Inorganica Chimica Acta, 56 (1981) 73–77
©Elsevier Sequoia S.A., Lausanne – Printed in Switzerland

Intramolecular Ring-Stacking Interaction Between Caffeine and Bis(1,10-phenanthroline)copper(II) Ion: ^{1}H NMR and Calorimetric Investigations

ANDREA DEI, ANDREA SCOZZAFAVA*
Istituto di Chimica Generale ed Inorganica della Facoltà di Farmacia, Università di Firenze, Florence, Italy

and GIOVANNI RENZI
Istituto di Chimica Farmaceutica, Università di Firenze, Florence, Italy

Received April 1, 1981

The interaction occurring in aqueous solution between caffeine and the bis(1,10-phenanthroline)-copper(II) cation has been investigated.

^{1}H NMR spectroscopy allowed us to establish that no coordinative bonding occurs between the copper atom and the caffeine molecule, the interaction being therefore of ring-stacking type.

Calorimetric and thermodynamic data are also reported and discussed in the light of the relevance of this type of interactions especially for biomolecules.

Introduction

The reactivity of chemical species in aqueous solutions has been deeply investigated by chemists, their attention being in particular focused on strong interactions such as covalent bond formation, ionic pairing and hydration energies [1, 2]. Aside from these dominant contributions which largely determine the relative stability of either reactants and products in a given chemical equilibrium, in the recent years other weak forces such those arising from hydrophobic and ring-stacking interactions have been found to affect the thermodynamics and the structural properties of chemical systems [3, 4].

The relevance of these interactions is apparent also in biology and in medicine. For example they are supposed to contribute to the tridimensional architecture of DNA and proteins as well as to determine in some cases the affinity of inhibitors and substrates towards the enzymes. Pharmacologically active molecules, such as caffeine, teobromine, AMP, have been also shown to interact through ring-stacking formation [5–8], and in principle this interaction might be also responsible of drugs to receptors binding. Finally these weak forces are such to affect deeply the solubility of a given substance in a given solvent, through the formation of molecular complexes. Typical is the case of caffeine whose solubility in water is highly enhanced in the presence of water soluble aromatic derivatives [9].

The detection and investigation of equilibria involving ring-stacking formation are often limited by experimental difficulties. In order to obtain more information on these systems, the use of more sensitive techniques would be desirable. With this in mind we have studied through ^{1}H NMR spectroscopy the equilibria involved in the formation of adducts between caffeine and the paramagnetic bis(phenanthroline)copper(II) cation. The use of paramagnetic probes has been often shown to be advantageous in obtaining structural information in solution [10]. Furthermore the energetics associated to these equilibria have been investigated through calorimetric studies.

Experimental

Materials

Commercially pure caffeine (ERBA-FU, 99.5%) was twice crystallized from water and dried *in vacuo*. Commercially very pure 1,10-phenanthroline·H_2O (ERBA-ACS) and copper(II) nitrate (ERBA-RPE) were used without further purification. The $Cu(phen)_2(NO_3)_2 \cdot H_2O$ complex was synthesized by mixing hot solutions containing stoichiometric amounts of the ligand and of the metal salt dissolved in the minimum amount of methanol. Crystals began to appear after cooling; they were filtered, recrystallized from methanol and dried *in vacuo*. The compound was satisfactorily analyzed for C, H, and N.

Physical measurements

^{1}H NMR spectra were recorded on a Varian CFT 20 spectrometer operating at 80 MHz. T_1 measurements were obtained by the inversion recovery

---

*Author to whom correspondence should be addressed.

method T_2 values were obtained from the linewidths at half peak height through the relation $T_2^{-1} = \pi\Delta\nu$

The evaluation of stability constant related to the formation of the paramagnetic copper(II)–phenanthroline–caffeine adduct was carried out by measuring the T_2 values of caffeine proton resonances upon addition of progressive amounts of the paramagnetic copper(II)–phenanthroline derivative D_2O solutions *ca* 5 × 10^{-3} *M* of caffeine were used The stability constant value was calculated through a least square analysis of the experimental data, by using an appropriate computer program In the calculation procedure it was assumed that both $Cu(phen)^{2+}$ and $Cu(phen)_3^{2+}$ species, which originates from the dissociation of the $Cu(phen)_2^{2+}$ species [11], exhibit the same π-donor properties of the $Cu(phen)_2^{2+}$ complex.

Calorimetric studies were carried out with a 10700-1 LKB flow calorimeter, equipped with a Keithley model 150B microvolt amplifier The details of the experimental procedure are reported elsewhere [12] Because of the small thermal effects occurring in the reaction, solution of the reactants not lower than 2 × 10^{-2} *M* were used In the elaboration of the experimental data it was assumed that the acceptor properties of the species of general formula $(caffeine)_n$ were the same

Results

According to previous investigations, the ^1H NMR spectra of D_2O solutions of caffeine are very concentration dependent, the four resonance signals being shifted upfield as the concentration increases [7, 9] This spectral behaviour is consistent with the presence of stacked self-associated caffeine molecules Further support to this hypothesis is given by the results of osmometric [13], cryoscopic [14], and calorimetric [15] measurements, which evidence the existence of self-association equilibria in aqueous solution A stability constant value of 8 6 M^{-1} for the reaction

$$2\ \text{caffeine} \rightleftharpoons (\text{caffeine})_2 \tag{1}$$

has been evaluated from ^1H NMR data [7] assuming the existence of the equilibrium (1) alone and neglecting the formation of more associated species A more accurate approach can be done under the hypothesis of an indefinite non-cooperative association model [16, 17] On this respect the fitting of the reported chemical shifts δ at different ceoncentrations C can be done using the relationship

$$\delta = \delta_\infty + (\delta_\infty - \delta_o)]\ 1 - (4kC + 1)^{1/2}]/2kC \tag{2}$$

where δ_o represents the chemical shift of a given proton at infinite dilution, δ_∞ is the shift of the same proton when the molecule belongs to an infinitely long stack, and K is defined as

$$K = \frac{|(\text{caffeine})_n|}{|(\text{caffeine})_{n-1}||\text{caffeine}|} = K_n = K_{n-1} \tag{3}$$

Using the relation (2) we have found a value of K equal to 9 7 ± 1 8, which has been used during this work As a result, it can be shown that for concentrations below 10^{-2} *M* the caffeine essentially exists as a monomeric species

Broadening of all the resonance signals as well as T_1^{-1} enhancements are observed when the solutions of caffeine in D_2O are mixed with solutions of $Cu(phen)_2(NO_3)_2 \cdot H_2O$ in the same solvent, the signal half-widths increasing with the concentration of the paramagnetic compound This is consistent with the existence in solution of equilibria of the type

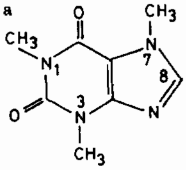
$$Cu(phen)_2^{2+} + (\text{caffeine})_n \rightleftharpoons Cu(phen)_2(\text{caffeine})_n^{2+} \tag{4}$$

which are fast on the ^1H NMR time scale In agreement to the hypothesis of an indefinite non-cooperative association model, we have assumed that all the K_n associated to type (4) equilibria have the same value

A typical pattern of T_1 and T_2 values corrected for the diamagnetic contribution, are reported in Table I The T_{1P}/T_{2P} ratios are similar for all of the caffeine protons, falling in the range 1 11–1 17 furthermore it should be noted that all T_1 and T_2 values are almost similar, differing less than a factor 2 Apparently the signal broadening is not accompanied by any shift of the resonances

TABLE I T_{1P} and T_{2P} Values for the Different Protons of Caffeine[a] in the Presence of Bis(phenanthroline)copper(II) [b]

	T_{1P}(s)	T_{2P}(s)	T_{1P}/T_{2P}
H(8)	0 155	0 138	1 12
CH_3(7)	0 147	0 133	1 11
CH_3(3)	0 095	0 083	1 14
CH_3(1)	0 095	0 081	1 17

[a]


[b] For a solution containing caffeine 4 4 × 10^{-2} *M* and bis-(phenanthroline)copper(II) nitrate 3 17 × 10^{-3} *M*

In order to evaluate the stability constant K associated with equilibrium (4), the half-widths of the four resonance signals of *ca.* 5 X 10 *M* solutions of caffeine in the presence of different amount of the paramagnetic copper(II) derivative were measured. These values, once corrected for the intrinsic half-width of the diamagnetic caffeine, were fitted with an appropriate computer program, leading to a K value of 10.1 ± 1.2 M^{-1}.

Both ESR and ligand field spectra of $Cu(phen)^{2+}$ solutions do not show any appreciable change upon addition of caffeine solutions. This suggests the lack of any direct interaction between the caffeine molecule and the metal ion. Furthermore no evidence could be drawn from ultraviolet spectroscopy because of the overlap of the absorption of the two reactants.

Significant thermal effects can be measured when caffeine solutions are mixed with pure water or solutions of $Cu(phen)_2(NO_3)_2$, H_2O. Using the above values of equilibrium constant, the following thermodynamic quantities were calorimetrically obtained: (i) $\Delta H = -3.1 \pm 0.1$ Kcal mol^{-1} and $\Delta S = -6 \pm 3$ e.u., for the self association of caffeine; (ii) $\Delta H = -2.0 \pm 0.1$ Kcal mol^{-1} and $\Delta S = -2 \pm 2$ e.u., for the reaction of caffeine with the copper(II)-phenanthroline complex. The low values of the standard deviations on the enthalpy changes values support the

Discussion

^1H NMR Spectra

When a diamagnetic ligand interacts with a paramagnetic ion the nuclear relaxation times T_{1M} and T_{2M} of nuclei nearby the paramagnetic center are drastically affected as a consequence of the fluctuating large magnetic field generated by the unpaired electrons. Under fast exchange conditions and in absence of chemical shift and outer sphere relaxation, the above parameters can be related to the experimentally observed T_1 and T_2 through the relation [18]

$$T_{iM}^{-1} = fT_{iP}^{-1} = f(T_i - T_{io})^{-1} \qquad i = 1, 2 \tag{5}$$

where f represents the molar fraction of the bound ligand and T_{io} the relaxation time of the diamagnetic ligand. On its turn T_{1M} and T_{2M} can be related to the distance *r* between the observed nuclei and the paramagnetic center, thus allowing to obtain structural information, through the well known Solomon-Blombergen-Morgan equations [19]

$$T_{1M}^{-1} = \frac{2S(S+1)\gamma_I^2 g^2 \beta^2}{15r^6}\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right) +$$

$$+\frac{2S(S+1)A^2}{3\hbar^2}\left(\frac{\tau_e}{1+\omega_S^2\tau_e^2}\right) \tag{6}$$

$$T_{2M}^{-1} = \frac{S(S+1)\gamma_I^2 g^2 \beta^2}{15r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13\tau_c}{1+\omega_S^2\tau_c^2}\right) +$$

$$+\frac{S(S+1)A^2}{3\hbar^2}\left(\tau_e + \frac{\tau_e}{1+\omega_S^2\tau_e^2}\right) \tag{7}$$

The first term in both the expressions represent the dipolar contribution to the relaxation mechanism, whereas the second is the so called 'Fermi contact' contribution. This second term is always negligible in the case of T_{1M}^{-1}, but it can be the dominant one in the case of T_{2M}^{-1}. Moreover when $\omega_S^2\tau_c^2 \gg 1$ and $\omega_I^2\tau_c^2 \ll 1$ as expected in this case (since $\omega_S = 3.29 \times 10^{11}$ s^{-1}, $\omega_I = 6.3 \times 10^8$ s^{-1} and $\tau_c \cong 10^{-10}$ s, see below), the above expressions (6) and (7) reduce to

$$T_{1M}^{-1} = 6kr^{-6}\tau_c \tag{8}$$

$$T_{2M}^{-1} = 7kr^{-6}\tau_c + \frac{S(S+1)A^2}{3\hbar^2}\tau_e \tag{9}$$

Under the hypothesis of negligible hyperfine exchange relaxation the ratio $T_{1M}/T_{2M} = T_{1P}/T_{2P}$ is equal to 7/6 (1.17). Larger values indicate that the fast exchange limit is not attained and/or the contact term is not negligible [20].

The T_{1P}/T_{2P} ratios of the system caffeine bis(phenanthroline)copper(II) (Table I) fall, within the experimental error, in the range expected when only dipolar mechanism is operative. This would exclude any direct interaction between the caffeine molecule and the paramagnetic metal ion, as supported also by the invariance of the ligand field and ESR spectra of the copper(II) complex upon addition of caffeine. These results indicate therefore that the caffeine molecule interacts with the paramagnetic complex without formation of any coordinative bonding between the caffeine itself and the copper(II) ion. This behaviour is markedly different from that shown by caffeine in the presence of copper(II) aquoion. In the latter case the caffeine is known to act as monodentate ligand and solid compounds showing caffeine coordinated to the metal ion have been also isolated [21]. In the case of the adduct between the $Cu(phen)_2^{2+}$ complex and caffeine the present data indicate that the interaction is of stacking type, through an interaction between the π-electron systems of the two aromatic rings, without any direct chemical bonding with the copper(II) ion. The occurrence of such type of interactions has been shown to occur in the adduct between caffeine and 5-chlorosalycilic acid, the X-ray diffraction data indicating that the two molecules are faced with a distance between the planes equal to 3.4 Å [22]. Also several molecules of biological relevance and with chemical structure similar to that of caffeine, have been shown to form stacked adducts; as a example many ternary complexes between nucleotide bases and aromatic ligands as bipyridyl or bipyridilamine have been shown to contain the purine and pyrimidine moieties of nucleotides engaged in stacking interactions with the aromatic ligands [23–27].

Further information on the nature of the adduct can be obtained by the analysis of the experimental T_1^{-1} values for any given proton. Since, as shown above, the relaxation rate enhancement is due only to the dipolar term of the Solomon-Blombergen-Morgan equations, it is consequent that the $T_1(H_i)/T_1(H_j)$ ratios are directly related to the $r^6(Cu-H_i)/r^6(Cu-H_j)$ ratios, thus allowing to obtain a direct information on the geometrical location of the caffeine molecule with respect to the paramagnetic center. From the data reported in Table I, it appears that all the hydrogen atoms show the same average distance within 10%. It should be observed that this result holds independently from the actual value of the τ_c parameter. It is apparent that the occurrence of similar distances cannot be explained assuming one stereoselective interaction mode, but it should be consistent with the existence of an averaged interaction mode, in which the caffeine molecules display different orientations with respect to the copper(II)-phenanthroline moiety.

The knowledge of the τ_c parameter would allow the calculation of the actual distance between the hydrogen atoms and the copper(II) ion. Unfortunately this would require T_1 measurements at different frequencies. However, if a τ_c value of 1.8×10^{-10} s is assumed, as can be derived from the Stokes–Einstein relationship, the following distances are obtained: Cu–H(8) 6.6 Å, Cu–CH_3(1) 6.1 Å, Cu–CH_3(3) 6.1 Å, Cu–CH_3(7) 6.6 Å. These distance values are in full agreement with the hypothesis inferred above of the existence of a stacked adduct with a distance between the planes of the aromatic ring similar to that found from X-ray diffraction data [19].

Calorimetric measurements

The obtained values for the free energy changes associated to interaction processes of caffeine with caffeine and the copper(II)-phenanthroline moiety are similar and of the same order of magnitude, as compared to those obtained on similar related systems [4, 17]. The reported data show that this kind of interactions are considerably stronger as the number of aromatic and pseudoaromatic fused rings increases. Also in the case of the caffeine however the stability constants of the adducts are not neglectable, since it can be shown that for concentrations larger than 10^{-2} *M* the concentration of the associated species is significant.

The thermodynamic quantities for the two investigated systems are quite consistent with those found for the formation of adducts between the caffeine molecule and the salicylate ion [15]. Since it has been shown that the adducts of this type are found to exist mainly in aqueous solution, the favourable enthalpy changes reasonably arise from the bonding interaction of faced aromatic rings and the favourable balance of hydration enthalpies of the reactants and products.

The first term is known to be operative in determining often the lattice arrangement of many aromatic compounds. This is supported also by theoretical calculations, which show that a favourable contribution ranging from 0.4 to 1.5 kcal mol^{-1} has to be expected for these interacting systems [28].

The latter term is associated with the formation of a greater hydrophobic hole in the structure of the solvent. The partial molal heat capacity value of caffeine has been found to be remarkably high [29], thus indicating a noticeable structuring capability of the molecule. This property should be reasonably enhanced in the case of associated species.

As usually observed in the case of hydration processes of nonpolar solutes, the formation of a greater cluster or water molecules surrounding the hydrophobic hole is associated with a favourable enthalpy change, but is also associated with a loss of translational and rotational freedom degrees of the molecules themselves. Therefore an unfavourable entropy change has to be expected, as observed in the present cases.

Unfortunately the lack of calorimetric data on similar related systems precludes any useful comparison. As mentioned before, we believe that our suggestions are accounted for by the fact that stacking adducts are found to exist mainly in aqueous solutions, where the balance of the hydration free energies of reactants and products can be sometime favourable. In other solvents no adducts were found in the case of caffeine. Similar conclusions have been recently suggested by Mitchell in order to explain the different values of stability constants of self association of 1,10-phenanthroline in water and aqueous mixtures [17].

Conclusions

The study of stacking interacting systems may be often advantageously carried out through UV spectroscopy and ^{1}H NMR investigations of diamagnetic samples. However these techniques are often inappropriate owing to the overlap of the absorption bands and/or to the difficulty of measuring very small chemical shifts. Moreover with these techniques the possibility of obtaining structural information on the nature of the adduct is very limited. The use of paramagnetic probes may provide in these cases an alternative powerful tool for detecting and investigating these interactions owing to the large sensitivity of this technique.

The calorimetric data reported here indicate that these equilibria are expected to occur mainly in aqueous solution, because of the peculiar balance of the free energy solvation terms between reactants and products. For this reason such interactions are thought to be relevant especially in biological systems and a more detailed knowledge of these forces appears necessary in order to understand the reactivity of biomolecules.

Attempts should also be made to obtain some appropriate paramagnetic probes for investigating the acceptor properties of many biologically and pharmaceutically interesting compounds in aqueous solution.

References

1 J. F. Coctzee and C. D. Ritchie, 'Solute-Solvent Interactions', M. Dekker, New York (1969).
2 S. J. Ashcroft and C. T. Mortimer, 'Thermochemistry of Transition Metal Complexes', Academic Press, New York (1970).
3 B. Pullman, Ed., 'Molecular Association in Biology', Academic Press, New York (1968).
4 H. Sigel, in 'Metal Ions in Biological Systems', vol. 2, pp. 63–125, H. Sigel, Ed., M. Dekker, New York (1973); H. Sigel, in 'Advances in Solution Chemistry', I. Bertini, L. Lunazzi, A. Dei, Eds., Plenum Press, New York (1981) in the press.
5 G. de Stevens, 'Diurectics, Chemistry and Pharmacology', Academic Press, New York (1963).
6 W. S. Hoffman, 'Biochemistry of Clinical Medicine', 4th edn., Year Book Medical Publisher, Chicago (1980).
7 A. L. Thakkar, L. G. Tensmeyer, R. B. Herman and W. L. Wilham, *Chem. Comm.*, 524 (1970).
8 N. A. Berger and G. L. Eichhorn, *Biochemistry, 10,* 1847 (1971).
9 H. Stamm, *Arch. Pharmac., 302,* 174 (1962) and references therein.
10 I. Bertini and R. S. Drago, Eds., 'ESR and NMR of Paramagnetic Species in Biological and Related Systems', D. Reidel Pub. Co., Dordrecht (1979).
11 From the reported values of the stability constants (L. G. Sillen and A. E. Martell, Stability Constants of Metal-ion Complexes, Special Publication No 25 of the Chemical Society, London (1971)) it appears that the percentage of $Cu(phen)^{2+}$ and $Cu(phen)_3^{2+}$ is of about 11%.
12 L. Fabbrizzi, M. Micheloni and P. Paoletti, *J. Chem. Soc. Dalton,* 1581 (1979).
13 F. M. Goyan and H. N. Borazam, *J. Pharm. Sic., 57,* 861 (1968).
14 M. Doubrow and Z. Z. Jan, *J. Chem. Soc.*, 3845 (1963).
15 J. H. Stern, J. A. Devore, S. L. Hansen, and O. Yavuz, *J. Phys. Chem., 78,* 1922 (1974).
16 M. P. Heyn and R. Bretz, *Biophys. Chem., 3,* 35 (1975).
17 P. R. Mitchell, *J. Chem. Soc. Dalton,* 1079 (1980).
18 T. J. Swift and R. E. Connick, *J. Chem. Phys., 37,* 307 (1962); Z. Luz and S. Meiboom, *J. Chem. Phys., 40,* 2686 (1964).
19 I. Solomon, *Phys. Rev., 99,* 559 (1955); N. Bloembergen, *J. Chem. Phys., 27,* 572 (1957).
20 W. G. Espersen and R. B. Martin, *J. Am. Chem. Soc., 98,* 40 (1976).
21 E. Shiefter, *J. Pharm. Sci., 57,* 1163 (1968).
21 A. Biagini Cingi, A. Chiesi Villa, A. Gaetani Manfredotti and C. Guastini, *Cryst. Struct. Commun., 1,* 363 (1972).
22 E. Shefter, *J. Pharm. Sci., 57,* 1163 (1968).
23 H. Sigel, *Angew. Chem., Int. Ed. Eng., 14,* 349 (1975); C. F. Naimann and H. Sigel, *J. Am. Chem. Soc., 96,* 2750 (1974).
24 P. Chandhmi and H. Sigel, *J. Am. Chem. Soc., 99,* 3142 (1977).
25 B. E. Fischer and R. Bau, *Inorg. Chem., 17,* 27 (1978).
26 K. Aoki, *J. Am. Chem. Soc., 100,* 7106 (1978).
27 R. W. Gellert and R. Bau, 'X-Ray Structural Studies of Metal–Nucleoside and Metal–Nucleotide Complexes', in 'Metal Ions in Biological Systems', Vol. 8, H. Sigel Ed., Marcel Dekker, Incl, New York and Basle, p. 1 (1979).
28 H. A. Scheraga, *Accounts Chem. Res., 12,* 7 (1979).
29 J. H. Stem and L. R. Beeninga, *J. Phys. Chem., 79,* 582 (1975).