Incorporation of C_{60} into Artifical Lipid Membranes

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Received December 11, 1992

Following the first characterization and development of a method for large-scale production of fullerenes, particularly C_{60},[1,2] an intense number of investigations has been published on the properties of this exciting class of molecules.[3] Recently, we reported on chemically induced radical reactions with C_{60} such as alkyl radical addition and one-electron redox processes in homogeneous solution.[4] In this study we found, for example, that electron transfer from $(CH_3)_2C^{\bullet}(OH)$ radicals to C_{60} was considerably slowed down upon embedding the C_{60} into γ-cyclodextrine (a water-soluble complex[5]) as compared to homogeneously dissolved C_{60}. This has now prompted us to further investigations in this direction by incorporating C_{60} into even more organized assemblies such as vesicular and micellar membranes in aqueous environment.

A solution of C_{60} (Kaesdorf, Munich) in an aqueous Triton X-100 (Aldrich)[6] micellar system (prepared by stirring) exhibits a brownish color. Incorporation of C_{60} into the micelle is concluded since C_{60} is not at all dissolved in water alone or below the critical micellar concentration (3.1×10^{-4} M). The UV part of the spectrum is identical with that for toluene or benzene solutions of C_{60} (including a characteristic small spike at 408 nm).[7] A weak vis band ($\epsilon \approx 700\ M^{-1}\ cm^{-1}$), however, showing up at about 540 nm in benzene, toluene, *n*-hexane, or 1,2-dichloroethane and seemingly being responsible for the purple color of C_{60} in these solvents, is apparently shifted to lower wavelengths and indicated under a much stronger UV band. A similar hypsochromic shift of this vis band but unaffected UV absorption is observed when C_{60} is dissolved in alcohols (vis band in ethanol at 445 nm). Since Triton X-100 is also an alcohol,[6] the observed color change from purple to brown may thus be caused by interaction of the polar polyethylene glycol units with the π-systems of C_{60}. This, in turn, would mean that the C_{60} is not located in the inner hydrophobic part of the micelle.

Vesicular solutions of C_{60} in DODAB (positively charged head group), DHP (negatively charged), and lecithin (zwitter ionic)[8] (prepared by standard methods)[9,10] all showed yellow-brownish colors, again suggesting incorporation of the fullerene into the membrane. Conclusive features in support are the following: (i) the yellow-brownish color emerges with formation of the vesicle during ultrasonication; (ii) gel exclusion chromatography (Sephadex G-50/Sephacryl S200, Pharmacia Fine Chemicals)[11] shows elution of the colored vesicle fraction within deadtime, while the nonincorporated and insoluble C_{60} remains at the top of the gel column; (iii) attempts to extract membrane-incorporated C_{60} from the colored vesicle fraction into toluene failed; (iv) extraction became possible, however, after destruction of the vesicle by addition of KCl, with the toluene phase showing again the characteristic features of C_{60} in this solvent including the purple color and the distinct narrow band at 408 nm.[7] This latter procedure allowed, by the way, determination of any vesicle-incorporated C_{60} concentration.

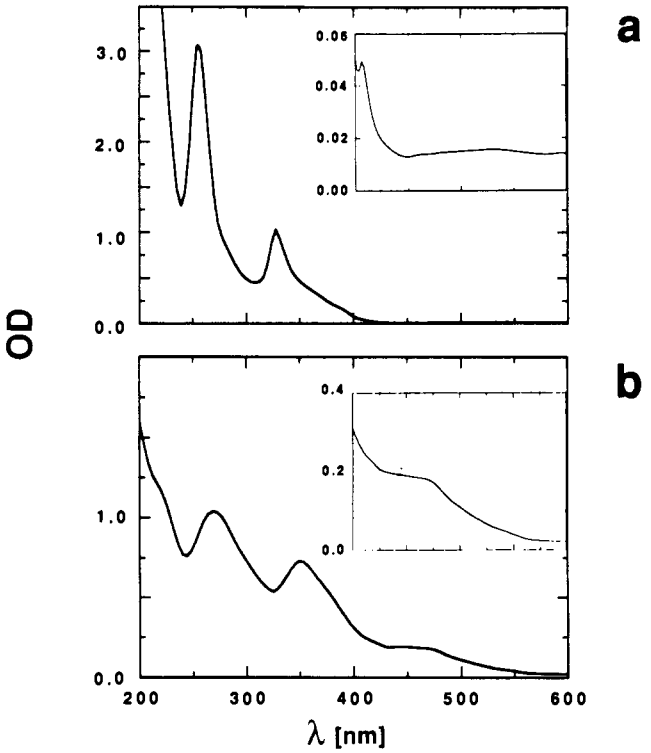

Figure 1. Absorption spectrum, in terms of OD, of 1.6×10^{-5} M C_{60} in (a) *n*-hexane and (b) aqueous solutions of lecithin vesicles (concentration of lecithin, 7.8×10^{-4} M). (1-cm cell).

The vis band of the vesicular C_{60} appears to be shifted to lower wavelengths and is of comparable intensity with micellar and homogeneous alcoholic solutions, accounting for the observable color change.[12] In analogy to the micellar system we consider this as evidence for a C_{60} location near an interaction with the vesicles' polar head groups. It is interesting to note that, e.g., pyrene (which could be regarded as a subunit of C_{60}) has also been proven to be accommodated preferentially near the polar surface of vesicles.[13]

A most significant result emerges with respect to the distinct 334-nm UV band, which undergoes remarkable changes in the vesicular environment as compared to homogeneous solutions and the micellar system. (The same holds for the 260-nm band which is, however, not well-resolved in the aromatic solvents). This is exemplified by the spectra shown in Figure 1 for C_{60} in

(1) Kroto, H. W.; Health, J. R.; O'Brien, S. C.; Curl, R. F.; Smalley, R. E. *Nature* **1985**, *318*, 162.
(2) (a) Krätschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature* **1990**, *347*, 354. (b) Krätschmer, W.; Fostiropoulos, K.; Huffman, D. R. *Chem. Phys. Lett.* **1990**, *170*, 167.
(3) See whole issue of *Acc. Chem. Res.* **1992**, *25*, and references therein.
(4) Guldi, D. M.; Hungerbühler, H.; Janata, E.; Asmus, K.-D. *J. Chem. Soc., Chem. Commun.* **1993**, 84.
(5) Andersson, T.; Nilsson, K.; Sundhal, M.; Westman, G.; Wennerström, O. *J. Chem. Soc., Chem. Commun.* **1992**, 604.
(6) Triton X-100 contains a benzene ring carrying an aliphatic and polyethylene glycol units in the 1- and 4-position, respectively.
(7) Creegan, K. M.; Robbins, J. L.; Robbins, W. K.; Millar, J. M.; Sherwood, R. D.; Tindall, P. J.; Cox, D. M. *J. Am. Chem. Soc.* **1992**, *114*, 1103.
(8) DODAB (dioctadecyldimethylammonium bromide; purchased from Kodak), DHP (dihexadecyl hydrogen phosphate; Fluka), Lecithin (dipalmitoyl phosphatidylcholine; Sigma).
(9) C_{60} and vesicular base units in concentrations of 4×10^{-6}–1×10^{-4} M and 5×10^{-4}–5×10^{-3} M, respectively, were dissolved in chloroform in order to obtain a homogeneous mixture. The solvent was then evaporated, and the remaining solid was dried under vacuum before water was added (in case of DHP addition of some base, NaOH, was necessary). The aqueous suspensions were kept for about 10 min at constant temperature (50–60 °C) to conduct soaking of the material. Vesicle formation was then achieved by ultrasonication with a KLN ultrasonic generator system 582 for about 30 min.
(10) (a) Fendler, J. H. *Acc. Chem. Res.* **1980**, *13*, 7; *Membrane Mimetic Chemistry*; Wiley: New York, 1982. (b) Huang, C. *Biochemistry* **1969**, *8*, 344. (c) Fuhrhop, J.-H.; Mathieu, J. *Angew. Chem.* **1984**, *96*, 124.
(11) Tyrrell, D. A.; Heath, T. D.; Colley, C. M.; Ryman, B. E. *Biochim. Biophys. Acta* **1976**, *457*, 259.
(12) A quantitative determination is hampered by stray light contributions which are relatively small in the practically transparent DODAB and lecithin systems but become visible in the DHP vesicle at higher concentrations of the latter.
(13) Schnecke, W.; Grätzel, M.; Henglein, A. *Ber. Bunsen-Ges. Phys. Chem.* **1977**, *81*, 821.

0002-7863/93/1515-3386$04.00/0

Table I. Optical Data of C_{60} Incorporated into a Variety of Vesicle Membrane Systems

vesicle	charge of head group	$[C_{60}]/[vesc]$	λ_{max}, nm	ϵ(at λ_{max}), M^{-1} cm^{-1}	ΔOD, arbitrary units	$\Delta OD/\epsilon \approx [C_{60}^{\bullet-}]$
DODAB	+	0.020	360	5000	−700	0.140
Lecithin	+/−	0.019	352	30000	−1700	0.057
DHP	−	0.011	378	2500	−100	0.035

n-hexane and in a lecithin vesicle, respectively. These UV bands are generally red-shifted with increasing $[C_{60}]/[vesicle]$ concentration ratio, with the extent depending on the nature of the vesicle. For example, at constant lecithin concentration (7.8×10^{-4} M) the 334-nm band shifts to 352 and 365 nm upon incorporation of 1.5×10^{-5} M and 4.1×10^{-5} M C_{60}, respectively. Increasing vesicle concentration at constant $[C_{60}]$, on the other hand, yields again a hypsochromic shift.

The bathochromic shifts are accompanied by line broadening and significantly decreasing extinction coefficients. The following representative data refer to [vesicle] = 8×10^{-4} M and varying $[C_{60}]$. For lecithin: incorporated $[C_{60}]$ $(1.5–4.1) \times 10^{-5}$ M; shift of 334-nm band to λ_{max} 352–365 nm; extinction coefficient ϵ(at λ_{max}) 32 000–25 000 M^{-1} cm^{-1}. For DODAB: $[C_{60}]$ $(0.5–2.5) \times 10^{-5}$ M; λ_{max} = 343–360 nm; ϵ(at λ_{max}) = 12 000–5000 M^{-1} cm^{-1}. For DHP: $[C_{60}]$ $(0.4–1.5) \times 10^{-5}$ M; λ_{max} = 355–378 nm; ϵ(at λ_{max}) = 10 000–4000 M^{-1} cm^{-1} (individual error limits estimated to ±20%). For comparison: ϵ(334 nm) 52 000 (±5000) M^{-1} cm^{-1} for C_{60} in *n*-hexane, chloroform, and 1,2-dichloroethane, and ϵ(334 nm) 65 000 (±5000) M^{-1} cm^{-1} for C_{60} in cyclohexane, benzene, and toluene (all concentration-independent).

We suggest that these concentration-dependent changes in the spectral characteristics of the 334-nm UV band are indicative of aggregation of C_{60} in the membranes. Such a conclusion is supported by corresponding findings in Langmuir–Blodgett films of C_{60} on phenolic aqueous solution or vapor deposition films of C_{60} on polar solid material.[14]

(14) Tomioka, Y.; Ishibashi, M.; Kajiyama, H.; Taniguchi, Y. *Langmuir* **1993**, *9*, 32 and references cited therein.

It is specifically noted that the characteristic 408-nm band (Figure 1a), observable in solution or heterogeneous systems which lack evidence for C_{60} aggregation, cannot be seen in the vesicular system. In the latter, this band may be obscured in the overall broad and comparatively high absorption in this wavelength range (Figure 1b). However, we can clearly exclude any disturbance by a possible epoxide since the typical bands of this oxidation product at 496 and 424 nm[7] did not show up in any of our solutions or heterogeneous systems.

Finally, C_{60} incorporated into the three types of vesicles discussed here can be reduced to $C_{60}^{\bullet-}$ radical anions by propan-2-ol radicals as described already for homogeneous solutions.[4] The reaction

$$(C_{60})_{vesicle} + (CH_3)_2C^{\bullet}OH \rightarrow (C_{60}^{\bullet-})_{vesicle} + (CH_3)_2CO + H^+ \quad (1)$$

was investigated in pulse radiolysis experiments of N_2O-saturated, aqueous solutions of the respective C_{60}-containing vesicles, containing propan-2-ol. The experiment showed changes with respect to the bleaching of the C_{60} UV band at the respective λ_{max} as in homogeneous solutions.[4] In view of a recent report on a possible and, in any case, presumably slow reaction of the reduced fullerene with water,[15] we did not notice any evidence in this direction during the ≤milliseconds time scale of our pulse radiolysis experiment. The yields of C_{60} reduction in the vesicles, as calculated from the measured changes in absorption and the respective extinction coefficients of C_{60} in terms of $\Delta OD/\epsilon$, are listed in Table I. They are highest for the positively charged vesicle DODAB and lowest for the negatively charged DHP. Assuming that the trends in ϵ parallel those in $\Delta\epsilon$, this finding would make sense in that the transfer of an electron from the propan-2-ol radical through the vesicle surface to the C_{60} would accordingly be most favored for the charge-wise least repelling head group barrier.

(15) Kalsbeck, W. A.; Thorp, H. H. *J. Electroanal. Chem.* **1991**, *314*, 363.