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Ascorbyl radical disproportionation in reverse micellar systems

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ABSTRACT

Ascorbyl radical was generated by the pulse radiolysis method and observed with the fast kinetic spectrophotometry within reverse micelles stabilized by AOT in n-heptane or by Igepal CO-520 in cyclohexane at different water to surfactant molar ratio, w_0. Rate constants for the disproportionation of the ascorbyl radicals were smaller than those for intermicellar exchange for both type of reverse micelles and slower than those in homogeneous aqueous solutions. However, they increased with increasing w_0 for AOT/n-heptane system, while they decreased for Igepal CO-520 system. The absorption spectra of ascorbic acid AOT/n-heptane reverse micellar system showed that the "pH" sensed by this molecule is lower than that in respective homogeneous aqueous solutions. The obtained results were rationalized taking into account three main factors (i) preferential location of ascorbic acid molecules in the interfacial region of the both types of reverse micelles; (ii) postulate that the pH of the interface is lower than that of the water pool of reverse micelles and (iii) different structure of the interface of the reverse micelles made by AOT in n-heptane and those formed by Igepal CO-520 I cyclohexane. Some possible consequences of these findings are discussed.

1. Introduction

Ascorbic acid (vitamin c, AsA) is naturally occurring molecule with a very rich chemistry given by its unusual structure: on a five-membered lactone ring there is a bifunctional enediol group with an adjacent carbonyl group. This property makes AsA an excellent reducing agent in both biological and industrial processes. It supports the synthesis of collagen, metabolism of fats and alcohols, prevents atherosclerosis and coronary heart diseases by reducing of LDL cholesterol, makes easier the assimilation of non-heme iron and takes part in erythrocyte formation, supports the action of other vitamins (e.g. folic acid and vitamin E), rises immunity to colds and other infections. Ascorbic acid is also used without restriction in food and cosmetics formulations. It is a powerful reducing agent in aqueous solutions, while this property is much less evident in non-aqueous media. It reacts both with low molecular oxidants as well as with enzyme proteins to form a relatively unreactive ascorbyl radical, $As^{-\bullet}$ with unpaired electron in a highly delocalized π-system (Packer and Fuchs, 1997). Behavior of the latter in aqueous solutions is relatively well known, while the influence of the local environment still needs more detailed research. For example the pH dependent mechanism of disproportionation of ascorbyl radical in water has been proposed by Bielski et al. (1981). As ascorbate reactions often take place within enzyme active sites (e.g. ascorbate peroxidase, coenzyme Q) or at membrane interfaces that are different from bulk water it is of common interest to understand better the effect of confinement on the reactivity of this important intermediate.

Reverse micelles (RM) are relatively simple ternary systems, in which an exterior organic solvent (continuous phase) surrounds the surfactant interfacial layer that separates the organic media from the inner polar water pool (Silber et al., 1999). Some parameters of the reverse micellar systems studied in this work are given in the Table 1.

Depending on the location of a solute, its environment may be hydrophobic in the outer organic layer or amphiphilic as in the interfacial layer, or hydrophilic as in the water pool. The properties of water confined within reverse micellar aggregates depend on size of the water pool, which can be varied by changing the molar water to AOT surfactant ratio, w_0 (Fletcher and Robinson, 1981; Pileni et al., 1982). Therefore, the effects of confinement of ascorbic acid on its reactivity can be explained based on its location within reverse micelle.

Here we show how reverse micelles, stabilized either by the anionic surfactant (AOT) in n-heptane or by the nonionic surfactants (Igepal CO-520) in cyclohexane, influence the decay of ascorbyl radicals generated by means of pulse radiolysis. Following factors are taken into account to rationalize the obtained results: intermicellar material exchange, local pH, as well as possible interactions of ascorbic acid/ascorbyl radical with surfactant molecules.

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Table 1
Concentration of micelles, [WP], aggregation number, N_{aggr}, overall concentration of ascorbic acid, $[AsA]_{ov}$, average number of ascorbic acid molecules per micelle, n, micellar outer radius, R_0, and second order rate constants of ascorbyl radicals decay observed at 360 nm in AOT/n-heptane reverse micellar system of different w_0 containing 0.2 mM AsA solution in water corrected for the *f* factor calculated as given in the text below.

[AOT], M	w_0	[WP][a], mM	N_{aggr}	$[AsA]_{ov}$ mM	n	R_0, nm	f	k_{corr}, 10^5 $M^{-1}s^{-1}$
0.05	20	0.185	270	0.036	1.95	3.5	50	1.08 ± 0.06
0.1	10	1.33	75	0.360	0.27	2.0	37	2.03 ± 0.03
	20	0.370	270	0.720	1.97	3.5	25	4.2 ± 0.03
	50	0.125	800	1.8	14.4	8.0	6.2	29.6 ± 0.07
0.2	10	2	100	0.72	0.36	2.0	25	4.44 ± 0.03
	20	0.74	270	1.44	1.95	3.5	12.5	9.36 ± 0.02
	50	0.185	1080	3.6	19.46	8.0	4.2	58.1 ± 0.11

[a] According to Lang et al. (1988).

2. Experimental

All chemicals were of at least analytical grade and used without further purification. AOT [sodium bis(2-ethylhexyl) sulfosuccinate] (Catalog No. D-4422), Igepal CO-720 [polyoxyethylene(12) nonylphenyl ether] (Catalog No. 23,865-1) and Ascorbic acid (Catalog No. A5960) were purchased from Sigma-Aldrich, Ascorbic acid (Catalog No. F689527) from Merck, n-Heptane (Catalog No. 51745) and Cyclohexane (Catalog No. 28930) from Fluka.

Ascorbyl radicals were generated via oxidation of ascorbic acid by hydroxyl radicals, $^{\bullet}OH$. The latter were the products of the radiolysis of bulk water or water confined within reverse micellar aggregates. In both cases the system was saturated with nitrogen monoxide, N_2O, to convert hydrated electrons into $^{\bullet}OH$ radicals and therefore to double the yield of the latter. Pulse radiolysis experiments were performed with a 6-MeV linear accelerator. Pulses of 17 ns delivering doses of 55–60 Gy or of 1 μs delivering doses of 300 Gy were applied. An optical path cell was 1 cm. The dose per pulse was determined with the thiocyanate dosimeter. The pulse radiolysis system including fast spectrophotometric equipment has been described elsewhere (Karolczak et al., 1992).

Steady state spectrophotometric measurements were taken using Specord S600 (Carl Zeiss Jena) diode-array spectrophotometer with a quartz cell of appropriate path length.

NMR measurements were undertaken to confirm interactions between AsA and Igepal molecules. The spin-lattice relaxation constants were measured by means of the inversion recovery experiment with the Bruker Avance II Plus 700 MHz spectrometer at 300 K in aqueous solutions of AsA and Igepal-720 micelles under following conditions: pH = 7.0 (100 mM phosphate buffer), [Igepal CO-720] = 20 mM and [AsA] = 5 mM. Samples were saturated with Ar.

Samples were prepared prior to an experiment to minimize the effect of ascorbic acid autooxidation. All measurements were carried out at 23 ± 1 °C except NMR experiments. Nano-pure water from MilliQ (Millipore) was used throughout.

All data were expressed as the means ± SD of three independent experiments.

3. Results and discussion

3.1. Formation of ascorbyl radicals ($As^{\bullet-}$) in reverse micellar systems

The transient absorption spectra observed in pulse-irradiated, N_2O-saturated reverse micellar solutions containing ascorbic acid stabilized either by AOT in n-heptane or by Igepal CO-520 in cyclohexane show the maximum peaking at 360 nm, characteristic for the ascorbyl radical ($As^{\bullet-}$), Fig. 1A and B, respectively. In the latter system the correction for the absorption of an adduct of OH radical to the Igepal CO-520 molecule (Perkowski et al., 1995) had to be done.

Ascorbyl radical can be formed exclusively within reverse micelles in the reaction of ascorbic acid with hydroxyl radical generated in water. Hence, the yield of ascorbyl radicals should be proportional to the water content in the system. Indeed this is the case as shown in the Fig. 2. The concentration of ascorbyl radicals is well below that of hydroxyl radicals due to the competitive scavenging of the latter by surfactant molecules. Similar behavior we have observed in reverse micelles filled with aqueous solutions of sodium thiocyanate (Gębicki, 2004; Gębicki and Gębicka, 1997). The inset in Fig. 2 shows that the absorption at 360 nm is proportional to the dose and hence can be used as a measure of ascorbyl radicals' concentration.

3.2. AOT/n-heptane reverse micelles

Fig. 3 shows transient absorption traces observed at 360 nm after electron pulses delivering different doses to the AOT/n-heptane reverse micellar system containing 0.2 mM ascorbic acid water solution. The half-life time of ascorbyl radicals determined from these traces depends linearly of the dose (see inset in the Fig. 3). The concentration of ascorbyl radicals was calculated assuming the absorbance coefficient identical with that in aqueous solution, i.e. 3300 $M^{-1}cm^{-1}$ (Bielski et al., 1981). The above strongly suggests that ascorbyl radicals formed within AOT/n-heptane reverse micelles decay in the second order disproportionation process. This process needs that at least two radicals are present in the same micelle.

It has been shown that the hydrophilic solute distributes between reverse micelles according to Poissonian equation (Pileni et al., 1984). Poissonian distribution of ascorbic acid molecules between reverse micelles is given by the equation:

$$P_j = \frac{n^j \cdot e^{-n}}{j!} \quad (1)$$

where *Pj* – probability of finding j molecules of AsA in a RM, $n = [AsA]_{ov}/[RM]$ – means the number of AsA molecules per RM. From the Eq. (1) one can calculate, that only for the largest micelles ($w_0 = 50$) almost every micelle contains at least two ascorbic acid molecules (Table 1). Moreover, the maximal overall concentration of ascorbyl radicals observed by us is around 5 μM, what is much less than the concentration of reverse micelles ranging from 0.185 to 2 mM (Table 1). It means that the intermicellar material exchange has to take place to enable radical disproportionation.

Transient absorption traces obtained at 360 nm for reverse micellar systems at different w_0 and different AOT content could be well fitted with the second order kinetic equation from which the overall decay rate constants were obtained. They fall in the range from $0.4 \cdot 10^7$ to $2.5 \cdot 10^7$ M^{-1} s^{-1}, while the rate constant of intermicellar material exchange is at least 10 times faster as determined by us earlier in AOT reverse micellar system (Gębicki and Gębicka, 1997) and also in Igepal CO-520 reverse micellar system (Gębicki, 2004). Hence, intermicellar exchange process cannot be a rate determining step for the disproportionation of ascorbyl radicals in reverse micellar systems studied by us. Taking into account the above and the low occupancy of micelles by the radicals the disproportionation has to proceed within reverse