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RESEARCH ARTICLE

Increased efficiency in biomimetic Lewis acid–base pair catalyzed monoacylation of diols by acyl phosphate monoesters

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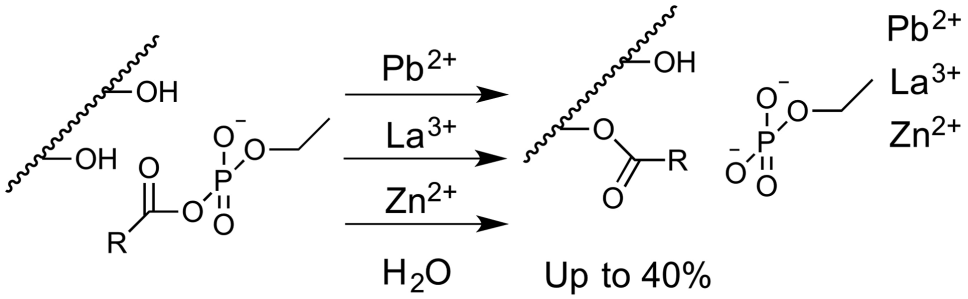
Abstract

Acyl phosphate monoesters are biomimetic acylation reagents that require coordination to metal ions to react with *cis*-diol substrates in water. With lanthanide catalysts, outcomes are compromised by (1) the competitive lanthanide-promoted hydrolysis of the acyl phosphate reagents as well as by (2) the high affinity of lanthanum ions for the phosphate monoester by-product. Based on analysis of the mechanism of the process, optimizing reaction conditions can selectively inhibit the lanthanum-promoted hydrolysis of acyl phosphate monoesters. Furthermore, using zinc salts and lead salts in place of lanthanides enhances the reactivity of the reactants and causes less complexation of the metal ion with the by-products.



Key words: biomimetic acylation, Lewis acid catalysis, acyl phosphate esters, zinc and lead ion catalysts, *cis*-diol substrates, monoesters

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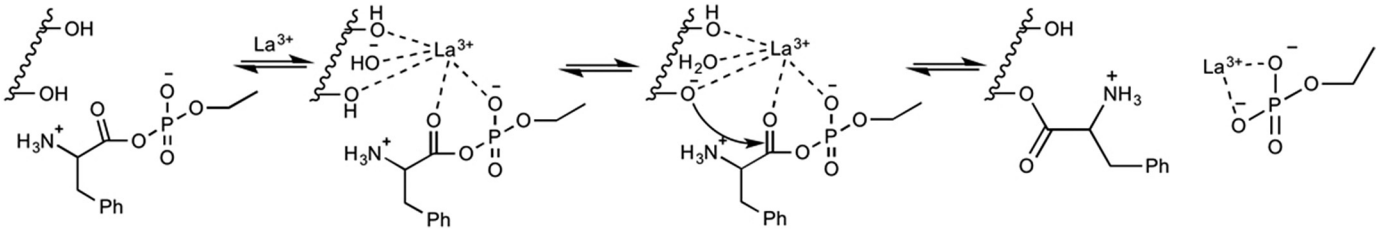
Introduction

The utility of acyl phosphates esters as biomimetic reagents for selective monoacylation of diols is a subject of continuing interest with important applications, including kinome interrogation (Patricelli et al. 2007; Nordin et al. 2015) and aminoacylation of the 3′-terminus of tRNA (Tzvetkova and Kluger 2007; Duffy and Dougherty 2010). We previously reported that lanthanide ions can serve as the chelated core to bind and activate the reacting species toward acyl transfer (Cameron et al. 2004; Tzvetkova and Kluger 2007; Her and Kluger 2011). Bidentate coordination of a *cis*-1,2-diol to a Lewis acid facilitates ionization of one of the coordinated diols while enhancing the activity of the bidentate-coordinate acyl phosphate (**Scheme 1**). The bis-bidentate array of



Scheme 1. Lanthanum-promoted monoacylation of the diol in a nucleotide via a chelate formation.

coordinated Lewis base (diol) and Lewis acid (acyl phosphate) promotes the ultimate combination that produces the acylated diol along with a phosphate monoester as a by-product. The arrangement can utilize the potential energy made available by the separation of the Lewis acid–base pair. Utilizing acyl phosphate monoesters as metal-coordinated acyl donors at the same Lewis acid where the ionized diol is coordinated promotes the reaction without involvement of the solvent. With a similar approach, regioselective monoacylation of carbohydrates in water can be based on the geometry of adjacent hydroxyl groups (Gray and Kluger 2007; Dhiman and Kluger 2010). However, although the system is set up for efficient acylation, the formation of the correct combination of ligands in the chelate for reaction is subject to competing homologous formation of the chelates as well as interference due to coordination to the lanthanide of the phosphate monoester product (see below).

An important potential use of the approach is for biomimetic aminoacylation of tRNA for the ribosomal introduction of unnatural amino acids into new proteins, which requires an aminoacylated reagent whose reactive functionality parallels that of the biological agents, aminoacyl adenylates (Schimmel 1987). However, complications arise from the competing effectiveness of the lanthanum ion in the hydrolysis of phosphate derivatives that are the acylation reagents (Hendry and Sargeson 1989; Kluger et al. 1997). Furthermore, transfer of the acyl group, whether to the hydroxyl or to water, releases ethyl phosphate, forming an insoluble complex with the metal ions that prevents further catalysis (Tzvetkova 2008).

In the present study, we assessed the effects of other metal ions for reactions in water that are reported to be effective in nonaqueous systems (Hikawa et al. 2014). We also assessed aqueous reaction conditions with added solvents and at lower reaction temperatures. In some cases, these provided significant improvements for the overall process.

Experimental

Preparation of N-deprotected phenylalanyl ethyl phosphate

BOC-PheEtP was formed in the DCC-mediated coupling of BOC-Phe and ethyl phosphate. PheEtP was produced by removal of the BOC protecting group using a minimal amount of trifluoroacetic acid. The N-deprotected product was precipitated from ice-cold dry acetone as a white powder (Kluger et al. 1996). ESI-MS [M − H^+] calculated $m/z = 272.0693$ and found $m/z = 272.0711$.

Analysis of the extent of PheEtP hydrolysis

A mixture of PheEtP (50 mmol/L) and lanthanum triflate (50 mmol/L) was incubated in an aqueous MES buffer (200 mmol/L, pH 6). We assessed the effects of conducting the reaction in a range of DMSO/buffer mixtures, as follows: 0/1, 2/3, 3/2, 9/1, 19/1, and 39/1 (*v*/*v*). The effect of temperature on product yield was studied by conducting reactions at 20 and 4 °C. Samples were analyzed after periodic additions of 20% volume of saturated EDTA solution until no further changes were observed. Hydrolysis of PheEtP was then tracked by the integrated peak area from UV-HPLC at 260 nm.

Monoaminoacylation of ethylene glycol

A mixture of ethylene glycol (60 mmol/L), PheEtP (50 mmol/L), and metal salt ($La(OTf)_3$ = $Zn(NO_3)_2$ = $Pb(NO_3)_2$ = 50 mmol/L) was stirred in an MES buffer (200 mmol/L, pH 6) at 4 °C for 24–72 h. Samples were analyzed after periodic additions of 20% volume of saturated EDTA solution until no further changes were observed. Products were isolated by reversed phase HPLC and lyophilized. A colorless solid was collected. The yield was estimated from the integration of the peak areas from UV-HPLC at 260 nm. ESI-MS [M + H^+] calculated m/z = 210.11 and found m/z = 210.1.

Monobenzoylation of ethylene glycol

A mixture of ethylene glycol (60 mmol/L), BMP (50 mmol/L), and metal salt ($La(OTf)_3$ = $Zn(NO_3)_2$ = $Pb(NO_3)_2$ = 50 mmol/L) was stirred in an EPPS buffer (200 mmol/L pH 8) at 25 °C for 24–72 h (Cameron et al. 2004). Samples were analyzed after periodic additions of 20% volume of saturated EDTA solution until no further changes were observed. Products were isolated by reversed phase HPLC and lyophilized. A colorless solid was collected. The yield was estimated from the integration of the peak areas from UV-HPLC at 230 nm.

Preparation of 5′-phospho-2′-deoxyribocytidylylriboadenosine

dCA was produced following published methods (Noren et al. 1989). ESI-MS [M + H^+] calculated m/z = 637.1167 and found m/z = 637.1173.

Aminoacylation of nucleotides

AMP (50 mmol/L) and dCA (1 mmol/L) were reacted with PheEtP (50 mmol/L) and metal salts (50 mmol/L) as described for the reaction with ethylene glycol. ESI-MS [M + H^+] for AMP-Phe: calculated m/z = 493.1242 and found m/z = 493.1197; for dCA-Phe: calculated m/z = 784.2000 and found m/z = 784.2000.

Results and discussion

An aminoacyl phosphate monoester, PheEtP, was used as the acylation agent for the evaluation of catalysts in this study. This reagent is a good model for the general approach to the formation of amino acids esters at the 3′-terminal of tRNA. We found that conducting reactions in mixtures of DMSO and water reduced the rate of hydrolysis of PheEtP in the presence of lanthanum ion compared with the reaction in water alone. In a 95/5 DMSO/aqueous buffer (v/v), the $t_{½}$ of PheEtP was about 30 min, whereas the $t_{½}$ in water was <2 min. Nonetheless, the yield of acylated products did not increase with the reduced hydrolysis rate of the reagent. Surprisingly, the yield of ester derivatives that were formed at the 2′- or 3′-ribosyl positions of AMP with PheEtP remained near 30% after 24 h, regardless of the amount of DMSO in the aqueous reaction mixture. This suggests that the reactants are locally solvated exclusively by water. The polarity of the reaction partners and their ability to form hydrogen bonds is consistent with this outcome. It is likely that the ionization of water coordinated to lanthanum ions is essential for the activation of PheEtP, whereas the reactivity of the hydroxyl group of diol toward the carboxyl carbon is increased as it is deprotonated by the lanthanum-coordinated hydroxyl (Kluger and Cameron 2002). Lowering the relative amount of water reduces the extent of ionization of the hydroxyl of the diol moiety, reducing the reactivity in the coordinated ligands.

The problematic competing hydrolysis of PheEtP occurs by the addition of non-chelated water. This will have a higher enthalpic barrier if it avoids the coordination sphere of the metal ion. Lowering the reaction temperature should reduce the rate of the reaction with water to a greater extent than it will reduce the desired acylation from reaction with the bis-bidentate coordinated chelate. As expected, at 4 °C, hydrolysis occurred at a significantly slower rate than at room temperature. At the lower

temperature, 15% of the initial PheEtP remained after 2.5 h, corresponding to a $t_{½}$ of 1 h under these conditions. Applying the same conditions to the lanthanum ion-promoted aminoacylation of AMP increased the yield from around 30% to 40%. For aminoacylation of dCA, a dinucleotide used by Robertson et al. (1989) in the chemical aminoacylation of tRNA, the combined yields of 2′ and 3′ esters doubled from 6.8% to 15% after 12 h of reaction. With PheEtP at its saturating concentration, the reaction produced the desired esters at a 40% yield after 12 h at 4 °C (Table 1). This establishes the importance of using lower reaction temperatures, consistent with the expected effect of selectively slowing the competing hydrolysis.

With this useful outcome, we optimized conditions for acylation with metal salts and determined the extent of the competing hydrolysis of PheEtP. The $t_{½}$ for catalyzed hydrolysis of PheEtP at 4 °C in the presence of zinc or lead salts were 60 and 5 min, respectively. The reaction in the presence of cupric salts is not effective, as it selectively promotes the hydrolysis of PheEtP. Based on these observations, we anticipated that Zn^{2+} and Pb^{2+} should catalyze aminoacylation of diols at or near 0 °C. We tested this approach by following the aminoacylation of ethylene glycol by PheEtP at 4 °C (Table 2).

Table 1. Yields for aminoacylation of AMP and dCA by PheEtP in the presence of Lewis acids.

Salt	Nucleotide	Temperature (°C)	Combined yield
$La(OTf)_3$	AMP	25	29%
		4	36%
	dCA	25	6.8%
		4	15% (40%)
$Zn(NO_3)_2$	AMP	4	31%
	dCA	4	19%
$Pb(NO_3)_2$	AMP	4	31%
	dCA	4	13%

Note: AMP, adenosine monophosphate; dCA, 5′-phospho-2′-deoxyribocytidylylriboadenosine; PheEtP, phenylalanyl ethyl phosphate.

Table 2. Summary of metal-mediated monoacylation reaction of ethylene glycol with PheEtP and BMP.

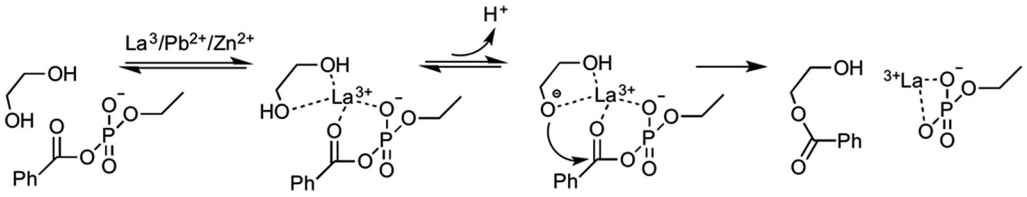
Acyl phosphate	Salt	[Salt] (mmol/L)	Yield
PheEtP	$La(OTf)_3$	50	64%
	$Zn(NO_3)_2$	50	23%
		150	24%
	$ZnSO_4$	50	22%
	$Pb(NO_3)_2$	50	67%
		150	63%
BMP	$La(OTf)_3$	50	3.5%
	$Zn(NO_3)_2$	50	3.6%
	$Pb(NO_3)_2$	50	7.5%

Note: PheEtP, phenylalanyl ethyl phosphate; BMP, benzoyl methyl phosphate.

In the presence of Zn^{2+} or Pb^{2+}, the reactions of ethylene glycol and PheEtP produced a mixture of the ester and the hydrolysis product, phenylalanine, within 1 min after addition to the solution. The Pb^{2+}-catalyzed reactions occurred with a half-life for PheEtP of <1 min. The yields are comparable with those from reactions conducted in the presence of La^{3+}. The Zn^{2+}-catalyzed reactions were slower and their overall yields were lower than with Pb^{2+} and La^{3+}.

We also investigated the reactions of ethylene glycol with BMP (Scheme 2). The $t_{½}$ for hydrolysis of BMP in the presence of Zn^{2+} and Pb^{2+} were 24 and 1 h, respectively. The Pb^{2+}-catalyzed monobenzoylation reactions of ethylene glycol gave the best yield of the monoester after 3 h, whereas reactions with Zn^{2+} required about 12 h to reach their maximum yield.

We also evaluated the effectiveness of Zn^{2+} and Pb^{2+} as catalysts for reactions of PheEtP with a mononucleotide and a dinucleotide. Both AMP and dCA were separately incubated with PheEtP in the presence of the metal ions at 4 °C, followed by analysis that used reverse-phase HPLC to separate the reactants and products. The reactions gave 2′ and 3′ nucleotidyl esters in each case. In reactions with dCA, the yield was 19% with Zn^{2+} and 13% with Pb^{2+} (Figures 1 and 2). These did not improve on the previously reported 15% yield with La^{3+}. We also monitored the reactions for an additional 72 h during which the distribution of products remained unchanged, establishing that the esters are stable under these conditions and that the reactions are self-limiting.



Scheme 2. Lanthanum/lead/zinc-mediated monobenzoylation of ethylene glycol.

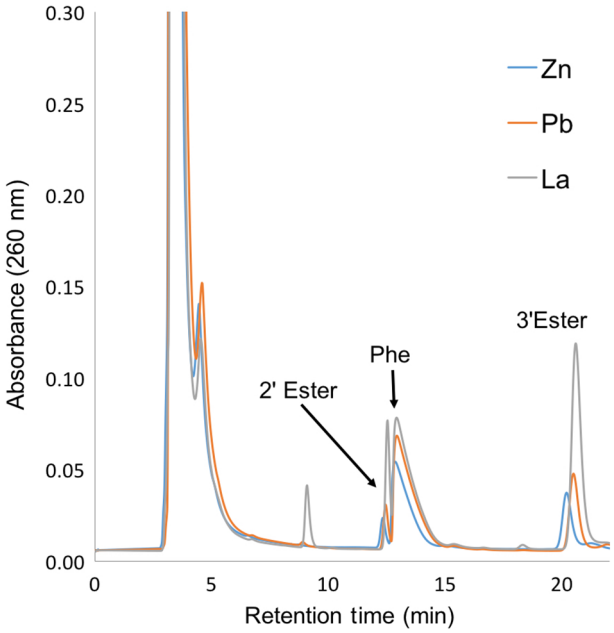


Fig. 1. Amionacylation reaction with adenosine monophosphate (AMP) and phenylalanyl ethyl phosphate (PheEtP) catalyzed by La^{3+}, Pb^{2+}, and Zn^{2+}.

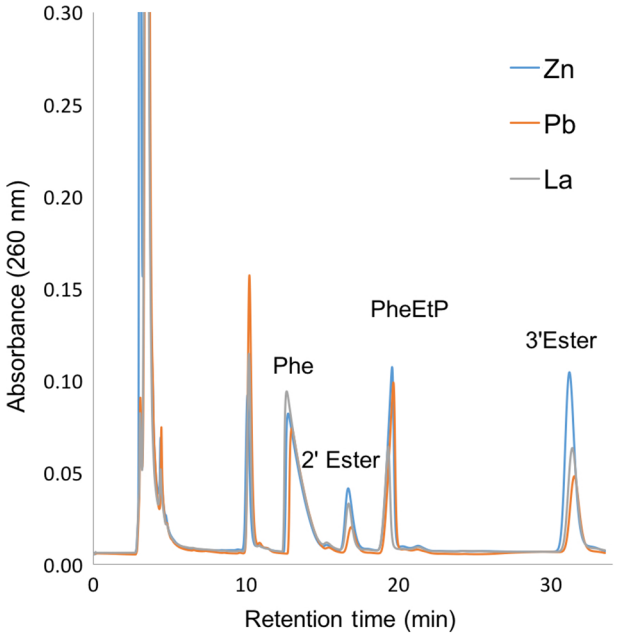


Fig. 2. Aminoacylation reaction with 5′-phospho-2′-deoxyribocytidylylriboadenosine (dCA) and phenylalanyl ethyl phosphate (PheEtP) catalyzed by La^{3+}, Pb^{2+}, and Zn^{2+}.

Reports of previous studies indicate that Pb^{2+} is among the most effective catalysts for site-specific cleavage of RNA (Huff et al. 1964; Brown et al. 1983). Lead hydroxide is an active catalyst for RNA hydrolysis (Brown et al. 1983) and for transesterification of a phosphate diester (Morrow et al. 1992). In reactions at pH around 7, where Pb^{2+}-promoted aminoacylation occurs, lead hydroxides are present (Perera et al. 2001). Lead hydroxides could assist in deprotonation of the incoming hydroxyl, further promoting acylation.

As noted earlier, a major problem with La^{3+}-promoted acylation is the high affinity of La^{3+} to phosphate monoesters. The resulting precipitate removes the La^{3+} catalyst from solution. As a result, La^{3+}-promoted acylation reactions require a large excess of the added metal ion (Gray and Kluger 2007). Reactions with Zn^{2+} and Pb^{2+} reduce the formation of insoluble metal phosphates, providing alternative catalysts for acylation reactions via acyl phosphates that can operate at lower concentrations.

Conclusions

We have demonstrated that lowering the reaction temperature improves lanthanide-ion-promoted aminoacylation reactions by selectively slowing the competing hydrolysis of activated amino acyl phosphates. Catalysis by Zn^{2+} and Pb^{2+} is effective, producing the desired aminoacylation products under conditions where the acyl phosphate reagent has improved stability.

List of abbreviations

AMP	adenosine 5′-monophosphate
BMP	benzoyl methyl phosphate
BOC	tertbutyl oxycarbonyl
dCA	5′-phospho-2′-deoxyribocytidylylriboadenosine
DCC	N, N′-dicyclohexylcarbodiimide
DMSO	dimethyl sulfoxide
EDTA	ethylenediamine tetraacetic acid

EPPS	N-(2-hydroxyethyl)piperazine-N′-(3-propane) sulfonic acid
ESI-MS	electrospray ionisation mass spectrometry
MES	2-(*N*-morpholino)ethanesulfonic acid
PheEtP	phenylalanyl ethyl phosphate
tRNA	transfer RNA
UV-HPLC	UV/Vis spectroscopic detection of output of high performance liquid chromatograph

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Author contributions

YL and RK conceived and designed the study. YL performed the experiments/collected the data. YL and RK analyzed and interpreted the data. RK contributed resources. YL and RK drafted or revised the manuscript.

Competing interests

RK is currently serving as a Subject Editor for FACETS, but was not involved in review or editorial decisions regarding this manuscript.

Data accessibility statement

All relevant data are within the paper.

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