ELSEVIER

Biophysical Chemistry 59 (1996) 133–138

Biophysical Chemistry

Binding of Ru(II) polyazaaromatic complexes to DNA: A ^{23}Na NMR spin-lattice relaxation study

M. Casu [a], G. Saba [a], A. Lai [a], M. Luhmer [b,*], A. Kirsch-De Mesmaeker [c,1], C. Moucheron [c], J. Reisse [b]

[a] *Universita di Cagliari, Dipartimento di Scienze Chimiche, 72 via Ospedale, 09100 Cagliari, Italy*

[b] *Université Libre de Bruxelles, Laboratoire de Chimie Organique E.P. (CP 165), 50 Avenue F.D. Roosevelt, 1050 Brussels, Belgium*

[c] *Université Libre de Bruxelles, Laboratoire de Chimie Organique Physique (CP 160 / 08), 50 Avenue F.D. Roosevelt, 1050 Brussels, Belgium*

Received 20 April 1995; revised 17 July 1995; accepted 25 July 1995

Abstract

The possibility of using sodium-23 spin-lattice relaxation rate measurements to probe the interaction modes of Ru^{II} polyazaaaromatic complexes with DNA is investigated. The following complexes are considered: $Ru(phen)_3^{2+}$ (phen = 1,10-phenanthroline), $Ru(phen)_2HAT^{2+}$ (HAT = 1,4,5,8,9,12-hexaazatriphenylene), and $Ru(diMeTAP)_3^{2+}$ (diMeTAP = 2,7-dimethyl-1,4,5,8-tetraazaphenanthrene). The addition of $Ru(diMeTAP)_3^{2+}$ to a solution of NaDNA leads to a decrease in the sodium-23 spin-lattice relaxation rate (R_1) similar to the effect observed upon addition of Mg^{2+}. This indicates that $Ru(diMeTAP)_3^{2+}$ interacts like Mg^{2+} with DNA and consequently that the electrostatic interaction dominates the association with DNA. $Ru(phen)_3^{2+}$ and $Ru(phen)_2HAT^{2+}$ diminish R_1 more efficiently than Mg^{2+}, in a manner similar to ethidium bromide, which is known for its intercalation properties. Thus interactions other than electrostatic occur between these two complexes and DNA. These results are in agreement with data obtained from other techniques, according to which $Ru(phen)_3^{2+}$ and $Ru(phen)_2HAT^{2+}$ are located partially inside the DNA double helix, in contrast to $Ru(diMeTAP)_3^{2+}$ which remains in the ionic atmosphere around the phosphate backbone.

Keywords: Ruthenium complex; DNA; Intercalation; Sodium-23; NMR spin-lattice relaxation

1. Introduction

The interactions of octahedral polyazaaromatic ruthenium(II) complexes with DNA have been the subject of numerous investigations [1–12]. Essentially three modes of binding to DNA have been considered: (i) the 'partial intercalation' where a ligand with an extended aromaticity is inserted into the stacking of bases, (ii) the surface binding of the octahedral species in the minor or major grooves of DNA, and (iii) a pure electrostatic binding of the complex in the ionic atmosphere surrounding the DNA double helix. For example, it has been demonstrated that for $Ru(phen)_2dppz^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine; a ligand with an extended aromaticity) intercalation occurs [10–12]. It is

* Corresponding author.

[1] Director of Research at the FNRS (Belgium).

SSDI 0301-4622(95)00114-X

worth pointing out that a battery of methods [1–3] such as absorption, luminescence and proton NMR spectroscopy [4–6], circular and linear dichroism [7], measurements of DNA unwinding [8,9] and viscosity experiments [2,3], is needed in order to describe realistically the non-covalent binding of these metallic species to DNA.

^{23}Na NMR spectroscopy has been extensively used to investigate the association of sodium ions, and other cationic species, with DNA in solution. Competitive interactions of Na^+ and cations such as alkali metal ions, Mg^{2+}, $Co(NH_3)_6^{3+}$ and charged polyamines with helical B-DNA have been studied by analyzing the ^{23}Na NMR line width [13–16]. Mariam et al. [17] observed a dramatic decrease in the ^{23}Na NMR linewidth upon addition of the intercalating ethidium bromide. More recently, Jacobsen and co-workers [18–21] devoted a series of papers to the effect of intercalation on the magnetic relaxation of ^{23}Na in NaDNA solutions. Most of this work concerns spin-lattice relaxation time (T_1) measurements and it was shown that '^{23}Na relaxation rate, $R_1 = 1/T_1$, is an extremely sensitive parameter for investigation of intercalation' [19]. Thus, the addition of an intercalator (such as ethidium bromide) to a solution of NaDNA leads to a decrease in R_1 which was more pronounced than the decrease observed in the case of titration with Mg^{2+} [18,19].

These findings urged us to investigate the interaction of Ru^{II} complexes with DNA by means of ^{23}Na R_1 measurements. For this study we tested Ru(diMeTAP)$_3^{2+}$ [22], Ru(phen)$_2$HAT^{2+} [23–25], and Ru(phen)$_3^{2+}$. According to luminescence data experiments, the first compound should exhibit a pure electrostatic association with the phosphate backbone of calf thymus DNA. Indeed, the luminescence intensity ratio (I/I_0, where I and I_0 are the luminescence intensities in presence and absence of polynucleotide, respectively) and the luminescence lifetime of the complex remain constant for increasing amounts of poly-d(AT) · poly-d(AT), or CT-DNA, added to the Ru(diMeTAP)$_3^{2+}$ solution (5 mM Tris buffer, 50 mM NaCl, to be published). This indicates that the complex is not protected from the aqueous environment while interacting with the polynucleotide. The same conclusion has been drawn by Mei et al. [26] for Ru(TMP)$_3^{2+}$ (TMP = 3,4,7,8-tetramethyl-1,10-phenantroline) in the presence of DNA. We have observed recently that Ru(phen)$_2$HAT^{2+} (and Ru(bpy)$_2$HAT^{2+}; bpy = 2,2′-bipyridine) induces a relative viscosity increase of DNA solutions (to be published). This result and luminescence data [23–25] suggest that Ru(phen)$_2$HAT^{2+} interacts by intercalation. For Ru(phen)$_3^{2+}$, luminescence intensities and lifetimes measurements [8,27] indicated protection of the complex inside the DNA double helix, suggesting that the compound is not only located in the ionic atmosphere. Moreover, DNA unwinding measurements [8,9] indicated the presence of intercalation. However, viscosity experiments [2,3] have recently shown that the complex does not produce an extension of the DNA helix, as expected for 'ideal' intercalators. It has therefore been concluded that Ru(phen)$_3^{2+}$ could intercalate 'partially' producing a DNA kinking or bending without elongation.

2. Theoretical aspects

The sodium-23 nucleus possesses a spin 3/2 and its magnetic relaxation is dominated by the quadrupolar mechanism which results from the interaction between the nuclear quadrupole moment and the fluctuating electric field gradients which, in the case of the spherical Na^+ cation, are of intermolecular origin. Outside the extreme narrowing conditions the relaxation of sodium-23 is biexponential [28,29]; both the longitudinal and transversal relaxations are theoretically described by two relaxation rates. However, the biexponential behaviour of the spin-lattice relaxation curve is not easily observed and an averaged relaxation rate (R_1) is then defined.

In polyelectrolyte solutions, the ^{23}Na relaxation rates are usually interpreted on the basis of a two-state model; the sodium ions are either in a free state (in the bulk), where the electric field gradients felt by the sodium nucleus are essentially not affected by the presence of the polyelectrolyte, or in a bound state in the vicinity of the polyelectrolyte. The free and bound states are characterized by different intrinsic relaxation rates. If the exchange between these states is fast on the NMR time scale, the observed spin-lattice relaxation rate is given by:

$$R_1 = p_f R_{1f} + p_b R_{1b} = R_{1f} + p_b(R_{1b} - R_{1f}) \qquad (1)$$

where R_{1f} and R_{1b} are the ^{23}Na longitudinal relaxation rates in the free and bound state respectively ($R_{1b} > R_{1f}$) and where p_f and p_b are the average fractions of free and bound sodium ions.

The decrease in the observed relaxation rate upon addition of a cationic species to a solution of NaDNA may be due to a lowering of the relaxation rate in the bound state and/or to a change in the distribution of sodium ions in favour of the free state (a release of sodium ions into the bulk). If electrostatic interactions dominate the association of the competing cation with DNA, the decrease in R_1 is primarily due to ion-exchange reactions. In the framework of the two-state model, the effect of a competing Z-valent ion, I^{Z+}, on the extent of sodium binding can be expressed as [14]:

$$R_1 = R_{1f} + (a^\circ - np_1 r)\frac{[P]}{[Na^+]}(R_{1b} - R_{1f}) \qquad (2)$$

where a° is the ratio $[Na^+]_b/[P]$ in the absence of the competing species, n is the number of sodium ions released from DNA by the association of a competing Z-valent ion, p_1 is the ratio $[I^{Z+}]_b/[I^{Z+}]$, r is the ratio $[I^{Z+}]/[P]$, [P] is the concentration of DNA phosphate, and $[Na^+]_b$, $[I^{Z+}]_b$, $[Na^+]$, $[I^{Z+}]$ are the bound and total concentration of Na^+ and I^{Z+}. By assumption R_{1b} is not affected by the ion-exchange processes and ΔR_1, the normalized enhancement of R_1 with respect to the free relaxation rate, can be written as:

$$\Delta R_1 = \frac{R_1 - R_{1f}}{R_1^\circ - R_{1f}} = 1 - \frac{np_1}{a^\circ} r \qquad (3)$$

where R_1° is the ^{23}Na longitudinal relaxation rate in the NaDNA solution before titration. At the initial stage of the titration, n and p_1 can be considered as constants and Eq. (3) predicts a linear dependence of ΔR_1 on the extent of the titration (r). If the linear fit to the initial portion of the titration curve is extented to intersect the horizontal line $\Delta R_1 = 0$ ($R_1 = R_{1f}$), it follows from Eq. (3) that the abscissa of the point of intersection is a°/np_1. For helical B-DNA in dilute solutions and in presence of an excess of uni–univalent electrolyte, the counterion-condensation hypothesis of Manning [30] predicts that $a^\circ = 0.76$ and $n = Z$ (the maximum value of n for a Z-valent competitor). If specific interactions are involved in the association of the competing cation with DNA, the charge density of the double helix may be affected. Intercalation of a cationic species reduces this charge density through an increase in the average phosphate to phosphate distance and by neutralizing some of the anionic charges of DNA [18–20]. It is known that both R_{1b} and p_b (Eq. 1) depend on the charge density of the double helix [30]. Therefore, the decrease in the observed relaxation rate is expected to be more important if intercalation occurs. In such a case, analysis of the initial portion of the titration curve by means of Eq. (3) gives an apparent value of n greater than Z.

3. Experimental

Calf thymus DNA, NaCl, $Mg(ClO_4)_2$ and ethidium bromide of guaranteed purity were purchased from Sigma and used without any further purification. The chloride salts of the Ru^{II} complexes were synthesized and purified as described previously [22–24]. 0.4 mg/ml DNA was dissolved in a pH 7.8 aqueous solution containing 0.1 M NaCl and 0.01 M tris(hydroxymethyl)aminomethane. After dissolution (48 h) the solution was sonicated for 8 h at 4°C with bursts of 2 s duration separated by 15 s pauses to avoid overheating the solution. After dialysis at 4°C (as described by Bleam et al. [14]) the DNA solution was freeze-dried. Stock solutions, approximately 3 mM and 6 mM in DNA phosphate, were prepared for NMR analysis in distilled water containing 20% D_2O. DNA phosphate concentrations, [P], were determined spectrophotometrically at 260 nm (ϵ_{260} = 6600 M^{-1} cm^{-1}). Sodium concentrations were measured from the integral of the ^{23}Na NMR signal by comparison with an external standard containing an aqueous NaCl solution of known concentration with a shift reagent (dysprosium(III) tripolyphosphate). The titration experiments were carried out in 10-mm NMR tubes by adding successive aliquots of a concentrated stock solution. The initial volume of the DNA solution was 3 ml; after completion of the titration, the volume increase never exceeded 10%. The ^{23}Na NMR experiments were performed on a Varian VXR-300 spectrometer at a resonance frequency of 79.35 MHz. The temperature was controlled at 25.0 ± 0.5°C. The D_2O resonance was

used for the lock signal. Longitudinal relaxation time measurements were made by the usual inversion-recovery (180°-τ-90°) pulse sequence with 10 to 16 values of τ. The average T_1 values were obtained by a three-parameter non-linear least square fitting procedure.

4. Results and discussion

The effect of $Ru(phen)_3^{2+}$, $Ru(phen)_2HAT^{2+}$, and $Ru(diMeTAP)_3^{2+}$ (present as racemic mixtures) on the ^{23}Na R_1 in DNA solutions was compared to the effect of ethidium and Mg^{2+}. DNA solutions of two different concentrations ([P] = 6.1 mM and 2.6 mM) but of constant sodium to phosphate ratio ($[Na^+]/[P] = 1.2$) were used. We measured ^{23}Na R_1 of 70 ± 2 s^{-1} and 59 ± 1 s^{-1} in the 6.1 mM and 2.6 mM solutions, respectively. A similar effect of [P] on R_1 had already been observed by Hald and Jacobsen [21]. The results of our titration experiments are given in terms of ΔR_1, defined in Eq. (3), using for R_{1f} the R_1 value measured in a 25 mM NaCl aqueous solution containing 20% D_2O: 18.0 s^{-1}. Fig. 1 illustrates that ΔR_1 is, within the experimental precision, independent of [P].

The results of the titration experiments are displayed in Fig. 2. The addition of $Ru(diMeTAP)_3^{2+}$ affects R_1 in a manner similar to the addition of Mg^{2+}. On the other hand, $Ru(phen)_3^{2+}$ and $Ru(phen)_2HAT^{2+}$ decrease R_1 more efficiently than

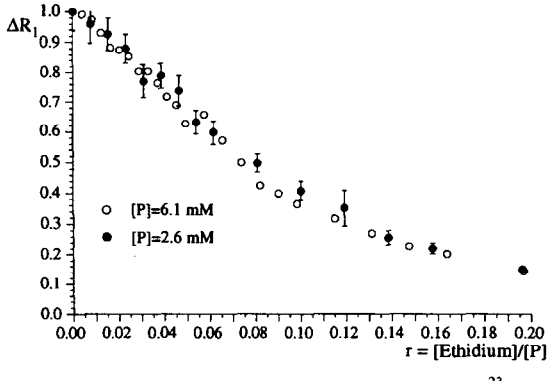

Fig. 1. Decrease of the normalized enhancement of the ^{23}Na R_1 (defined by Eq. 3) upon addition of ethidium bromide in 2.6 mM and 6.1 mM NaDNA solutions. The errors have been estimated from fitting errors on R_1.

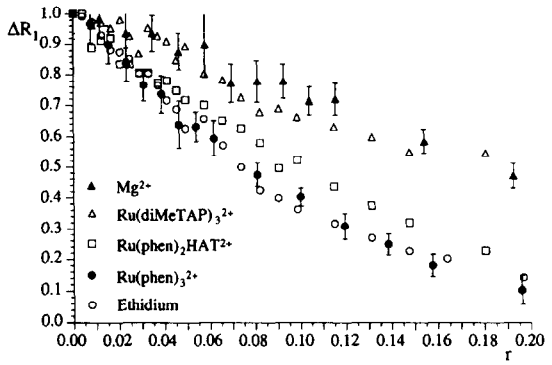

Fig. 2. Decrease of the normalized enhancement of the ^{23}Na R_1 (defined by Eq. 3) upon titrations; r is the ratio of the concentration of the added species to the DNA phosphate concentration. Filled symbols refer to the 2.6 mM DNA phosphate NaDNA solutions while open symbols refer to the 6.1 mM NaDNA solutions. The errors have been estimated from fitting errors on R_1 and are only shown on the 2.6 mM titrations for reasons of clarity.

Mg^{2+}; moreover, the reduction of R_1 upon addition of $Ru(phen)_3^{2+}$ is as important as the effect due to ethidium. The analyses of the initial portion of the titration curves by means of Eq. (3) lead to the data quoted in Table 1, i.e. the values for the slope np_1/a°, the ratio a°/n (given for comparison with previously reported values), and n. For the sodium to phosphate ratio used in these experiments, the p_1 values over the concentration range corresponding to

Table 1
Results of the analyses of the initial portion of the titration curves ($r < 0.07$) according to Eq. (3). The ratios a°/n have been obtained assuming $p_1 = 1$ and n has been estimated with $a^\circ = 0.76$ [30]

Competitor	[P] (mM)	np_1/a°	a°/n	n
Mg^{2+}	2.6	2.9 ± 0.6	0.35 ± 0.07	2.2 ± 0.5
			0.29 ± 0.02 [14]	
			0.26 ± 0.03 [15]	
			0.23 [18]	
$Ru(diMeTAP)_3^{2+}$	6.1	3.1 ± 0.4	0.32 ± 0.04	2.4 ± 0.3
$Ru(phen)_2HAT^{2+}$	6.1	5.1 ± 0.3	0.20 ± 0.01	3.9 ± 0.3
$Ru(phen)_3^{2+}$	2.6	7.0 ± 0.4	0.14 ± 0.01	5.3 ± 0.3
	6.1	6.3 ± 0.3	0.16 ± 0.01	4.8 ± 0.2
Ethidium	2.6	6.6 ± 0.4	0.15 ± 0.01	5.0 ± 0.3
	6.1	6.8 ± 0.3	0.15 ± 0.01	5.2 ± 0.2
			0.11 [18]	

the linear portion of the titration curves can be equated to unity (a p_1 value of 0.94 has been estimated for Mg^{2+} in NaDNA solutions of sodium to phosphate ratio twice as large as in the present case [15]). The ratios a°/n in Table 1 have thus been obtained assuming $p_1 = 1$; n has been estimated using $a^\circ = 0.76$ [30]. For Mg^{2+}, the ratio a°/n obtained in the present work (0.35 ± 0.07) is in agreement with the data from Bleam et al. (0.29 ± 0.02) [14] but significantly larger than the value obtained by Eggert et al. (0.23) [18]. Our result for ethidium (0.15 ± 0.01) is also significantly larger than the value reported by Eggert (0.11) [18]. This disagreement might be due to the determination of the DNA phosphate concentration possibly originating from protein contamination of the starting material. Indeed, the ratios of the a°/n value for Mg^{2+} relative to the corresponding value for ethidium are approximately the same for both sets of data (0.23/0.11 and 0.35/0.15).

For $Ru(diMeTAP)_3^{2+}$ (and Mg^{2+}) n is close to 2. This result indicates that electrostatic interactions with the phosphate backbone dominate the association of $Ru(diMeTAP)_3^{2+}$ with DNA and is in agreement with the conclusion of the luminescence experiments (see the Introduction). As proposed by Mei et al. [26] for $Ru(TMP)_3^{2+}$, the complexes with methyl substituents on each ligand are sterically hindered versus their penetration inside the major or minor grooves of DNA so that they remain in the ionic atmosphere around the double helix. For the titrations with $Ru(phen)_3^{2+}$ and $Ru(phen)_2HAT^{2+}$ (and ethidium) the use of Eq. (3) leads to n values significantly larger than the valence of the competitor. These observations mean that interactions other than purely electrostatic are present between these compounds and DNA; the same conclusion was drawn from luminescence data and viscometry measurements (see the Introduction). Viscometry results suggest that $Ru(phen)_2HAT^{2+}$ intercalates. Indeed the HAT ligand with its extended aromatic plane can easily be inserted into the stacking of bases.

It is not straightforward to conclude from these ^{23}Na R_1 measurements whether intercalation occurs, however. The difficulty essentially lies in the lack of a reliable theoretical model describing the dependence of R_b on the concentration of the added substance; though Jacobsen and co-workers [18] tried to interpret titration experiments with intercalators in order to determine quantities such as helix extension, they recently advised that their results should be interpreted with great care [21]. Moreover, if the helix extension is the most efficient process in decreasing the relaxation rate of the bound sodium ions [18–20], the fact that $Ru(phen)_3^{2+}$ reduces the ^{23}Na R_1 more efficiently than $Ru(phen)_2HAT^{2+}$ is unexpected (cf. Fig. 2 and n values in Table 1). Indeed, viscometry measurements indicate that, unlike $Ru(phen)_2HAT^{2+}$, $Ru(phen)_3^{2+}$ induces a bending of DNA. As explained by Satyanarayana et al. [2,3], this would be due to the fact that the phen ligand, with a smaller aromatic plane that the HAT or DPPZ ligand, cannot really intercalate and therefore induces, via the other ligands, a kink in the DNA helix. For the same reason the striking similarity between the effect on the ^{23}Na R_1 of $Ru(phen)_3^{2+}$ (not a real intercalator) and ethidium (real intercalator) is intriguing. In contrast, if we remember that both species produce very similar degree of unwinding of the DNA double helix [8,9], our results would indicate that processes other than helix extensions, such as the unwinding of the double helix, significantly modify the electric field gradients felt by the sodium nucleus in the vicinity of the polyelectrolyte and thus affect R_1. Therefore, the similarity between the ^{23}Na relaxation results should not be surprising. We propose to further investigate the effect of $Ru(phen)_3^{2+}$ and $Ru(phen)_2HAT^{2+}$ via the measurement of longitudinal and transversal relaxation rates outside the extreme-narrowing conditions. Indeed, these quantities give access to correlation times and quadrupolar coupling constants which could highlight differences between the interaction of ethidium, $Ru(phen)_3^{2+}$ and $Ru(phen)_2HAT^{2+}$ with DNA and establish the real sensitivity of the ^{23}Na relaxation rates to the mode of binding.

In conclusion, the measurements of the ^{23}Na longitudinal relaxation rate of NaDNA solutions constitute a method for the study of the interactions of Ru^{II} complexes with DNA which is as good as most of other existing techniques. Indeed, it allows the distinction between an interaction in the atmosphere of ions around DNA ($Ru(diMeTAP)_3^{2+}$ and Mg^{2+}) and an interaction inside the double helix. However, whether the interaction mode corresponds to intercalation ($Ru(phen)_2HAT^{2+}$ and ethidium) or surface

binding ($Ru(phen)_3^{2+}$) cannot be concluded up to now from this NMR method.

Acknowledgements

M.L., A.K.-D.M., and J.R. thank the 'Communauté Française de Belgique' (ARC 91/96-149) for financial support. M.C., G.S. and A.L. thank the italian M.U.R.S.T. (fondi 40 %) for financial support.

References

[1] E.C. Long and J.K. Barton, Acc. Chem. Res., 23 (1990) 271–273.
[2] S. Satyanarayana, J.C. Dabrowiak and J.B. Chaires, Biochemistry, 31 (1992) 9319–9324.
[3] S. Satyanarayana, J.C. Dabrowiak and J.B. Chaires, Biochemistry, 32 (1993) 2573–2584.
[4] B. Nordén, N. Patel, C. Hiort, A. Gräslund and S.K. Kim, Nucleos. Nucleot., 10 (1991) 195–205.
[5] M. Eriksson, M. Leijon, C. Hiort, B. Nordén and A. Gräslund, J. Am. Chem. Soc., 114 (1992) 4933–4934.
[6] J.P. Rehman and J.K. Barton, Biochemistry, 29 (1990) 1710–1717.
[7] C. Hiort, B. Nordén and A. Rodger, J. Am. Chem. Soc., 112 (990) 1971–1982.
[8] J.K. Barton, A.T. Danishefsky and J.M. Goldberg, J. Am. Chem. Soc., 106 (1984) 2172–2176.
[9] J.M. Kelly, A.B. Tossi, D.J. McConnell and C. Oh Uigin, Nucleic Acids Res., 13 (1985) 6017–6034.
[10] A.E. Friedman, J.C. Chambron, J.P. Sauvage, N.J. Turro and J.K. Barton, J. Am. Chem. Soc., 112 (1990) 4960–4962.
[11] A.E. Friedman, C.V. Kumar, N.J. Turro and J.K. Barton, Nucleic Acids Res., 19 (1991) 2595–2602.
[12] C. Hiort, P. Lincoln and B. Nordén, J. Am. Chem. Soc., 115 (1993) 3448–3454.
[13] M.L. Bleam, C.F. Anderson and M.T. Record Jr., Proc. Natl. Acad. Sci. USA, 77 (1980) 3085–3089.
[14] M.L. Bleam, C.F. Anderson and M.T. Record Jr., Biochemistry, 22 (1983) 5418–5425.
[15] W.H. Braunlin, C.F. Anderson and M.T. Record Jr., Biopolymers, 25 (1986) 205–214.
[16] W.H. Braunlin, C.F. Anderson and M.T. Record Jr., Biochemistry, 26 (1987) 7724–7731.
[17] Y.H. Mariam and W.D. Wilson, J. Am. Chem. Soc., 105 (1983) 627–628.
[18] H. Eggert, J. Dinesen and J.P. Jacobsen, Biochemistry, 28 (1989) 3332–3337.
[19] J. Dinesen, J.P. Jacobsen, F.P. Hansen, E.B. Pedersen and H. Eggert, J. Med. Chem., 33 (1990) 93–97.
[20] M. Hald and J.P. Jacobsen, Biophys. Chem., 41 (1991) 113–124.
[21] M. Hald and J.P. Jacobsen, Chem. Phys., 159 (1992) 257–267.
[22] A. Kirsch-De Mesmaeker, D. Maetens and R. Nasielski-Hinkens, J. Electroanal. Chem., 182 (1985) 123–132.
[23] A. Kirsch-De Mesmaeker, G. Orellana, J.K. Barton and N. Turro, Photochem. Photobiol., 52 (1990) 461–472.
[24] L. Jacquet and A. Kirsch-De Mesmaeker, J. Chem. Soc. Faraday Trans., 88 (1992) 2471–2480.
[25] F. de Buyl, A. Kirsch-De Mesmaeker, A.B. Tossi and J.M. Kelly, J. Photochem. Photobiol. A, Chem., 60 (1991) 27–45.
[26] H.-Y. Mei and J.K. Barton, Proc. Natl. Acad. Sci. USA, 85 (1988) 1339–1343.
[27] J.K. Barton, J.M. Goldberg, C.V. Kumar and N.J. Turro, J. Am. Chem. Soc., 108 (1986) 2081–2088.
[28] P.S. Hubbard, J. Chem. Phys., 53 (1970) 985–987.
[29] T.E. Bull, J. Magn. Resonance, 8 (1972) 344–353.
[30] G.S. Manning, Q. Rev. Biophys., 11 (1978) 179–246.