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Phosphodiesterolytic activity of alkaline-earth cations in aqueous DMSO†

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Rate of the hydrolysis of bis(*p*-nitrophenyl) phosphate in 90% v/v aqueous DMSO increases 10^3–10^5 times in the presence of 1–2 mM alkaline-earth cations, which in the same concentration range do not affect the reaction rate in water.

Current efforts in the development of chemical nucleases are mostly concentrated on lanthanide and transition metal complexes.[1] Alkaline-earth cations among which Mg^{2+} and Ca^{2+} are frequent components of active sites of natural nuclease and phosphatase enzymes[2] show by themselves very low if any phosphoesterase activity,[3–5] although recently larger accelerations by Mg^{2+} in the hydrolysis of oligoribonucleotides[6] and Co(III)-bound ATP[7] were reported. An important question is therefore what factors make these cations so efficient in biological systems. Recently, fairly strong alkali metal[8] and strontium[9] ion catalysis in the cleavage of respectively phosphonate/phosphate and carboxylic acid esters in ethanol was described. These findings indicate the suitability of non-aqueous media, which imitate the apolar microenvironment of enzyme active sites, for improvement of catalysis by s-block cations. In this paper we report a dramatic increase in the phosphodiesterolytic activity of alkaline-earth cations in DMSO solvent with low water content. The speciation and preliminary mechanistic results are provided for the most active Sr^{2+} cation.

Kinetic studies were performed with often used model substrate bis(*p*-nitrophenyl) phosphate (BNPP), monitoring its hydrolysis spectrophotometrically by the appearance of *p*-nitrophenolate anion.‡ The rate of alkaline hydrolysis of BNPP initially decreases on increase in DMSO content but then sharply increases in the range 80–90% v/v DMSO. All further experiments were performed in 90% v/v DMSO. The rate of BNPP hydrolysis in this medium was *ca*. 20 times higher with NaOH than with $(n\text{-Bu})_4NOH$ base, Table 1. Thus, the rate increase was *ca*. 3-fold with $(n\text{-Bu})_4NOH$ and *ca*. 60-fold with NaOH in comparison with that in water.§ In accordance with this, addition of NaCl in the presence of $(n\text{-Bu})_4NOH$ strongly accelerated the reaction, Table 1. Addition of LiCl and KCl produced much smaller accelerations (see Supplementary Information, Fig. 1S†).

Salts of alkaline-earth metals produced large rate enhancements. The largest catalytic effect was observed with Sr(II): in the presence of 1 mM $SrCl_2$ the rate of hydrolysis by $(n\text{-Bu})_4NOH$ increases by a factor of 10^5 (*cf*. lines 1 and 12 in Table 1). The efficiency of Sr(II) with NaOH was somewhat lower. Also large accelerations were observed with Mg(II), but in this case the catalytic activity was much higher with NaOH or $(n\text{-Bu})_4NOH$ + NaCl than with $(n\text{-Bu})_4NOH$ alone (Table 1, lines 7–9). The catalytic activity of Ca(II) was lower than that for Sr(II) and Mg(II) (Table 1, lines 10 and 11), but the reaction rate was still 10^3 times higher in the presence of $CaCl_2$ than in the presence of equal amount of free hydroxide. Studies with $BaCl_2$ were limited by low solubility of $Ba(OH)_2$ in the mixed solvent, but qualitatively the activity of Ba(II) was close to that of Ca(II). In water all these cations at similar metal and base concentrations did not affect the reaction rate.

Rate-concentration profiles were studied in detail for Na^+, Mg^{2+} and Sr^{2+} cations. Additions of increased amounts of NaCl up to 0.06 M produced monotonic linear increase in k_{obs} (Fig.1S†), but both with $MgCl_2$ and $SrCl_2$ sharp rate maxima were observed at the metal-to-base ratios between 1:1 and 1:2. Similarly, on varying the base concentration at a fixed metal salt concentration we observed plots with maxima. A typical behavior is illustrated in Fig. 1 for $SrCl_2$ (results for $MgCl_2$ and $CaCl_2$ are shown in Figs. 2S and 3S†).

The existence of optimum metal/base ratios indicates that besides an active metal hydroxo complex other inactive species of different stoichiometries are formed in the solution. To obtain an information on composition and stability of metal hydroxo complexes we performed potentiometric titrations of 1–2 mM $SrCl_2$ and $MgCl_2$ by $(n\text{-Bu})_4NOH$ in 90% v/v DMSO (titration curves are shown in Supplementary Information, Figs. 4S and 5S†).¶ First, titrations of dilute HCl solutions were performed for the electrode calibration and from these results the apparent autoprotolysis constant $pK_{w,app}$ = 21.3 ± 0.2 was obtained in a reasonable agreement with published values in water–DMSO

Table 1 Selected kinetic results for the hydrolysis of BNPP in 90% v/v DMSO at 25 °C

	$(n\text{-Bu})_4$-NOH mM	NaOH mM	NaCl mM	$MgCl_2$ mM	$CaCl_2$ mM	$SrCl_2$ mM	k_{obs}, s^{-1}
1	5						4.5×10^{-8}
2	30						5.1×10^{-7}
3		5					1.7×10^{-6}
4		30					1.1×10^{-5}
5	30		5				2.0×10^{-6}
6	30		30				1.3×10^{-5}
7	5			2			1.7×10^{-5}
8		5		2			1.0×10^{-3}
9	5		5	2			8.4×10^{-4}
10	2				1		6.0×10^{-5}
11		2			1		3.9×10^{-5}
12	2					1	1.0×10^{-3}
13		2				1	4.9×10^{-4}

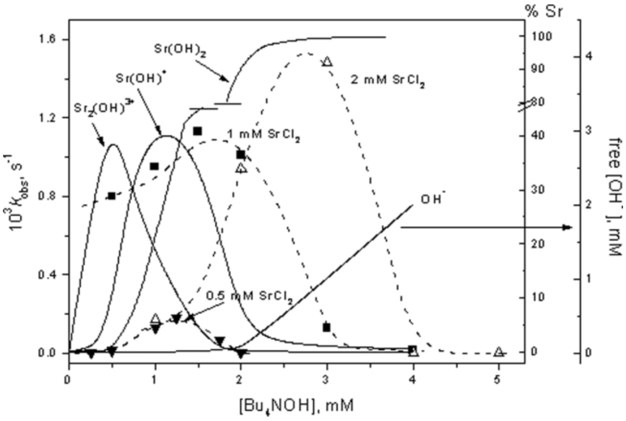

Fig. 1 Observed first-order rate constants for the BNPP hydrolysis at 25 °C in 90% v/v DMSO in the presence of increased amounts of $SrCl_2$ *vs*. the base concentration concentration and the species distribution diagram for hydroxo-complexes of Sr(II) (dashed lines) calculated in accordance with eqns. (1)–(3) for 1 mM $SrCl_2$.

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† Electronic supplementary information (ESI) available: Figs. S1–S5. See http://www.rsc.org/suppdata/cc/b4/b402432j/

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mixtures.[10] Titration results for $SrCl_2$ required for the best fit a set of the following equilibria (uncertainty in $\log\beta \pm 0.05$):

$$2\,Sr^{2+} \rightleftharpoons Sr_2OH^{3+} + H^+,\ \log\beta_{2\text{-}1} = -9.85 \tag{1}$$

$$Sr^{2+} \rightleftharpoons SrOH^+ + H^+,\ \log\beta_{1\text{-}1} = -14.65 \tag{2}$$

$$Sr^{2+} \rightleftharpoons Sr(OH)_2 + 2\,H^+,\ \log\beta_{1\text{-}2} = -31.31 \tag{3}$$

The species distribution diagram calculated in accordance with eqns. (1)–(3) is superimposed with the kinetic results for 1 mM $SrCl_2$ in Fig. 1. The experimental points (solid squares) follow the curve for $SrOH^+$. The curve for free OH^- shows that at optimum metal/base ratios the concentration of free OH^- is very low. Thus, the fact that these systems use added alkali does not mean that the reactivity is observed in strongly basic conditions. In fact, addition of the metal ion completely converts free OH^- into hydroxo-complexes and the observed increase in reactivity by several orders of magnitude indicates that the metal hydroxo-complex reacts with BNPP much faster than the free hydroxide.

Rate constants measured in the range of 0.5–1.5 mM Sr(II) and 0.5–5.0 mM $(n\text{-}Bu)_4NOH$ were correlated with calculated in accordance with eqns. (1)–(3) concentrations of all three hydroxo species and contributions of Sr_2OH^{3+} and $Sr(OH)_2$ appeared to be negligible. The dependence of k_{obs} on $[SrOH^+]$ shown in Fig. 2 was of the Michaelis–Menten type:

$$k_{obs} = k_cK_S[SrOH^+]/(1 + K_S[SrOH^+]) \tag{4}$$

with $k_c = (1.2 \pm 0.2) \times 10^{-3}\ s^{-1}$ and $K_S = (4.6 \pm 1.9) \times 10^3\ M^{-1}$. The reaction proceeds therefore *via* the following steps:

$$Sr^{2}+OH^{-} \underset{}{\overset{K_{OH}}{\rightleftharpoons}} Sr(OH)^{+}, Sr(OH)^{+} + BNPP^{-} \overset{K_S}{\rightleftharpoons} Sr(OH)(BNPP) \xrightarrow{k_c} \text{hydrolysis products} \tag{5}$$

Similar mechanism operates also for metal ion catalysis in water,[1] but for alkaline earth cations both K_{OH} and K_S are very small, *e.g.* for Sr^{2+} $K_{OH} = 6.3\ M^{-1}$[11] and $K_S \approx 1\ M^{-1}$.‖ Estimated with these association constants value of k_{obs} in water (assuming the same value of k_c) for a mixture of 1 mM Sr^{2+} and 2 mM OH^- equals $1.5 \times 10^{-8}\ s^{-1}$ (essentially coincides with k_{obs} without metal ion) instead of $1.0 \times 10^{-3}\ s^{-1}$ in 90% DMSO (Table 1). This indicates that the major factor responsible for larger catalytic activity in DMSO is strongly increased affinity of the reactants to the catalyst providing much higher efficiency of the template effect of the metal ion.

Potentiometric titrations of $MgCl_2$ revealed formation of a single species:

$$Mg^{2+} \rightleftharpoons Mg(OH)_2 + 2\,H^+,\ \log\beta_{1\text{-}2} = -21.86 \tag{6}$$

As one can see from Table 1, Mg(II) in the presence of $(n\text{-}Bu)_4NOH$ is *ca.* 100 times less active than Sr(II). Since the activity of $Sr(OH)_2$ is also low one may conclude that the neutral hydroxo species are generally low active. Titration of $MgCl_2$ in the presence of added NaCl showed a complex behavior (see Supplementary Information†) and did not allow us to analyze the system quantitatively, but it clearly showed formation of mixed hydroxo complexes $MgNa(OH)_2^+$ and $MgNa(OH)_3$, one of which may be responsible for the increased catalytic activity in the presence of sodium cations.

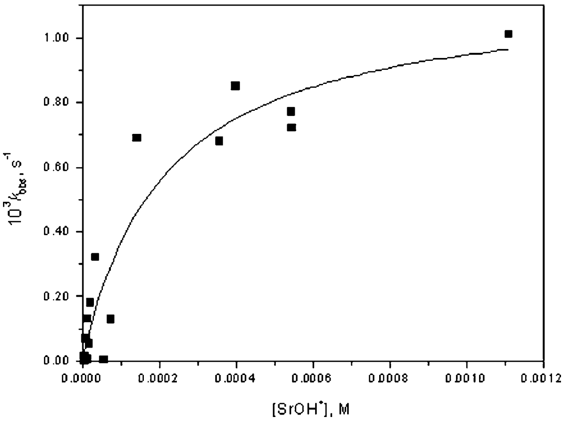

Fig. 2 Plot of k_{obs} *vs.* the concentration of $SrOH^+$ species calculated in accordance with eqns. (1)–(3).

In conclusion, the use of a dipolar aprotic co-solvent allows one to greatly improve the catalytic activity of alkaline-earth cations in the phosphodiester hydrolysis. The activity of lanthanides decreases in water–DMSO mixtures,[12] but rate constants for the BNPP hydrolysis with simple hydroxo complexes of Sr(II) and Mg(II) in 90% v/v DMSO are even higher than those reported for lanthanide aqua-ions in water.[1a,b,13] This result indicates the important role of a medium of low water content, which allows rather weak Lewis acids like the alkaline-earth cations to activate efficiently the substrate and the nucleophile.

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Notes and references

‡ Kinetic measurements used 20–100 μM BNPP and varied concentrations of metal chlorides and $(n\text{-}Bu)_4NOH$ or NaOH in the range 1–5 mM at 25 °C.

§ The positive solvent effect of DMSO on the rate of alkaline hydrolysis of BNPP is much lower than that on the hydrolysis of the dianion of *p*-nitrophenyl phosphate,[14] which proceeds *via* a dissociative-type mechanism.

¶ Measurements of pH were taken on an Orion Model 710-A research digital pH meter equipped with an Orion standard research grade combination electrode with Ag/AgCl reference cell as carbonate-free $(n\text{-}Bu)_4NOH$ 0.1 M solution in 90% v/v DMSO was added to the system in small increments and with sufficient time intervals (15–20 min between additions in pH range 10–16, but only 2–3 min in more acidic and more basic solutions) to allow the equilibration of the system and obtaining stable pH readings. Careful removal of carbonate and exclusion of CO_2 absorption during the titration were extremely important for obtaining reproducible titration curves. In all titrations 1 mM HCl was added to ensure that at the beginning no hydroxide complexes of alkaline-earth cations were present and to check additionally the electrode calibration in each run. No supporting electrolyte was employed to minimize the ion-pairing of divalent cations with electrolyte anions, which can be significant in this medium. The program Hyperquad[15] 2000 Version 2.1 NT was used to calculate all equilibrium constants.

‖ The stability constant of the complex of Sr^{2+} with $H_2PO_4^-$, a monoanion similar by charge and type of donor atoms to BNPP, equals $2\ M^{-1}$,[11] but for binding to $Sr(OH)^+$ one should expect a smaller value.

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