Brief Articles

Highly Stable C_{60}/Poly(vinylpyrrolidone) Charge-Transfer Complexes Afford New Predictions for Biological Applications of Underivatized Fullerenes

Cezar Ungurenasu* and Anton Airinei

"P. Poni" Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda 41-A, 6600 Iasi, Romania

Received October 11, 1999

The equilibrium constants for complexation of C_{60} with poly(vinylpyrrolidone) (PVP) in water have been determined by UV−visible spectroscopy. The magnitude of the equilibrium constants was found to describe the formation of a charge-transfer (CT) saturated 1:1 complex for [PVP] = 2.62−5.25 × 10^{-2} M (K = 1331.3 M^{-1}), and a contact-pairs complex for [PVP] = 7.0−12.25 × 10^{-2} M (K = 20.64 × 10^{-2} M^{-1}). These results indicate that the binding affinity of C_{60} for drug receptors, base pairs in double-stranded DNA, or AT-rich segments of its minor groove to form CT complexes is limited by the strong coordination in the C_{60}/PVP-saturated CT complex or envelopment by the polymer ligand in the contact CT complex.

Introduction

It is now widely accepted that molecular complexation is one of the driving forces that lead to the selective binding of a substrate to a receptor in aqueous solutions.[1,2] Taking into account that fullerene C_{60} molecule forms intramolecular and intermolecular charge-transfer (CT) complexes with aromatic rings[3,4] and tertiary amines,[5,6] one can suppose that it also can interact with side chains of aromatic amino acids incorporated into a receptor and base pairs in double-stranded DNA or AT-rich segments of its minor groove to form CT complexes. To achieve this, the C_{60} molecule must be reasonably free or weakly precomplexed with a dissolving agent. On the other hand, the C_{60} molecule can react with those protein family members which share several regions of highly conserved amino acids in their amino termini to form covalently linked C_{60} adducts.

Here we report that C_{60}/poly(vinylpyrrolidone) (PVP) complexes have the highest equilibrium constant K found so far for organic CT complexes. These findings may have direct implications for the biological effects of C_{60} when dissolved in water with excess PVP.

Thus far, fullerenes have proved useful for a wide variety of biological applications.[7] However, the pharmacological effects of C_{60} are scarcely studied. Satoh et al.[8] found that direct effect by C_{60} was not observed in guinea pig trachea, heart, and ileum or rat stomach, vas deferens, and uretus when dissolved in water with excess PVP. These results and the above considerations prompted us to investigate the complexation of C_{60} with PVP and 1-methyl-2-pyrrolidone (NMP) in water by UV/vis absorption spectroscopy.

Results and Discussion

The reported chemistry of the C_{60} tertiary amine complexes is limited to aromatic amines[5,6] and organic solvents. Values of K = 0.047−0.28 M^{-1} have been evaluated for C_{60}/*N*,*N*-dialkylaniline CT complexes by using the Benesi−Hildebrand equation and assuming a 1:1 complexation. In the present study we have extended the equilibrium constant measurements to investigate the C_{60}/PVP and C_{60}/NMP systems by electronic absorption spectroscopy in water at 20 °C.The spherical C_{60} molecule exposes more coordination sites than the relatively flat organic acceptors to form CT complexes. Thus, the C_{60}/NMP and C_{60}/PVP CT complexes formed in aqueous medium may have either one or multiple coordinating nitrogens, depending on the concentration of the ligand and its ionization potential. However, this multiple coordination ability of the C_{60} molecule is limited because of its small surface area, especially when the ligand provides a complex steric environment near the coordinating nitrogen, like in PVP.

Fullerene C_{60} and PVP (K25, M_w = 24 000 kDa) were obtained from Fluka. The studied ranges of [PVP] were required by the low solubility of C_{60} in water for [PVP] < 2 × 10^{-2} M and the segregation phenomenon which occurs for [PVP] >13 × 10^{-2} M.

The C_{60}/PVP complex samples were prepared by addition of C_{60} (0.8 mg) in toluene (1 mL) to a stirred solution of PVP (calculated amount) in chloroform (3 mL). After stirring for 1 h the solvent was thoroughly evaporated under vacuum (30 °C), and the residue was redissolved by stirring in water (final volume 25 mL) to produce a clear yellow solution. The C_{60}/NMP complex samples were prepared as above, except that a calculated amount of NMP was added to C_{60} (0.8 mg) in the absence of solvents.

Representative UV/vis absorption spectra of the C_{60}/PVP complexes are shown in Figure 1. As seen from the spectra, it should be noted that the formation of the C_{60}/PVP complex is characterized by a new absorption band located at about 415 nm.

The corresponding CT absorption band was observed

* To whom correspondence should be addressed. Tel: 40-32-144909. Fax: 40-32-211299. E-mail: rbsscung@ichpp.tuiasi.ro.

10.1021/jm991175q CCC: $19.00
Published on Web 07/27/2000

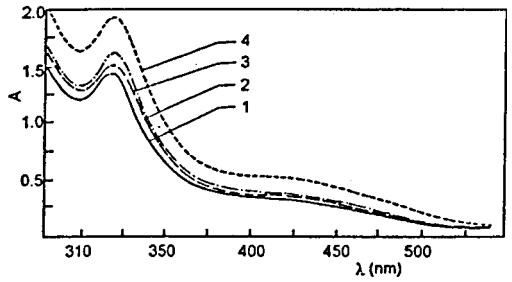

Figure 1. Representative electronic absorption spectra of C_{60}/PVP complexes in H_2O: $[C_{60}] = 4.4 \times 10^{-5}$ M; [PVP] = 1−7.0 $\times 10^{-2}$, 2−8.75 $\times 10^{-2}$, 3−10.5 $\times 10^{-2}$, 4−12.25 $\times 10^{-2}$ M.

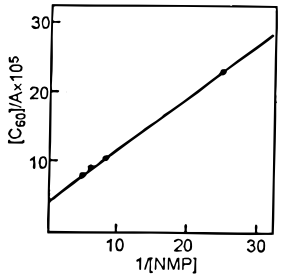

Figure 2. Benesi−Hildebrand plot for complexation of C_{60} and NMP in water: $[C_{60}] = 4.4 \times 10^{-5}$ M; [NMP] = 4−20 $\times 10^{-2}$ M.

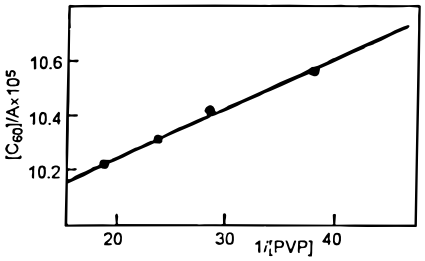

Figure 3. Benesi−Hildebrand plot for complexation of C_{60} and PVP in water: [PVP] = 2.62−5.25 $\times 10^{-2}$ M.

at 425 nm for the C_{60}/NMP complex. A monotonic growth of the CT absorbance at 415 nm with increasing C_{60}/PVP complex was observed. The position of the absorption band at 330 nm for C_{60}/PVP complex is shifted a little toward longer wavelengths, as compared with that of a solution of C_{60} in hexane. The equilibrium constant K for complexation was established from concentration dependence data, using the Benesi−Hildebrand equation:

$$\frac{[A]\cdot l}{A} = \frac{1}{K\epsilon_{CT}} \cdot \frac{1}{[D]} + \frac{1}{\epsilon_{CT}}$$

where $[D]$ is the donor concentration, $[A]$ is the acceptor concentration, l is the thickness of the absorbent layer, ϵ_{CT} is the molar absorptivity of the CT complex, and A is the absorbance of the CT complex.

Data were taken at 415 nm (C_{60}/PVP) and 425 nm (C_{60}/NMP), and the results of the analysis are shown in Figure 2 for C_{60}/NMP and Figures 3 and 4 for C_{60}/PVP systems. Over the [NMP] studied range (4−20 $\times 10^{-2}$ M) the continuous linear regression of the plot in Figure 2 led to a value of $K = 5.23$ M^{-1}.

However, a nonlinear $[C_{60}]/A$ vs 1/[PVP] relationship was found for the C_{60}/PVP system over the range of [PVP] = 2.62−12.25 $\times 10^{-2}$ M. Attempts were made at

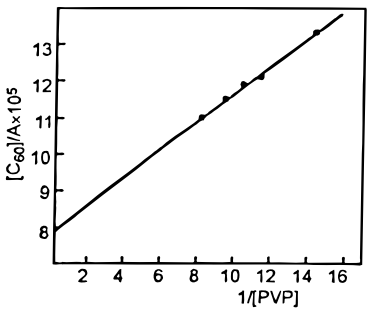

Figure 4. Benesi−Hildebrand plot for complexation of C_{60} and PVP in water: [PVP] = 7.0−12.25 $\times 10^{-2}$ M.

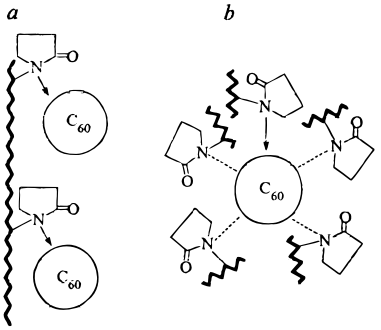

Figure 5. Schematic models of C_{60}/PVP CT complexes: (a) CT-saturated 1:1 complex; (b) CT-unsaturated "contact-pairs" complex.

rationalizing this behavior by plotting C_{60}/A vs 1/[PVP] over separate ranges of [PVP], within 2.62−5.62 and 7.0−12.25 $\times 10^{-2}$ M. Indeed, we found two linear plots for the [PVP] ranges given in Figures 3 and 4.

The calculated K for [PVP] ranging within 2.62−5.25 $\times 10^{-2}$ M (Figure 3) is 1331.3 M^{-1}, a value much higher than that observed for the C_{60}/NMP system. The formation of a CT-saturated "1:1" complex should account for this value which compares favorably with that evaluated for Cu-O_2 complexes implicated in biological oxidation processes.[9] However, for [PVP] = 7.0−12.25 $\times 10^{-2}$ M (Figure 4) K is 20.64 M^{-1}, indicating that the "contact" CT absorption[10] occurs during random encounters, when the C_{60} acceptor and pyrrolidone ligand are sufficiently close to one another, that is when the PVP concentration is sufficiently high.

The equilibrium constant K describing the part of the CT interaction in which mechanistic pathways for PVP diverge shows a large effect of polymer concentration: the value for [PVP] = 7.0−12.25 $\times 10^{-2}$ M is reduced by around 65-fold from that for [PVP] = 2.62−5.25 $\times 10^{-2}$ M. Monomeric NMP is likely to much more easily form "contact-pairs" with C_{60} than PVP, the K value being reduced by around 250-fold from that for [PVP] = 2.62−5.25 $\times 10^{-2}$ M. This equilibrium constant is now likely to describe chiefly the contact complexation event.

The schematic models presented in Figure 5 afford several predictions. Although weakly coordinated to PVP the C_{60} molecule of the "contact" complexes is severely hindered by the macromolecular chains from binding with a receptor.

Depending on PVP concentration the binding affinity of C_{60} for acceptors is limited by the strong coordination (CT-saturated complexes) or envelopment (CT-unsaturated contact-pairs). However, to fall in the physiological requirements, the samples containing "contact" C_{60}/PVP

species have to be diluted up to a level at which CT-saturated species prevail and the effect of C_{60} envelopment by PVP is small. On the basis of this hypothesis, we estimate that saturated CT C_{60}/PVP species may function as antagonists on all classes of receptors. When used as a free antagonist, the C_{60} molecule must be solubilized by a weakly coordinating agent having an ionization potential below those exhibited by the bases involved in receptors. Liposomes appear to be very attractive C_{60}-solubilizing agents for this purpose.

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JM991175Q