused by the »adherence« of the methyl groups of the two caffeine molecules during association. This leads to displacement of some of the water molecules localized near each methyl groups, and to their inclusion in the general net of the H-bonds. Thus, the number of contacts of methyl groups with water decreases, in agreement with the known hydrophobic properties of these groups.

The observed »adherence« of methyl groups of caffeine molecules during stack formation is a typical manifestation of the hydrophobic effect. Unlike the classical case, however, our calculated enthalpy term contributes considerably to the stabilization of a caffeine stack.

For the system D2-200 H_2O the changes of the energy for the association reaction are maximal in absolute value (see Tables I and II). Analysis of the energetical and structural characteristics for this system has shown that the energetic preference for the D2 configuration was mainly determined by the water structure in the layers more distant from the sphere centre. However, the analysis of the energetic characteristics for the systems of D1-70 H_2O and D2-70 H_2O in which surface effects are estimated suggests an energetic preference for the D1 configuration. Comparison of the vacuum geometry of D2 configuration with the geometry in water cluster has explained this result (see Figure 2). The upper molecule in stack has been considerably removed relative to the lower one after being transfered from vacuum to water cluster which leads to the displacement of some water molecules to the layers more distant from the spehere centre. As a result, the net of H-bonds has been reconstructed only in these layers (after 7.3 Å from the sphere centre).

The D3 configuration is energetically the least favourable in the cluster of 200 water molecules though it is energetically most favourable in the vacuum (see Tables I and II). The geometry of D3 configuration in vacuum and in water cluster have not been considerably distinguished (see Figure 3). Hydrophilic and hydrophobic groups of caffeine molecules in stack are covered again. The hydrophobic sphere was formed because the methyl groups of two monomers were filled in the sphere with a radius of 5.3 Å. As a result, the water molecules are displaced up to boundaries of the whole sphere (up to 22 Å).

The existence of competition of three interactions (water — water, water — caffeine and caffeine — caffeine) probably leads to transformation of the energetically most favourable configuration in vacuum to the least favourable in the cluster of 200 water molecules.

It should be also noted that, despite the fact that the contribution of the U_{ww}, U_{wc} and U_{cc} interactions differs for each of the three studied configurations, a process of association is mainly determined by the interaction of water molecules with each other.

It is interesting to compare our theoretical data with the results of experimental studies of analogous systems. For this purpose we used the data of SHF-dielectrometry and IR-spectroscopy, as well as the experimental data reported in literature.

By means of SHF-dielectrometry the integral characteristics for the degree of caffeine hydration in solution were determined, first of all the number of water molecules most tightly »connected« with caffeine. Analysis of these data allowed the conclusion that a caffeine molecule connects with up to three water molecules, while in a stacked associate the degree of hydration decreases to 1—1.5 water molecules.

Results of the SHF-dielectrometry of caffeine in aqueous solutions confirm our conclusion regarding the different character of the interaction of monomer and stacked dimer with solvent that were obtained from the simulation of the hydration of caffeine molecules and stacks.

Comparison of the frequencies of the main bands in the IR-spectra of caffeine water solution shows a high frequency shift of the absorption bands of carbonyl groups with an increase in concentration. This is a spectroscopic manifestation of stacked association.[3]

Using the equilibrium configurations of caffeine dimer found theoretically and applying a dipole-dipole approximation, we calculated the resonance frequencies of carbonyl oscillations. They are given in Table III. Analogous experimental data are also listed there. A comparison shows agreement between the observed ($\Delta\bar{\nu} = -21$ cm^{-1} for C_2O groups and $\Delta\nu = 11$ cm^{-1} for the C_6O groups of caffeine) and calculated shifts of the frequencies of carbonyl oscillation absorption bands in dimer D1 and D3 during the transition from monomer to dimer. This indicates that the molecules in solution are in the associated form.

TABLE III

Resonance frequencies and displacements of resonance frequencies for different dimers (carbonyl)

Dimer	Carbonyl	frequency	Calculated and (cm^{-1})	displacement
D 1	$C_2=O_{11}$	1659		18
	$C_6=O_{13}$	1699,5		8
D 2	$C_2=O_{11}$	1654		10
	$C_6=O_{13}$	1693,5		2
D 3	$C_2=O_{11}$	1661		20
	$C_6=O_{13}$	1703,5		12

On the basis of the NMR-spectroscopy study of the equeous solutions of caffeine, some models of its stacked dimer were suggested.[1,2] According to the one that best describes these data, the six-member rings of the caffeine molecules are considerably overlapped, while the five-member rings are drawn away. Methyl groups are drawn considerably nearer. This geometry of caffeine dimers closest to the D1 configuration was obtained in the Monte Carlo simulation.

Thus, an agreement between the results of the theoretical and experimental study of the association of caffeine molecules in water indicates a similarity in the geometry of the Monte Carlo equilibrium configurations and the geometry of the caffeine dimers formed in solutions.

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SAŽETAK

Interakcija molekulskih nakupina kofeina s vodom: Teorija i eksperiment

Anna V. Shestopalova

Proces asocijacije molekula kofeina u vodi razmatran je pomoću Monte Carlo simulacije. Pokazano je da se metilne skupine prilikom stvaranja grozdova ponašaju hidrofobno. Rezultati su uspoređeni s eksperimentom.