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Invited Paper

Temperature and Solvent Effects on the Europium(III)-catalyzed Hydrolysis of Bis-(nitrophenyl) Phosphate

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Dedicated to Professor Eiichi Kimura

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The hydrolysis of bis-(nitrophenyl) phosphate (BNPP) as model for secondary phosphate esters is analyzed at six different concentrations of Eu(III) ions and four temperatures between 303 and 343 K. Eyring plots yield activation enthalpy parameters which with, e.g. [Eu^{3+}] between 0.40 and 10.0 mM drop from 130 to 74 kJ mol^{-1}, respectively, with a relatively small drop in the opposing entropy contributions. The observed saturation profiles allow for the first time to evaluate the influence of ΔH and ΔS separately on the Michaelis–Menten values K_M and k_{cat}, showing that the catalytic metal-ion effects are largely due to changes in k_{cat} and dominated by favorable ΔH changes. Preliminary studies of the solvent influence show a surprising difference between water mixtures with either ethanol or DMSO. With, e.g. 40% ethanol, one observes a doubling of the rate constant, with 40% DMSO an almost 10-fold rate decrease. In both cases, a linear correlation with the solvent polarity parameter E_T is found.

Keywords: Hydrolysis; Lanthanide; Catalysis; Parameters

INTRODUCTION

In recent years, the development of artificial enzymes for the hydrolytic cleavage of nucleic acids has received much attention [1–8]. The most often-studied model reaction is the hydrolysis of the activated secondary ester bis-(nitrophenyl) phosphate (BNPP), which is not only convenient to follow by liberation of nitrophenolate, but is also of interest in view of its resemblance to biocidic phosphates, including pesticides and chemical weapons. The results obtained with lanthanide-catalyzed hydrolysis of BNPP are often found to parallel those with, e.g. plasmid DNA cleavage. In this context, it is remarkable that the absence of nitrogoups in the less activated bis(phenyl) phosphate ester leads to even slightly higher catalytic efficiency of Eu(III) salts [9,10], pointing once more to the suitability of BNPP as model for the extremely stable dialkyl ester present in nucleic acids.

Lanthanide catalysis of ester hydrolysis must be seen in the context of many investigations on artificial metallo-hydrolases, for which significant insight is available already from several reviews [11–14]. It is, however, surprising that even with the longer-known metal complexes comprising, e.g. Cu or Zn ions relatively few studies have been devoted to temperature and solvent effects of such reactions. Such studies are of obvious practical importance in view of the variable temperatures often necessary for such measurements, as well as of the need to use mixed organic solvents due to solubility problems. In addition, the data obtained from temperature and medium effects can shed light on the mechanistic origin of catalytic efficiency, including those in metallo-enzymes for which the possible range of temperature and solvent is obviously much more limited than with the more stable artificial analogues.

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TABLE I Rate constants k_{obs} (in $10^7 s^{-1}$ units) at different temperatures (in K) and catalyst concentrations ($[Eu^{3+}]$ in 10^4 M units) in water*

$[Eu^{3+}]$	k at 303 K	k at 323 K	k at 333 K	k at 343 K
4.0	2.7†	92	423	1040
8.0	16.9	200	701	1860
16	52.7	480	1100	3150
32	73.9	636	1520	3700
64	110	949	1840	4100
100	125	1090	2070	4120

* k from nonlinear least square fits of double runs(except for †, linear fitting); reactions followed usually over at least one half life times; correlation coefficient $r > 0.998$ unless noted otherwise. † $r = 0.987$; standard error in k (deviation between two measurements ±2%).

The extremely slow rates of secondary phosphate ester hydrolysis at neutral pH are characterized by activation enthalpies ΔH reaching, e.g. 130 kJ/mol [15,16]. The activation entropy is also quite unfavorable, in contrast to monoesters [17] which in the case of monoanions react with a proton transfer to the leaving group and subsequent charge delocalization. This then can lead to significant rate increases with less polar solvents, reaching several orders of magnitudes with corresponding thioesters [16]. Strongly negative ΔS values have also been found for the hydrolysis of triesters with special Zn(II) complexes [18]. Lower activation parameters were as expected observed at lower and higher pH, in particular with cyclic phosphates [19,20], but are difficult to compare with data under physiological conditions at pH 7. The catalysis of triester hydrolysis by imidazole has been found in some cases to be essentially due to more favorable entropies [21].

RESULTS

Hydrolysis of BNPP was followed at pH 7.0 as described before [22,23], with perfect pseudofirst order non-linear fitting over usually at least one half-life time (Table I). In the case of very slow reaction, linear fitting based on known end absorbancies of the liberated nitrophenolate was applied, with the end absorbance measured independently in the used solvent. Eyring plots were linear over the observed temperature range from 303 to 343 K (Fig. 1).

The observed temperature effects on the rates can be represented in different ways. The traditional interpretation with Eyring plots for each catalyst concentration is shown in Fig. 2, the results (see also Table II) show, that the rate acceleration by lanthanide is essentially due to lowered ΔH values, with smaller changes in $T\Delta S$. A plot of $T\Delta S$ vs. ΔH (Fig. 3) is quite linear, which, however, should not be over-interpreted in view of the known systematical errors [24,25] in such correlations. This is particularly so in enzyme- or enzyme-analog catalysis, where the observed apparent rate constants are composites of k_{cat} and K_M factors.

Also for this reason a more meaningful, but perhaps less practical representation is based on saturation kinetics at the different temperatures, which allowed us to extract for the first time ΔH and ΔS separately for k_{cat} and K_M. At all temperatures, good fitting to the Michealis–Menton equation was observed (Fig. 4), yielding pre-transition state equilibrium K_M values between Eu^{3+} and the BNPP around mM (Table III), in accordance with our earlier results. The k_{cat} values show a satisfactory fit with the

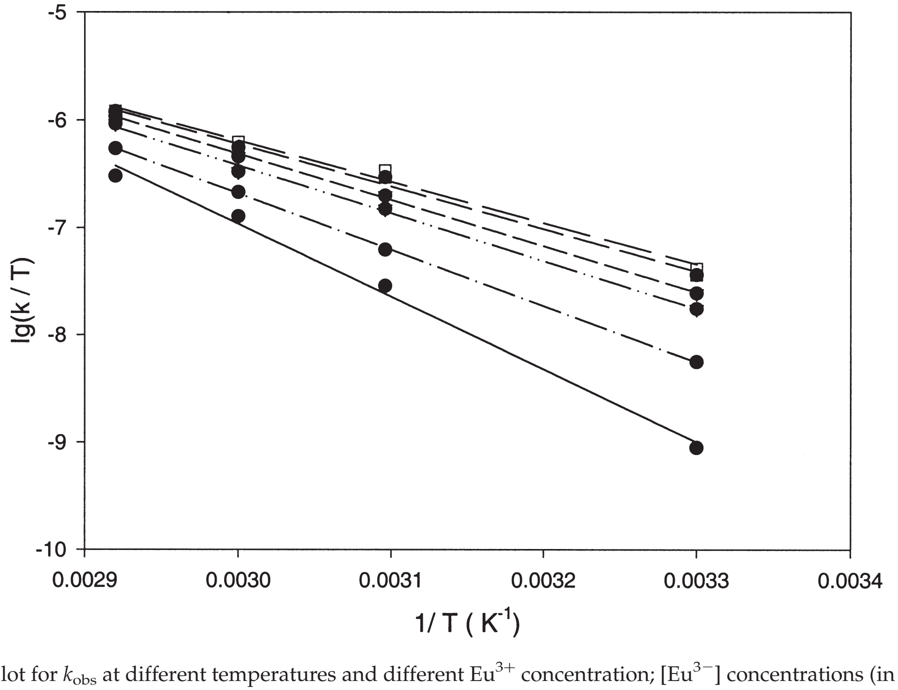

FIGURE 1 Eyring plot for k_{obs} at different temperatures and different Eu^{3+} concentration; $[Eu^{3-}]$ concentrations (in $\times 10^4$ M units) from bottom to top: 4.0; 8.0; 16.0; 32.0; 64.0; 100.0.

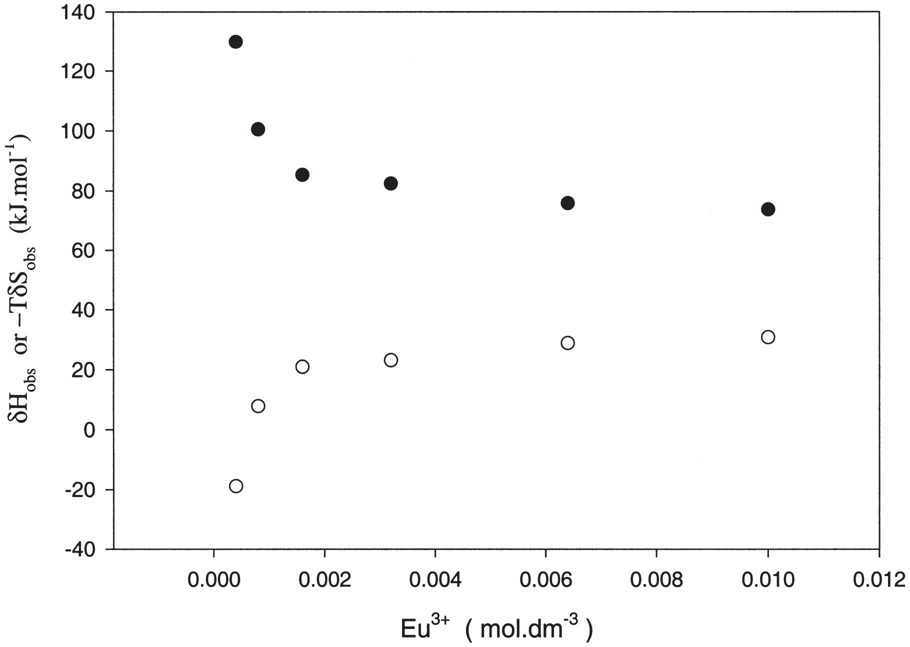

FIGURE 2 Activation enthalpy (ΔH_{obs}, in kJ mol^{-1}) and entropy term ($-T\Delta S_{obs}$, in kJ mol^{-1}) contributions as function of catalyst concentration at 323 K. (filled circles ΔH_{obs}, open circles $-T\Delta S_{obs}$).

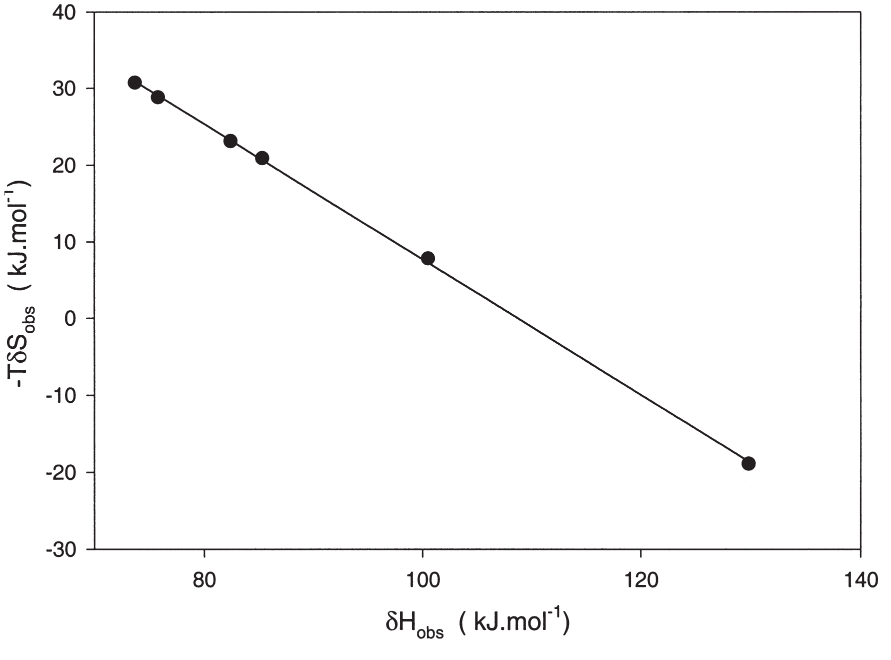

FIGURE 3 Compensation between $-T\Delta S$ and ΔH; ($-T\Delta S = 95.9 - 0.882\Delta H$, correlation coefficient $r = 0.9998$).

TABLE II Activation parameters for different catalyst Eu^{3+} concentrations*

$[Eu^{3+}] \times 10^4$ M	ΔH (kJ mol^{-1})	$-\Delta S$ (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})	$T\Delta S$ (kJ mol^{-1})	r
4.0	129.8	58.4	−18.9	110.9	0.994
8.0	100.5	−24.2	7.8	108.3	0.999
16.0	85.3	−64.7	20.9	106.2	0.997
32.0	82.4	−71.4	23.1	105.5	0.999
64.0	75.8	−89.3	28.8	104.6	0.994
100.0	73.7	−95.1	30.7	104.4	0.991

* From data in Table I and Eyring plots; r, linear correlation coefficient; $T\Delta S$ calc. for 323 K.

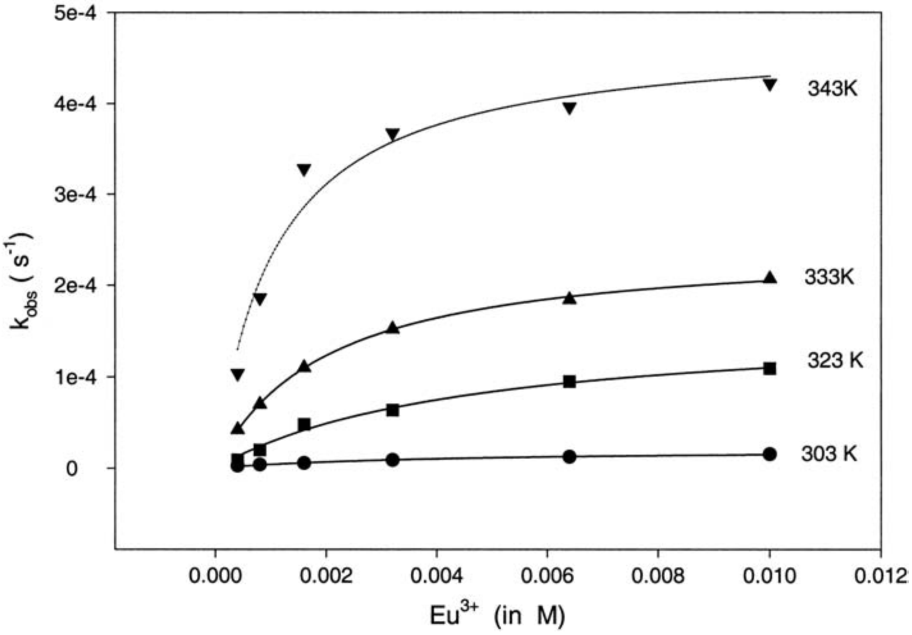

FIGURE 4 Saturation curves at different temperatures and different catalyst concentrations.

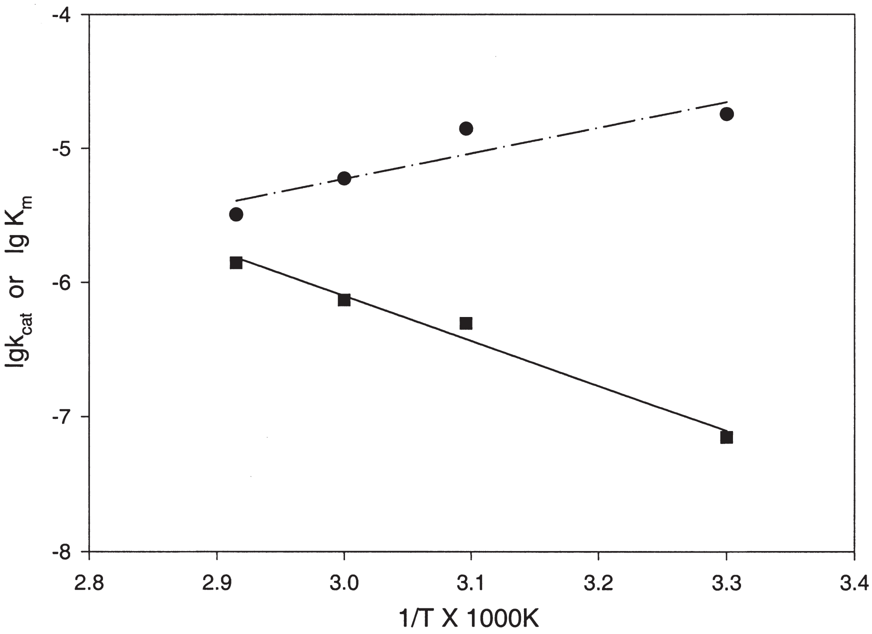

FIGURE 5 Eyring plot for k_{cat}, van't Hoff plot for K_M; circles, K_M; squares, k_{cat}.

TABLE III Michealis–Menton K_M and k_{cat} values at different temperatures*

T (K)	k_{cat}	K_M	R
303	2.12	4.25	0.993
323	15.94	4.52	0.990
333	24.51	1.98	0.960
343	47.78	1.09	0.971

* From saturation curves (Fig. 4); R, regression coefficient; k_{cat} in $(s^{-1}) \times 10^5$; K_M in $\times 10^3 M^{-1}$ units.

Eyring equation, as do the K_M values with the van't Hoff equation (Fig. 5). The resulting parameters (Table IV) show, that the major catalytic effect of Eu^{3+} is due to a lowered free activation enthalpy ΔH, partially compensated by a considerable entropic disadvantage. The K_M values decrease with the temperature, but only to a smaller degree (Table III). In view of the different reaction mechanism of the uncatalyzed and the metal-catalyzed reaction, and of course the absence of pre-transition state complexes in the normal reaction it difficult to draw parallels.

TABLE V Hydrolysis rate constants measured in EtOH–H$_2$O or DMSO–H$_2$O mixtures*

EtOH–H$_2$O system				DMSO–H$_2$O system			
EtOH%	$E_T(30)$	k (s^{-1}) × 10^5	r	DMSO%	$E_T(30)$	k (s^{-1}) × 10^5	r
0	63.1	7.17	0.999	0	63.1	10.5	0.998
10	61.6	8.60	0.998	10	61.3	6.80	0.998
20	60.5	10.1	0.992	20	59.5	3.50	0.999
30	59.4	11.7	0.999	40	55.9	1.25	0.990
40	58.3	14.1	0.996				

* Solvent polarity values $E_T(30)$ from Reichardt, C. (1978) *Solvent Effects in Organic Chemistry*, Verlag Chemie, Weinheim, New York. $E_T(30)$ values for DMSO–H$_2$O from linear extrapolations between 0% and 100% DMSO.

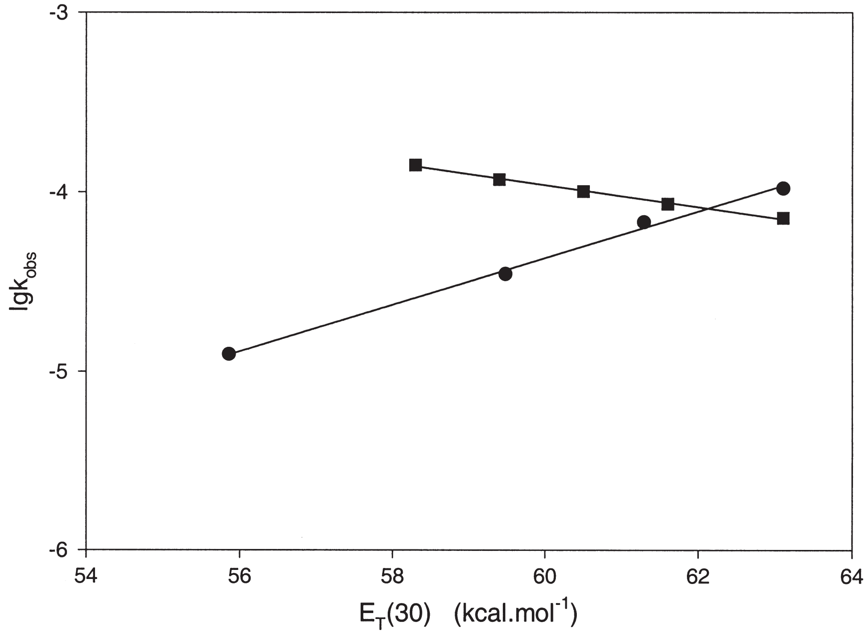

FIGURE 6 Rate constants (lg$k$) as function of solvent polarity ($E_T(30)$ values, see Table V); EtOH–H$_2$O, cicles; DMSO–H$_2$O, squares. (linear fit of lgk_{obs} *vs.* $E_T(30)$: lgk_{obs}(EtOH–H$_2$O) $= -0.061E_T - 0.29$, correlation coefficient $r = 0.998$; lgk_{obs}(DMSO–H$_2$O) $= 0.13E_T - 12.2$, with $r = 0.998$).

One can, however, see the role of the metal ion by the observed rate changes with increasing temperatures in presence of different Eu^{+3} concentrations (see Table I, Fig. 4).

Solvent effects were investigated with binary water mixtures containing up to 40% ethanol or DMSO. Particularly with percentages of DMSO above 40% the necessary pH control turned out to be difficult, for which reason we restricted these preliminary medium effect studies to solutions containing at least around 60% water, with higher buffer concentration (0.1 M) for the more difficult to handle DMSO mixtures. The observed rate constants showed a small increase with ethanol, reminiscent of results with monoesters [16,17], but a surprising opposite behavior with DMSO mixtures; here we observe a rate constant decrease by one order of magnitude with 40% organic solvent. Further investigations at different catalyst concentrations, pH values and possibly temperatures will be

TABLE IV Activation parameters for k_{cat}, K_M and the uncatalyzed reaction*

For k_{cat}	For K_M	uncatalyzed reaction
$\Delta H^{\neq} = 78.2$ kJ.mol^{-1}	$\Delta H = -28.3$ kJ.mol^{-1}	$\Delta H^{\neq} = 103.7$ kJ.mol^{-1}
$\Delta S^{\neq} = -80.1$ e.u.	$\Delta S = -136.9$ e.u.	$\Delta S^{\neq} = -107$ e.u.
$-T\Delta S^{\neq} = 23.9$ kJ.mol^{-1}	$-T\Delta S = 40.8$ kJ.mol^{-1}	$T\Delta S^{\neq} = 34.6$ kJ.mol^{-1}
$\Delta G^{\neq} = 102.1$ kJ.mol^{-1}	$\Delta G = 12.5$ kJ.mol^{-1}	$\Delta G^{\neq} = 69.1$ kJ.mol^{-1}

* From nonlinear fitting (Fig. 5); coefficient $r > 0.99$ for both of K_M and k_{cat}; errors in ΔH: ± 15%; in ΔS and TΔS: ± 20% on the average; values for uncatalyzed reaction after data from literature, see text.

necessary to elucidate the origin of this opposing solvent effects.

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