Stable β-phosphorylated cyclic aminoxyl radicals in SDS micelles

Cécile Rizzi, Corinne Mathieu, Béatrice Tuccio,* Robert Lauricella, Jean-Claude Bouteiller and Paul Tordo

UMR-CNRS 6517 'Chimie, Biologie et Radicaux Libres', Universités d'Aix-Marseille 1 et 3, Av. Escadrille Normandie-Niemen, 13397 Marseille Cedex 20, France

Received (in Cambridge, UK) 4th June 1999, Accepted 27th September 1999

The behaviour of a series of stable cyclic aminoxyl radicals in the presence of SDS micelles was studied by EPR spectroscopy. Six different compounds, *i.e.* two non-phosphorylated and four β-phosphorylated, were investigated. Except in the case of the strongly hydrophilic 3-CP **1**, which always remained in the bulk aqueous phase, all the radicals were found to exchange between water and micelles. Their partition coefficients were evaluated from computer simulations of the EPR spectra and in the case of two aminoxyls TOMER-Et **5** and TOMER-Pr^{i} **6**, the variation of the rate constant with temperature allowed us to estimate the exchange activation energy.

Introduction

Stable aminoxyl free radicals are being used for an increasing number of applications in various fields, particularly in biology. These compounds have been widely used as EPR spin probes in biological systems to determine the molecular dynamics of various macromolecules,[1] to study membrane permeability and structure,[2,3] to follow drug delivery to organs *via* liposomes,[4] as SOD mimics,[5] or in oximetry.[6] Aminoxyls have also been successfully used as contrast agents in magnetic resonance imagery to characterise diseases or to investigate affected organs.[7] Such a variety in potential applications explains the great diversity of available stable aminoxyl radicals, especially concerning their lipophilicity and their EPR parameters.

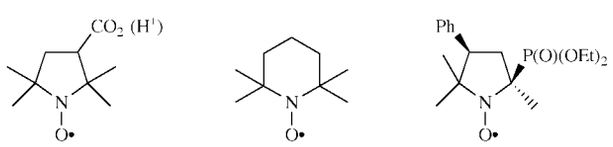
Previous studies were devoted to the behaviour of non-phosphorylated aminoxyl radicals in water/micelle heterogeneous media, in particular in order to elucidate micelle structure and properties.[8–10] A few papers also deal with the determination of aminoxyl radical penetration depth, location and mobility in the presence of sodium dodecyl sulfate (SDS) micelles and of a polymer, since these radicals can be used to control polymerisation in the presence of micelles.[11,12]

Recently the behaviour of persistent β-phosphorylated aminoxyls generated by spin-trapping in the presence of SDS micelles was studied by EPR spectroscopy[13] and the phosphorus coupling constant was found to be a good indicator of the spin-adduct location in the water/micelle heterogeneous system. A new technique was thus elaborated to determine spin-adduct partition coefficients and it would now be interesting to extend this method to stable β-phosphorylated aminoxyl radicals, in order to improve its validity.

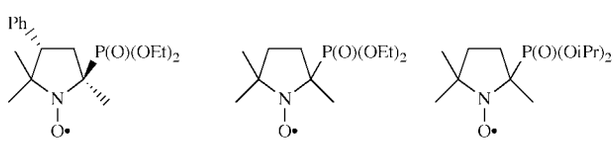
A few years ago, stable aminoxyl radicals of the pyrrolidinoxyl series were generated in our laboratory.[14] The mechanisms of reduction of these compounds by various biological agents, such as ascorbic acid or flavins, have already been determined and acceptable reduction rates have been found.[15,16] In addition, their EPR spectra present a large phosphorus hyperfine splitting constant (hfsc), which was found to be very sensitive to the pyrrolidinyl ring conformation, and which is thus expected to be a useful probe for investigating the behaviour of the aminoxyls in heterogeneous systems. The β-phosphorylated aminoxyl EPR spectra were thus recorded in the presence of aqueous solutions of sodium dodecyl sulfate (SDS) micelles. Although micelles do not have a bilayer structure, this work can be considered as a very first step in the study of β-phosphorylated aminoxyl behaviour in the presence of various biomembrane models. In the next step, the same kind of study will be realised with more complicated and more reliable membrane models, such as liposomes.

Results

The six stable aminoxyl radicals studied, the structures of which are given below, could be divided into two groups: two non-phosphorylated probes, *i.e.* 3-carboxy-2,2,5,5-tetramethylpyrrolidine-*N*-oxyl **1** (3-CP), and 2,2,6,6-tetramethylpiperidine-*N*-oxyl **2** (TEMPO), and four β-phosphorylated compounds, *i.e.* *r*-2-diethoxyphosphoryl-*c*-4-phenyl-2,5,5-trimethylpyrrolidine-*N*-oxyl **3** (TOBER-36), *r*-2-diethoxyphosphoryl-*t*-4-phenyl-2,5,5-trimethylpyrrolidine-*N*-oxyl **4** (TOBER-53), 2-diethoxyphosphoryl-2,5,5-trimethylpyrrolidine-*N*-oxyl **5** (TOMER-Et), and 2-di(isopropyloxy)phosphoryl-2,5,5-trimethylpyrrolidine-*N*-oxyl **6** (TOMER-Pr^{i}).



1: 3-CP **2**: TEMPO **3**: TOBER-36



4: TOBER-53 **5**: TOMER-Et **6**: TOMER-Pr^{i}

When the aminoxyl EPR spectra were recorded in the presence of SDS (concentrations ranging from 0 to 150 mmol dm^{-3}), three different cases were observed.

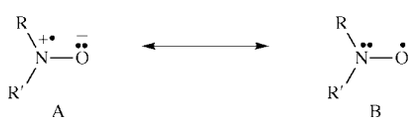
First, for 3-CP **1,** the hfsc with the nitrogen nucleus (a_N = 1.62 mT) remained unchanged whatever the SDS concentration was, indicating that this compound remained in the bulk aqueous phase. This result was expected since **1** has been reported to be strongly hydrophilic, with an octanol–phosphate buffer partition coefficient of 0.08 at pH = 7.[17] Furthermore, it has been demonstrated that this compound was unable to enter the micelles because of electrostatic repulsions between the polar heads of the SDS molecules associated in micelles and the charge of the anionic 3-CP.[18–20]

Table 1 EPR parameters in pure water and in micelles, distribution coefficients (K_d) and partition coefficients (K_p) between water and micelle phases of the aminoxyls **1**–**6**

Aminoxyl	a_{Pw}/mT	a_{Nw}/mT	a_{Pm}/mT	a_{Nm}/mT	$10^{-3}\,K_d$	K_p
TEMPO **2**	—	1.723[a]	—	1.685 ± 0.004[c]	3.04 ± 1[c]	91[c]
TOBER-36 **3**	3.638[a]	1.520[a]	3.520 ± 0.001[c]	1.487 ± 0.001[c]	70.75 ± 10[c]	2122[c]
TOBER-53 **4**	5.452[a]	1.524[a]	5.305 ± 0.004[c]	1.492 ± 0.001[c]	27.10 ± 4[c]	813[c]
TOMER-Et **5**	4.799[b]	1.533[b]	4.510[b]	1.490[b]	3.16 ± 0.2[d]	95[d]
TOMER-Pr^i **6**	4.741[b]	1.538[b]	4.394[b]	1.500[b]	15.00 ± 0.5[d]	450[d]

[a] Determined from EPR spectra recorded in aqueous media. [b] Evaluated by simulation of the EPR spectra exhibiting two separated species. [c] Evaluated using computer modelling of the average hfsc *vs.* ([SDS] − cmc) following eqn. (1). [d] Calculated using linear regression on $K_p \approx 0.03\,K_d$.

In the case of TEMPO **2**, TOBER-36 **3**, and TOBER-53 **4**, modifications in the EPR spectra occurred as soon as micelles were formed, *i.e.* at SDS concentrations higher than the critical micelle concentration (cmc *ca.* 8.2 mmol dm^{-3}). For these three compounds, the nitrogen hfsc (a_N) decreased slightly when the SDS concentration was raised. For example, in the case of TEMPO, a_N varied from 1.73 mT to 1.69 mT when the SDS concentration was increased from the cmc to 50 mmol dm^{-3}. Such a phenomenon has already been mentioned for different aminoxyl radicals in previous studies,[12,13,21] and this a_N modification was explained by a decrease in the radical environment polarity. Actually, aminoxyl radicals can be represented by the two mesomeric forms A and B, as indicated in Scheme 1,



Scheme 1 Representation of the two limiting mesomeric forms of an aminoxyl radical. The A form is favoured in polar media.

and the a_N value is expected to increase when the A form is favoured, for example in polar media. However, this variation was always lower than 0.04 mT and in the case of **3** and **4**, the main change observed in the EPR spectra, when the SDS concentration was raised, resulted from a larger decrease of the phosphorus hfsc (a_P). For example, in the case of TOBER-36, a 0.1 mT sudden a_P decrease was observed as soon as the cmc was reached. As previously mentioned, this a_P variation corresponded to a modification in the compound conformation, which, along with the a_N decrease, confirmed a partial location of the aminoxyl radical in micelles. Thus, the EPR signals observed above the cmc corresponded to the averaged spectra of aminoxyl radicals in exchange between aqueous and micellar media, that is to say that a_N and a_P values obtained for different SDS concentrations should be considered as mean values and designated as $\langle a_N \rangle$ and $\langle a_P \rangle$, respectively.

In a previous work, the aminoxyl radical affinity for a micellar pseudophase has been evaluated by a micelle–water distribution coefficient K_d,[13] which was defined as indicated in eqn. (1), in which n_{Am} and n_{Aw} represent the number of

$$K_d = \frac{n_{Am}/n_{SDS}}{n_{Aw}/n_w} \qquad (1)$$

aminoxyl radical moles in micelles and in water, respectively, n_{SDS} being the number of SDS moles associated in micelles and n_w the number of water moles in the medium. Note that K_d is directly related to the micelle–water partition coefficient K_p. As previously reported in the case of SDS micelles in water[13] $K_p \approx 0.03\,K_d$.

The distribution coefficients were thus calculated from the average coupling constants, $\langle a_X \rangle$, X being either a nitrogen or a phosphorus. Computer modelling of $\langle a_X \rangle$ variation *vs.* the concentration of SDS monomers associated in micelles was carried out using eqn. (2), in which a_{Xw} and a_{Xm} are the hfscs for the

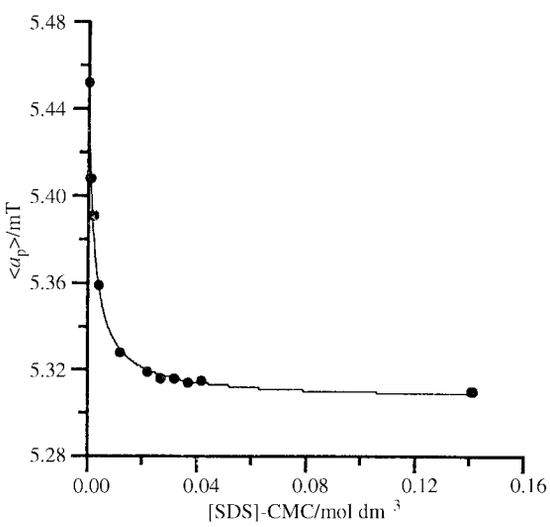


Fig. 1 Modelling of TOBER-53 $\langle a_P \rangle$ variation *vs.* concentration of SDS monomers associated in micelles, ([SDS] − cmc). The a_P value for the aminoxyl radical in water (a_{Pw} = 5.452 mT) was obtained by simulating the EPR spectrum at [SDS] = 8 mmol dm^{-3}. The modelling led to the following parameters: a_{Pm} = 5.305 mT and K_d = 27100.

$$\langle a_X \rangle = \frac{(a_{Xm} - a_{Xw})K_d}{K_d + \dfrac{n_w}{n_{SDS}}} + a_{Xw} \qquad (2)$$

aminoxyl radical in water and micelles respectively. As an example, the $\langle a_P \rangle$ variation observed for TOBER-53 has been plotted *vs.* SDS concentration in Fig. 1. In this case, modelling the $\langle a_P \rangle$ decrease led to a_{Pm} = 5.305 mT and K_d = 27100 (see Table 1).

As can be seen from Table 1, K_d evaluation was much less reliable for TEMPO than for compounds **3** and **4**. For the former, the calculation was carried out from a_N variations, while $\langle a_P \rangle$ was used to determine K_d for **3** and **4**. Thus, the larger variation observed for $\langle a_P \rangle$ allowed a more precise K_d evaluation, and this should be regarded as being an advantage of β-phosphorylated aminoxyls.

For the three aminoxyl radicals, satisfactory simulations of the observed EPR spectra could not be achieved using conventional simulation software, certainly because of modifications in the line shape induced by the partition equilibrium. Nevertheless, these average spectra recorded at various SDS concentrations were satisfactorily simulated by introducing an exchange between two paramagnetic species (namely aminoxyl in water and in micelles) using the program elaborated by Rockenbauer.[22] As an example, Fig. 2 shows the TOBER-36 spectrum recorded at [SDS] = 12 mmol dm^{-3} and the superimposed simulated spectrum. The calculated exchange correlation time, 1.7×10^{-7} s, was in the range of well determined kinetics of a SDS monomer exchanging between the micelle structure and the bulk environment.[8,23] Moreover, the hfscs in

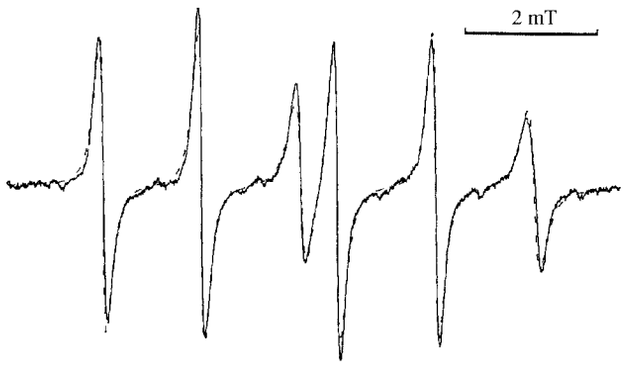


Fig. 2 TOBER-36 experimental EPR spectrum (solid line) recorded in the presence of 12 mmol dm^{-3} SDS and simulation of this signal (dotted line) obtained by considering two paramagnetic species in rapid equilibrium, the minor (16%) being TOBER-36 in water (a_{Nw} = 1.502 mT and a_{Pw} = 3.661 mT), the major (84%) being the same aminoxyl radical in micelles (a_{Nm} = 1.496 mT and a_{Pm} = 3.527 mT). The exchange correlation time was evaluated to be 1.7×10^{-7} s.

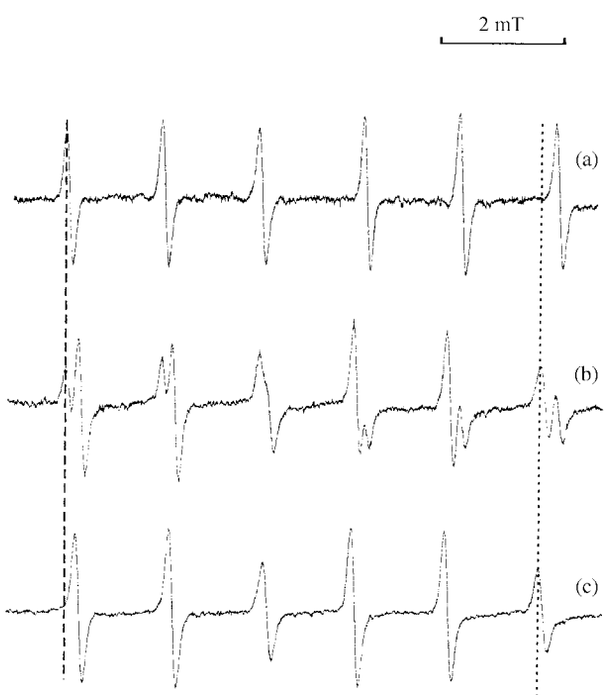


Fig. 3 TOMER-Pr^i EPR spectra recorded at various SDS concentrations: (a) [SDS] = 0.004 mol dm^{-3}; (b) [SDS] = 0.012 mol dm^{-3}; (c) [SDS] = 0.050 mol dm^{-3}. The two vertical lines indicate the position of the first low-field peak of TOMER-Pr^i in water (dashed line) and of the first high-field peak of TOMER-Pr^i in micelles (dotted line).

micelles, calculated with this program, agreed nicely with the previous values obtained from eqn. (1).

Finally the EPR spectra of TOMER-Et and TOMER-Pr^i showed the presence of two species each exhibiting a six line signal in the presence of micelles. The hfscs of the two species remained unchanged, even at high SDS concentrations. For both radicals, one of the species always exhibited the same hfscs as those found for aminoxyl radical in pure water, and was thus assigned to the aminoxyl in the bulk aqueous phase. Increasing the SDS concentration resulted in modification of the relative intensities of the two signals. Thus in the case of **5**, aminoxyl radical in water became the minor compound, while the second species signal, which appeared from [SDS] = 15 mmol dm^{-3}, grew regularly until [SDS] = 150 mmol dm^{-3}. In the case of **6**, the signal of aminoxyl in water vanished as soon as a 50 mmol dm^{-3} SDS concentration was reached (see Fig. 3).

This could indicate that this second species corresponded to the aminoxyl radical in micelles. Moreover, the a_N measured for

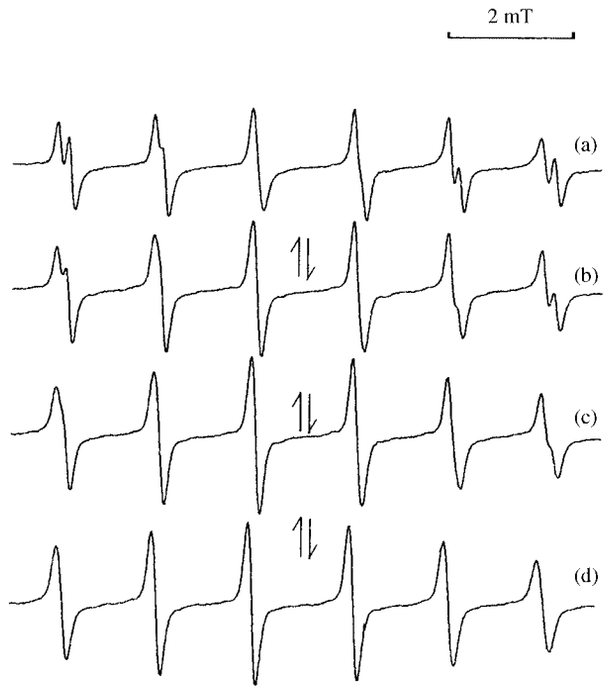


Fig. 4 TOMER-Et EPR spectra recorded in presence of micelles ([SDS] = 40 mmol dm^{-3}) at various temperatures: (a) T = 298 K; (b) T = 318 K; (c) T = 333 K; (d) T = 353 K.

5 and **6** second species (see Table 1) were found to be smaller than for the aminoxyl radical in water, just as in the case of **3** and **4**. Nevertheless, in order to corroborate this hypothesis, TOMER-Et **5** and TOMER-Pr^i **6** spectra were recorded at a given SDS concentration ([SDS] = 40 mmol dm^{-3} for TOMER-Et and [SDS] = 15 mmol dm^{-3} for TOMER-Pr^i), and at various temperatures between 298 K and 353 K. The temperature increase resulted in important modifications in the spectrum shape, and initial spectra were recovered by cooling the media back to 298 K, which showed that the phenomenon observed was perfectly reversible. We verified that these modifications could not be simply explained by a change of the EPR parameters with temperature by recording spectra of **5** and **6** at various temperatures in pure water. The decrease of a_P thus determined by increasing the temperature from 298 K to 363 K was only 8.71×10^{-4} mT K^{-1} for **5** and 6.71×10^{-4} mT K^{-1} for **6**, that is to say much too weak to explain the temperature dependance of the spectra recorded in the presence of micelles. As an example of the signal modifications observed by varying the temperature, TOMER-Et spectra recorded at 298 K, 318 K, 333 K and 353 K are given in Fig. 4.

Rockenbauer's program[22] was used to simulate all spectra and gave values of the exchange rate constant k. These values have been plotted *vs.* the temperature using a logarithmic Arrhenius equation, which permitted us to evaluate the activation energy E_a of the exchange phenomenon for both **5** and **6**. These calculations led to $E_a = 37.8 \pm 1.3$ kJ mol^{-1} and $E_a = 32.4 \pm 4.1$ kJ mol^{-1}, for **5** and **6** respectively, and it is noteworthy that these results are similar to those previously published for di-*tert*-butylaminoxyl in SDS micelles (E_a = 36.4 kJ mol^{-1}).[24] The signal simulations made also indicated that a temperature rise resulted in a significant decrease of the aminoxyl population in micelles, especially in the case of **6**, thereby indicating that the enthalpy of the radical considered was lower in micelles than in water.

Furthermore, in order to calculate K_d for **5** and **6**, the ratio of the population of aminoxyl radical in micelles to that in water (*i.e.* n_{Am}/n_{Aw}) was directly obtained for each SDS concentration by computer simulations of the various spectra at room temperature. Assuming that the solution density was close to that of pure water, eqn. (3) was then derived from eqn. (1). A

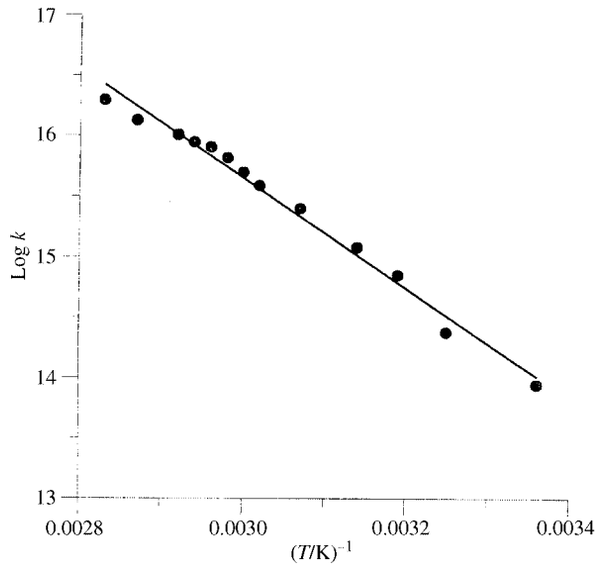


Fig. 5 Logarithmic Arrhenius plot of exchange rate constant k for TOMER-Et: activation energy $E_a = 37.8$ kJ mol^{-1}, pre-exponential factor $A = 5.3 \times 10^{12}$ s^{-1}.

$$\frac{n_{Am}}{n_{Aw}} = 0.018K_d([SDS] - CMC) \quad (3)$$

linear regression on the plot of n_{Am}/n_{Aw} *vs.* ([SDS] − CMC) gave the K_d values reported in Table 1 for **5** and **6**.

Discussion

The K_d values determined for the various aminoxyls reported in Table 1, are in good agreement with their structures. Generally speaking, the more hydrophobic the compound, the higher is its affinity for the micellar phase. For example, if we compare **5** and **6**, it appears that replacing two ethyl groups by more hydrophobic isopropyl groups results in increasing K_d from 3.1×10^3 to 15×10^3. Note also that the aminoxyl radical affinity for the micellar phase may strongly depend on its stereochemistry. Thus, K_d was found to be nearly three times higher for **3** than for **4**, although these two diastereoisomeric radicals differ only in the *cis* or *trans* position of the phenyl group on the pyrrolidinyl ring.

It is well known that a solute can penetrate more or less deeply the hydrophobic core of micelles. In order to determine the precise location of the phosphorylated aminoxyls in the micelle, their a_N values evaluated in micelles were compared to those determined in various solvents. In the case of TEMPO **2**, the uncertainty of the a_N value determined in micelles was too great to conclude unambiguously its location in the micelle structure. However, the small a_N variation between water and micelles could be explained by the aminoxyl being located in the surface of the micelles, as previously published.[25] For the four phosphorylated compounds, the a_N values evaluated in micelles were halfway between those measured in water and in methanol. In the case of TOMER-Et **5** for example, a_N was 1.533 mT in water, 1.445 mT in methanol and 1.490 mT in micelles. This clearly shows that, whatever the hydrophobicity and the micelle–water partition coefficient of the compound considered, its aminoxyl moiety was always located in an environment more polar than methanol, probably in the surface of the micelle near the sulfate head groups.

A comparison of a_P values determined for compounds **3**–**6** in micelles (a_{Pm}) to those obtained in various solvents also gave information about the ring conformations of these compounds. It appeared that a_P was of the same order in micelles and in heptane for TOBER-53 **4** (5.305 mT in micelles and 5.287 mT in heptane), indicating that the predominant conformers of **4** could be the same in micelles and in apolar solvents. For the three other phosphorylated radicals, this phosphorus coupling was always found to be lower in micelles than in all the most common solvents. For example, in the case of TOMER-Et **5**, a_P was 5.1 mT in heptane, 4.8 mT in water and 4.51 mT in micelles. It can thus be concluded that the conformational equilibria were greatly modified when the aminoxyl radical entered the micelles.

As can be seen from Fig. 3 for example, the line width observed for the aminoxyl in micelles was only slightly higher than that of the aminoxyl in water, showing that the radical motions were not strongly restricted in the micelles, as previously observed for di-*tert*-butylaminoxyl.[24] However, since too many parameters, such as the medium viscosity, the exchange rate or radical and oxygen concentrations, can contribute to the line broadening in our experiments, the computer program used to simulate the various spectra did not permit us to determine the rotational correlation time for the radicals studied.

As previously specified in this text, TOMER-Et and TOMER-Pr^i EPR spectra recorded in the presence of micelles clearly exhibited two separated signals, while mean spectra were always observed with compounds **2**–**4**. This apparent difference in the behaviour of **5** and **6** is surprising and we first wondered whether this was the result of a much slower exchange of these aminoxyl radicals between water and the micelle pseudophase. However the computer simulations of **5** and **6** spectra did not give significantly lower values for the exchange rate constant, which were always in the range of 10^6–10^7 s^{-1}, and this first hypothesis was then rejected. It should be kept in mind that the EPR signal general shape depends not only on the exchange rate of the radical between the two phases, but also on the relative position of each spectrum line of this compound, on the one hand in micelles and on the other hand in the bulk aqueous phase. The position of each line toward the EPR signal centre B_0 can be calculated using eqn. (4), where a_i is the hyperfine

$$B = B_0 + \sum_i a_i m_{Ii} \quad (4)$$

coupling constant and m_{Ii} is the nuclear magnetic quantum number relative to the nucleus i, i being either a nitrogen or a phosphorus. In the case of TOMER-Et for example, the signal recorded can be considered as a superposition of two spectra, both having roughly the same g factor. Thus using eqn. (4) and the values listed in Table 1 to calculate the gap between the EPR lines, it appears that the clearer splitting of the two spectra can be seen either on the first low field lines or on the first high field lines, which are separated by 0.332 mT. This line splitting is much less obvious on the second low field or high field lines, which are separated by 0.289 mT only, and this phenomenon is hardly seen on the other lines, for which the calculated splitting became 0.246 mT only. The same analysis can be made for the TOMER-Pr^i spectrum. If we now consider compounds **3** and **4**, we can observe that the gap between the EPR lines of the aminoxyl in water and in micelles is always lower than 0.2 mT, which explains why mean lines were always observed for these compounds. As for TEMPO **2**, it should be mentioned that mean spectra were always observed, since the gap calculated between the EPR lines was never higher than 0.04 mT. Because of this weak gap, the simulation program used did not yield a reliable value for the exchange rate constant. Thus, the method employed in this study did not permit us either to confirm or to validate the results previously published[25] concerning the behaviour of TEMPO in the presence of micelles (*i.e.* a slow exchange of TEMPO between water and micelles, with an exchange rate constant in the range 10^4–10^6 s^{-1}). However, we can reasonably assume that the exchange kinetics are not very different for TEMPO than for the other aminoxyls. Note also that the uncertainty about the parameters determined by computer simulation of EPR spectra was weak in the case

of compounds **3**–**6**, just because of the presence of a strong coupling with the phosphorus.

Molecular mechanics calculations performed on compounds **3**–**6** have shown that both **5** and **6** exist as an equilibrium between many conformers, for which a_P can be very different, while only one predominant conformer is populated for both TOBER-36 and TOBER-53, just because of the presence of a phenyl group on the ring.[26] Thus the major conformers of TOBER-36 and TOBER-53 in micelles and in water could be only slightly different. On the contrary, the pyrrolidinyl ring is much more floppy in the case of compounds **5** and **6**, which results in the larger difference between a_{Pw} and a_{Pm} observed for **5** and **6**.

Conclusion

The present study allowed us to determine both aqueous and micellar hfscs of stable aminoxyl free radicals. In addition, the method described permitted us to evaluate the partition coefficients of stable aminoxyl radicals between water and micelles and to confirm that the aminoxyl affinity for the micellar pseudophase was higher when the compound considered was more lipophilic. Except in the case of the strongly hydrophilic 3-CP, all the aminoxyl radicals studied were found to exchange between water and micelles. Thus, considering the micelles as an extremely simplified biomembrane model, aminoxyls **2**–**6** could be expected to be able to enter the cells. However, this is to be confirmed either by using these compounds in biological media, or by studying their behaviour in the presence of more reliable biomembrane models, such as liposomes. As already mentioned in this text, K_d evaluation was less accurate for TEMPO than for **3**–**6**. It is obvious that the existence of a strong coupling with the phosphorus allowed a better evaluation not only for K_d but also for all the other parameters obtained by simulating EPR spectra, in particular when the aminoxyl pyrrolidinyl ring is not locked by the phenyl group. This emphasises the key role of the phosphorylated group of compounds **3**–**6** as an efficient probe of the aminoxyl radical environment, and should be regarded as a major advantage of β-phosphorylated aminoxyl radicals.

Experimental

Aminoxyls **1**, **2** and SDS have been purchased from Sigma Chemical Co. and used without further purification. Compounds **3**–**6** were synthesised and purified in our laboratory as previously described.[14]

Various SDS concentrations (0 to 150 mmol dm^{-3}) were added to a 10^{-4} mol dm^{-3} aqueous solution of stable aminoxyl radicals for EPR experiments. EPR spectra were recorded at 293 K using a computer-controlled Bruker EMX spectrometer operating at the X-band with 100 kHz modulation frequency, and equipped with an NMR gaussmeter for magnetic field calibration. The following conditions were used: microwave power 10 mW; modulation amplitude 0.1 mT; receiver gain 1.6×10^3; scan time from 82 s to 328 s; time constant from 0.64 s to 2.56 s. Temperature studies were achieved on EPR sealed tubes with the aid of a Bruker Eurotherm B-VT 2000 variable temperature unit using a N_2 stream cooling system.

EPR simulations were performed using two programs. The first was a standard simulation software elaborated by Dulling.[27] The second was elaborated by A. Rockenbauer,[22] and permitted simulation of exchanging species spectra.

Acknowledgements

We are very grateful to Professor Antal Rockenbauer, from the Technical University of Budapest, who kindly provided us with a new version of his EPR simulation computer program.

References

1 M. F. Ottaviani, E. Cossu, N. J. Turro and D. A. Tomalia, *J. Am. Chem. Soc.*, 1995, **117**, 4387.
2 C. Altenbach, D. A. Greenhalgh, H. G. Khorana and W. L. Hubbell, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 1667; A. Ivancich, L. I. Horvath, M. Droppa, G. Horvath and T. Farkas, *Biochim. Biophys. Acta*, 1994, **1196**, 51.
3 M. Balakirev and V. Khramtsov, *J. Chem. Soc.*, *Perkin Trans. 2*, 1993, 2157.
4 M. Federico, A. Iannone, H. C. Chan and R. L. Magin, *Magn. Reson. Med.*, 1989, **10**, 418.
5 M. C. Krishna, R. F. Halevy, R. Zhang, P. L. Gutierrez and A. Samuni, *Free Radical Biol. Med.*, 1994, **17**, 379.
6 C. S. Lai, L. E. Hopwood, J. S. Hyde and S. Lukiewicz, *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 1166; H. C. Chan, J. F. Glockner and H. M. Swartz, *Biochim. Biophys. Acta*, 1989, **1014**, 141; J. E. Baker, W. Froncisz, J. Joseph and B. Kalyanaraman, *Free Radical Biol. Med.*, 1997, **22**, 109.
7 K. Mader, G. Bacic, A. Domb, O. Elmanak, R. Langer and H. M. Swartz, *J. Pharm. Sci.*, 1997, **86**, 126.
8 A. M. Wasserman, *Russ. Chem. Rev.* (*Engl. Transl.*), 1994, **63**, 373.
9 S. Weber, T. Wolff and G. von Bünau, *J. Colloid Interface Sci.*, 1996, **184**, 163.
10 E. Szadjzinska-Pietek, R. Maldonado, L. Kevan, R. R. M. Jones and M. J. Coleman, *J. Am. Chem. Soc.*, 1985, **107**, 784.
11 Y. S. Kang and L. Kevan, *J. Phys. Chem.*, 1994, **98**, 7624.
12 P. C. Griffiths, C. C. Rowlands, P. Goyffon, A. M. Howe and B. L. Bales, *J. Chem. Soc.*, *Perkin Trans. 2*, 1997, **12**, 2473.
13 C. Rizzi, R. Lauricella, B. Tuccio, J. C. Bouteiller, V. Cerri and P. Tordo, *J. Chem. Soc.*, *Perkin Trans. 2*, 1997, **12**, 2507.
14 A. Mercier, Y. Berchasdsky, Badrudin, S. Pietri and P. Tordo, *Tetrahedron Lett.*, 1991, **32**, 2125; F. Le Moigne, A. Mercier and P. Tordo, *Tetrahedron Lett.*, 1991, **32**, 3841; L. Dembkowski, J. P. Finet, C. Fréjaville, F. Le Moigne, R. Maurin, A. Mercier, P. Pages, P. Stipa and P. Tordo, *Free Radical Res. Commun.*, 1993, **19**, S23; V. Roubaud, F. Le Moigne, A. Mercier and P. Tordo, *Phosphorus Sulfur*, 1994, **86**, 39.
15 C. Mathieu, A. Mercier, D. Witt, L. Dembkowski and P. Tordo, *Free Radical Biol. Med.*, 1997, **22**, 803.
16 C. Mathieu, B. Tuccio, R. Lauricella, A. Mercier and P. Tordo, *J. Chem. Soc.*, *Perkin Trans. 2*, 1997, **12**, 2501.
17 R. J. Mehlhorn and I. Probst, *Methods Enzymol.*, 1982, **88**, 334; U. G. Eriksson, T. N. Tozer, G. Sosnovsky, J. Lukszo and R. C. Brasch, *J. Pharm. Sci.*, 1986, **75**, 334.
18 Z. Gao, R. E. Wasylishen and J. C. T. Kwak, *J. Phys. Chem.*, 1989, **93**, 2190.
19 P. J. Bratt, H. Choudhury, P. B. Chowdhury, D. G. Gillies, A. M. L. Krebber and L. H. Sutcliffe, *J. Chem. Soc.*, *Faraday Trans.*, 1990, **86**, 3313.
20 Z. Gao, R. E. Wasylishen and J. C. T. Kwak, *J. Chem. Soc.*, *Faraday Trans.*, 1991, **87**, 947.
21 F. M. Witte, P. L. Buwalda and J. B. F. N. Engberts, *Colloid Polym. Sci.*, 1987, **265**, 42.
22 A. Rockenbauer and L. Korecz, *J. Magn. Reson.*, 1996, **10**, 29.
23 E. A. G. Aniansson, S. N. Wall, M. Almgren, H. Hoffman, I. Kielmann, W. Ulbricht, R. Zana, J. Lang and C. Tondre, *J. Phys. Chem.*, 1976, **80**, 905.
24 N. Atherton and J. Strach, *J. Chem. Soc.*, *Faraday Trans. 2*, 1972, **68**, 374.
25 J. Oakes, *J. Chem. Soc.*, *Faraday Trans. 2*, 1972, **68**, 1464.
26 A. Gaudel, P. Tordo and D. Siri, unpublished work.
27 D. R. Dulling, *J. Magn. Reson.*, 1994, **104**, 105.

Paper 9/04479E