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Lanthanide ion-induced hydrolyses of alkyl esters and amides of α-amino acids†

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ABSTRACT: Lanthanide ion-induced hydrolyses of methyl esters, ethyl esters, and amides of α-amino acids were systematically studied. In the hydrolysis of the alkyl esters, all the lanthanide ions are effective and the catalytic activities decrease in the order Ce(III), Nd(III) > Sm(III) > Eu(III) > Gd(III), Ce(IV) > Pr(III) > Dy(III), Tb(III), Er(III), Ho(III), Tm(III) > La(III), Lu(III), Yb(III). For the hydrolysis of the amides, however, the Ce(IV) ion is overwhelmingly more active than other lanthanide(III) and non-lanthanide ions. The results are interpreted in terms of the difference in the rate-limiting step for these two reactions. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: lanthanide ion; hydrolysis; α-amino acid esters; α-amino acid amides

INTRODUCTION

There has been increasing interest in the molecular design of catalysts for the chemical transformation of peptides, nucleic acids and other biomaterials. These catalysts should be potentially applicable to molecular biology, biotechnology and therapy. They should be also useful for a better understanding of the mechanisms of bioreactions. Various catalysts for these bioreactions have been proposed.[2,3] However, still more active catalysts, which can promptly transform biomaterials into the desired forms under physiological conditions, are required for further development in this field.

Recently, remarkable catalytic activities of the lanthanide ions for the hydrolysis of peptides,[1] DNA,[3,4] RNA[3,5] and adenosine 3′,5′-cyclic monophosphate[6] were evidenced. Artificial nucleases, which selectively hydrolyse the target phosphodiester linkage in DNA, were prepared by the attachment of a Ce(IV) complex to synthetic DNA oligomers[7]. These metal ions are a number of orders of magnitude more active than non-lanthanide ions, and thus are promising for future applications. However, the mechanisms of the catalysis by these metal ions have not yet been sufficiently clarified. Undoubtedly, information on the lanthanide ion-induced hydrolysis of alkyl esters, which have much better leaving groups than those in proteins and nucleic acids, should shed light on the mechanisms.

Here we report on the lanthanide ion-induced hydrolysis of alkyl esters of α-amino acids. The catalytic activities of a series of lanthanide ions were compared with those for the hydrolysis of amides of α-amino acids. Spontaneous hydrolysis of the alkyl esters and the amides in the absence of metal ions was also investigated in order to obtain fundamental information. Mechanisms of the catalysis by lanthanide ions are proposed on the basis of the kinetic evidence obtained.

EXPERIMENTAL

Materials. All the methyl esters, ethyl esters, and amides of α-amino acids and their derivatives, were purchased from Sigma (except for *N*-acetyl-L-phenylalanine amide from Bachem). The lanthanide(III) salts (in the form of the chlorides) (Soekawa) and $Ce(NH_4)_2(NO_3)_6$ (Nacalai Tesque) were used without further purification. D_2O (99.9 atom% D) and DCI (99.5 atom% D) were obtained from Aldrich. Highly purified water was sterilized immediately before use. Throughout the study, great care was taken to avoid contamination by natural enzymes. Absence of such contamination was further confirmed by careful control experiments.

Kinetic analysis of the hydrolysis of alkyl esters and amides of α-amino acids. Hydrolysis in the presence and absence of lanthanide ions was carried out at pH 7.0 (0.1 mol dm^{-3} Tris buffer) and 50 °C unless noted otherwise. The initial concentration of the substrate was 0.01 mol dm^{-3}. After an appropriate interval, the mixtures were

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Table 1. First-order rate constants for the lanthanide ion-induced hydrolysis of L-Phe-Me at pH 7.0 and 50°C[a]

Metal ion	$10^4 k_{obs}(s^{-1})$	Metal ion	$10^4 k_{obs}(s^{-1})$
La(III)	1.1	Tb(III)	1.5
Ce(III)	4.0	Dy(III)	1.6
Ce(IV)	2.4	Ho(III)	1.4
Pr(III)	1.9	Er(III)	1.5
Nd(III)	3.8	Tm(III)	1.4
Sm(III)	3.3	Yb(III)	1.0
Eu(III)	3.0	Lu(III)	1.1
Gd(III)	2.5	None	0.57

[a] $[\text{L-Phe-Me}]_0 = [\text{metal ion}]_0 = 0.01$ mol dm^{-3}.

analysed by reversed-phase HPLC [Merck LiChrospher RP-18(e) ODS column, water–acetonitrile (92:8, v/v) as element]. Assignment of the HPLC peaks was achieved by co-injection with authentic samples. All the reactions were followed for three or more half-lives, and satisfactorily showed first-order kinetics. Neither covalent intermediates nor unidentified by-products were detected.

For D_2O solvent isotope effect experiments, pD values were determined using the equation pD = pH meter reading + 0.41.[8] The pD of the reaction mixtures was adjusted with D_2O solutions of DCI and NaOD.

RESULTS

Catalytic activities of lanthanide ions for the hydrolysis of alkyl esters of α-amino acids

As indicated in Table 1, all the lanthanide ions are effective for the hydrolysis of L-phenylalanine methyl ester. The magnitude of acceleration is 2 – 7-fold. The catalytic activity decreases in the orders Ce(III), Nd(III) > Sm(III) > Eu(III) > Gd(III), Ce(IV) > Pr(III) > Dy(III), Tb(III), Er(III), Ho(III), Tm(III) > La(III), Lu(III), Yb(III). Most of the reactions proceed in virtually homogeneous solutions [for Ce(III), Ce(IV),Sm(III),Eu(III),Gd(III) and Tb(III), the reaction mixtures were slightly turbid]. The corresponding ethyl ester (L-Phe-Et) was hydrolysed by the metal ions at similar rates to the methyl ester.

In contrast with the significant activities of the lanthanide ions for the hydrolysis of L-Phe-Et, its *N*-acetyl derivative (Ac-L-Phe-Et) was not hydrolysed to a measurable extent by any of these metal ions under the same conditions. The primary amino groups in L-Phe-Me and L-Phe-Et are essential for efficient catalysis by the lanthanide ions.

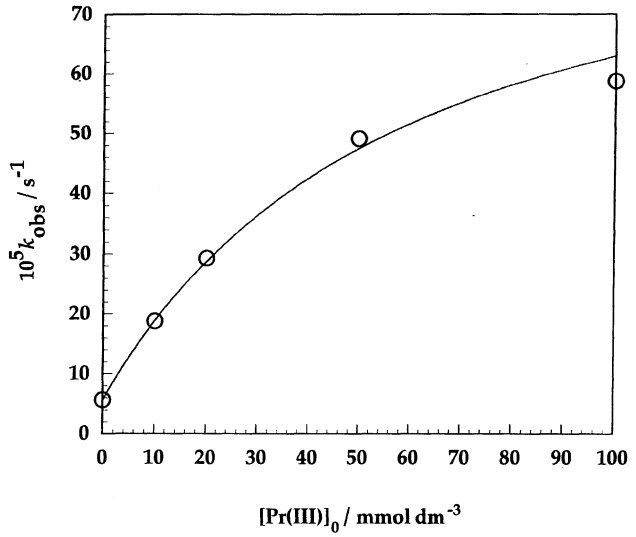

Figure 1. Plot of the rate constant for the hydrolysis of L-Phe-Me vs $[\text{Pr(III)}]_0$ at pH 7.0 and 50°C: $[\text{L-Phe-Me}]_0 = 0.01$ mol dm^{-3}. The solid line is the theoretical relationship calculated by use of equation (1) ($k_c = 9.7 \times 10^{-4}$ s^{-1} and $K = 17.0$ mol^{-1} dm^3)

Kinetic study of the Pr(III)-induced hydrolysis of L-Phe-Me

Figure 1 depicts the rate constant of the hydrolysis (k_{obs}) as a function of the concentration of Pr(III) ion. The initial concentration of L-Phe-Me was kept constant at 0.01 mol dm^{-3}. The rate constant increases monotonically with increasing $[\text{Pr(III)}]_0$ up to $[\text{Pr(III)}]_0$ / $[\text{L-Phe-Me}]_0 = 5$, but shows a gradual saturation at higher ratios. Apparently, the reaction involves complex formation between the substrate and the metal ion. The data were analysed in terms of the equation

$$1/(k_{obs} - k_{un}) = 1/(k_c - k_{un}) + 1/[(k_c - k_{un})K] \times 1/[\text{Pr(III)}]_0 \quad (1)$$

which is based on the assumption that 1 : 1 complex between them is responsible for the reaction. Here k_{un} and k_c are the rate constants for the spontaneous hydrolysis in the absence of Pr(III) and for the hydrolysis of L-Phe-Me in the complex, respectively, and K is the formation constant of the complex. A fairly straight line was obtained from this Lineweaver–Burk-type plot (correlation coefficient = 0.998), substantiating that the hydrolysis proceeds via the 1 : 1 complex between the Pr(III) ion and L-Phe-Me. The values of k_c and K were determined as 9.7×10^{-4} s^{-1} and 17.0 mol^{-1} dm^3, respectively (the solid line in Figure 1 is the theoretical relationship, calculated by use of these parameters).

The logarithm of the rate constant of the hydrolysis increases almost linearly with increasing pH up to 8 (Figure 2). The slope is 0.7. At a higher pH, the rate constant gradually decreases. Probably the protonation of the amino residue of L-Phe-Me ($pK_a = 7.6$; see Table 4) prevents the formation of the substrate–Pr(III) complex, which is essential for the catalysis. Furthermore, the formation of metal hydroxide aggregates which prevails when the pH is greater than the pK_a (8.5)[9] of the Pr(III)-bound water, is also responsible for the suppression.

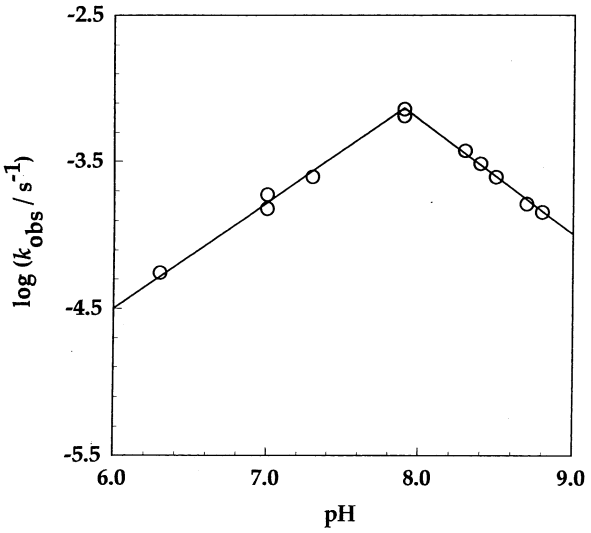

Figure 2. pH–rate constant profile for the Pr(III)-induced hydrolysis of L-Phe-Me at 50 °C: [L-Phe-Me] = [metal ion] = 0.01 mol dm^{-3}

Catalytic activities of lanthanide ions for the hydrolysis of amides of α-amino acids

For the hydrolysis of L-Phenylalaninamide (L-Phe-NH_2), only the Ce(IV) ion is efficient (see Table 2). The acceleration by the Ce(IV) ion (0.01 mol dm^{-3}) is 180-fold. Other lanthanide(III) ions show much poorer activity. Even the Eu(III) ion, which is the second best, is more than 20 times less active than the Ce(IV) ion. This is in great contrast with the results for the hydrolysis of the alkyl esters, since all the lanthanide ions are active there (compare Table 2 with Table 1). The non-lanthanide ions investigated [Zn(II), Cu(II) and Ni(II)] were virtually inactive for the hydrolysis of L-Phe-NH_2. The reactions are totally hydrolytic, as confirmed by HPLC. Although the Ce(IV) ion is a well-known oxidant, no by-products assignable to the oxidative cleavage of the substrates were detected.

N-Acetyl-L-phenylalaninamide (Ac-L-Phe-NH_2) was not hydrolysed to a measurable extent by any of the lanthanide ions. The primary amino groups in the amides of α-amino acids are crucially important for the lanthanide ion-induced hydrolysis, as is also the case for the alkyl esters.

Table 2. First-order rate constants for the lanthanide ion-induced hydrolysis of L-Phe-NH_2 at pH 7.0 and 50 °C[a]

Metal ion	$10^7 k_{obs}$ (s^{-1})	Metal ion	$10^7 k_{obs}$ (s^{-1})
La(III)	0.53	Ho(III)	1.4
Ce(III)	0.38	Tm(III)	1.4
Ce(IV)	60	Lu(III)	0.63
Pr(III)	1.2	None	0.33
Eu(III)	2.5		

[a] [L-Phe-NH_2]$_0$ = [metal ion]$_0$ = 0.01 mol dm^{-3}.

Table 3. First-order rate constants for spontaneous hydrolysis of the alkyl esters and amide of L-phenylalanine and their *N*-acetyl derivatives at pH 7.0 and 50 °C[a]

Substrate	k_{obs} (s^{-1})
L-Phe-Me	5.7×10^{-5}
L-Phe-Et	1.5×10^{-5}
Ac-L-Phe-Et	$<1.3 \times 10^{-7}$
L-Phe-NH_2	3.3×10^{-8}
Ac-L-Phe-NH_2	–[b]

[a] [Substrate]$_0$ = 0.01 mol dm^{-3}.
[b] No measurable hydrolysis took place.

Spontaneous hydrolysis of alkyl esters and amides of α-amino acids in the absence of lanthanide ions

The rate constants for spontaneous hydrolysis of the esters and the amides at pH 7.0 and 50 °C in the absence of metal ions are presented in Table 3. Significantly, the rates of spontaneous hydrolysis of L-Phe-Me and L-Phe-Et are more than 100 times greater than the corresponding value for Ac-L-Phe-Et. Similarly, L-Phe-NH_2 is hydrolysed at a reasonable rate at pH 7.0 and 50 °C, although Ac-L-Phe-NH_2 remains intact under the identical conditions. It is conclusive that the primary amino groups in both of the substrates greatly promote the intrinsic reactivities of the ester and the amide moieties therein. Consistently, ethyl acetate is far more stable than L-Phe-Et (the rate constant for its hydrolysis at pH 7 and 50 °C is only 10^{-7} s^{-1}),[10] and acetamide is not hydrolysed to a measurable extent under the conditions used.

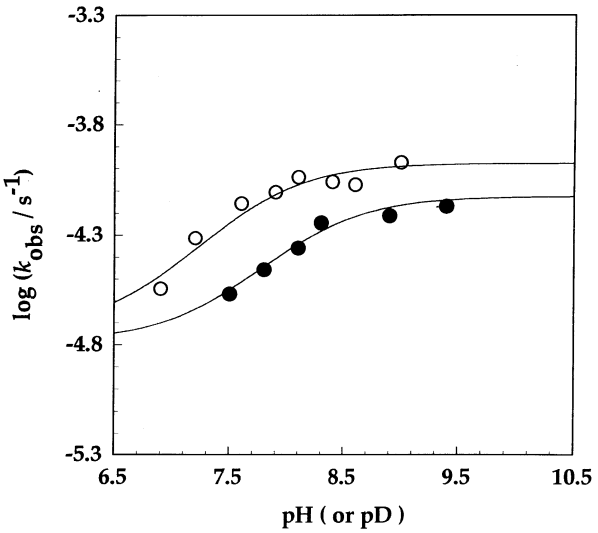

Figure 3. pH–rate constant profiles for the spontaneous hydrolysis of L-Phe-Me in the absence of lanthanide ions at 50 °C. The open and the closed circles are for the reactions in H_2O and D_2O, respectively; the solid lines are the theoretical relationships calculated by use of equation (2) and the parameters in Table 4

Table 4. Partial rate constants and apparent pK_a for spontaneous hydrolysis of L-Phe-Me at 50°C[a]

Solvent	$10^6 k_1$ (s^{-1} mol^{-1} dm^3)	$10^7 k_2$ (s^{-1} mol^{-1} dm^3)	pK_a
H_2O	1.9 ± 0.1	3.3 ± 1.0	7.6 ± 0.1
D_2O	1.5 ± 0.1	3.3 ± 0.7	8.1 ± 0.1

[a] The values of k_1, k_2 and pK_a were determined by fitting the curves in Figure 3 to equation (2) (see text for details).

The pH–rate constant profile for spontaneous hydrolysis of L-Phe-Me

The open circles in Figure 3 show the pH–rate constant profile for the spontaneous hydrolysis of L-Phe-Me. The profile is composed of two components:(1) the pH-dependent region at pH 6.5 – 8.5 (slope < 1) and (2) the pH-independent region at pH 8.5 – 9.5 (alkaline hydrolysis was explicit only when pH > 10.5). Thus the profile was analysed in terms of the equation

$$k_{obs} = k_1 ([RNH_2]/[RNH_2]_0)[H_2O] + k_2([RNH_3^+]/[RNH_2]_0)[H_2O]$$
$$= k_1K_a[H_2O]/([H^+] + K_a) + k_2[H^+][H_2O]/([H^+]+K_a) \quad (2)$$

Here the first and the second terms correspond to the reactions of the neutral species of L-Phe-Me (RNH_2) and its protonated form (RNH_3^+) with H_2O, respectively (k_1 and k_2 are the rate constants). K_a is the acid dissociation constant of RNH_3^+. All the experimental points fit the theoretical line (the solid line, calculated by use of the parameters in Table 4).

D_2O solvent isotope effects for spontaneous hydrolysis of L-Phe-Me

The kinetic parameters for the reactions in D_2O were similarly determined by fitting the closed circles in Figure 3 to equation (2). A small but notable D_2O solvent isotope effect (1.3) was observed for k_1 (Table 4), showing a rate-limiting proton transfer in the reaction. However, the solvent isotope effect was zero for k_2.

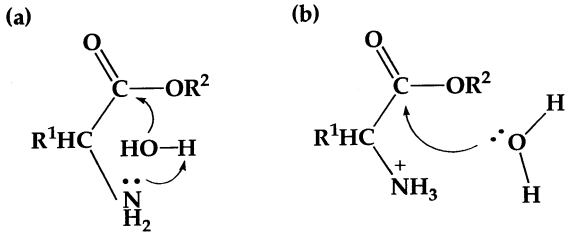

Figure 4. Proposed mechanisms of spontaneous hydrolysis of the alkyl esters of -amino acids: (a) is for the first and (b) is for the second term in equation (2) (see text for details)

DISCUSSION

Mechanism of spontaneous hydrolysis of alkyl esters of α-amino acids

The kinetic evidence in Figure 3 and Table 4 indicates that the spontaneous hydrolysis of L-Phe-Me proceeds as depicted in Figure 4. Mechanism (a) corresponds to the first term in equation (2), in which the primary amino residue in the neutral substrate shows an intramolecular general base catalysis. The result of the D_2O solvent isotope experiment in Table 4 agrees fairly well with the mechanism. In mechanism (b), which is for the second term in equation (2), a non-activated water molecule attacks the protonated substrate. Here, the ammonium cation electrostatically stabilizes the negatively charged transition state of hydrolysis. A similar electrostatic enhancement of ester hydrolysis was previously achieved with positively charged micelles[11] and cyclophanes.[12] These two types of catalyses (general base catalysis and electrostatic catalysis) greatly accelerate the hydrolysis (note that L-Phe-Me is hydrolysed more than 100 times faster than Ac-L-Phe-Me). The contributions of the first and the second terms to the hydrolysis at pH 7 are 59% and 41%, respectively, as estimated from the parameters in Table 4.

The mechanism in which the neutral amino residue shows an intramolecular nucleophilic attack towards the carbonyl carbon atom is unlikely, since then a stable covalent four-membered amide intermediate would be formed and should be accumulated in the reaction mixture. The intermediate, if formed, should be hydrolysed much more slowly than are the alkyl esters of α-amino acids (the rate constant for the hydrolysis of the four-membered ring amide in penicillin derivatives is around 10^{-8} s^{-1}, [13] which is 1000 times smaller than the values for the hydrolysis of L-Phe-Me and L-Phe-Et).

The proposed mechanism is consistent with the fact that the hydrolysis of L-Phe-Me and L-Phe-Et in water is much faster than that in ethanol–water mixtures. The first-order rate constant for the hydrolysis of L-Phe-Et in an 85:15 ethanol–water mixture is about 10^{-9} s^{-1}, [14] which is 10^4 times smaller than the value in water (1.5×10^{-5} s^{-1}). In these less polar solvent systems, general base catalysis is not favourable since it involves the partial dissociation of a water molecule to more polar species. Furthermore, protonation of the primary amino residues in the substrates is greatly suppressed, resulting in inefficient electrostatic catalysis.

Mechanism of the lanthanide ion-induced hydrolysis of alkyl esters of α-amino acids

The proposed mechanism is depicted in Figure 5. The substrates are activated by the coordination to the

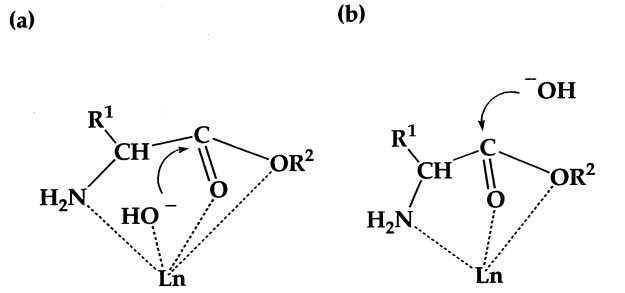

Figure 5. Proposed mechanisms of the lanthanide ion-induced hydrolysis of the alkyl esters of -amino acids: (a) intramolecular attack by the metal-bound hydroxide; (b) intermolecular attack by hydroxide ion. The hydroxide ions can be replaced by water

lanthanide ion through the primary amino group and the ester moiety. The coordination is apparently essential, since the corresponding *N*-acetyl derivatives are hardly hydrolysed by the metal ions. The metal-bound hydroxide (or the metal-bound water) as an intramolecular nucleophile can attack the substrate coordinating to the same metal ion [mechanism (a)]. Alternatively, hydroxide ion or water in the solutions functions as an external nucleophile [mechanism (b)]. The slope of 0.7 for the pH–rate constant profile (pH < 8) in Figure 3 indicates that both hydroxide ion (either free or metal-bound) and water participate in the reactions.

In both of the mechanisms, the positive charges on the metal ion electrostatically stabilize the negatively charged transition state, in the same way as does the ammonium cation in spontaneous hydrolysis. The small catalytic effects of the lanthanide ions are attributable to the fact that both intramolecular general base catalysis and electrostatic catalysis by the ammonium ion can no longer work in the lanthanide-induced reactions.

Comparison of the catalytic activities of lanthanide ions for ester hydrolysis and amide hydrolysis

In the alkyl ester hydrolysis, the leaving groups (alkoxide ions) are sufficiently good so that the tetrahedral intermediate, formed by the nucleophilic attack by either hydroxide ion or water, is promptly converted to the final hydrolysis products. The essential role of the metal ion is to promote the formation of the tetrahedral intermediates by either providing the metal-bound hydroxide and water as efficient nucleophiles or stabilizing the transition state as an electrostatic catalyst.

The hydrolysis of the amides of α-amino acids by lanthanide ions proceeds by a similar mechanism. However, the leaving group ($^{-}$NHR′) is so poor that the decomposition of the tetrahedral intermediate to the products, rather than the formation of the intermediate, is rate-limiting. Hence the main role of the lanthanide ions is to stabilize the leaving group as an acid catalyst. In spontaneous hydrolysis of the amides of α-amino acids, the ammonium cations in the substrates probably function as intramolecular general acid catalysts to stabilize the leaving groups. The overwhelmingly greater activity of the Ce(IV) ion than those of other lanthanide(III) ions is consistent with this argument, since its acidity is far greater: the pK_a value of the coordination water of the Ce(IV) ion is around zero, whereas the corresponding values for other lanthanide(III) ions are 7 – 9.[9] In the ester hydrolysis, however, the catalytic activity of the Ce(IV) ion is comparable to those of the other trivalent lanthanide ions (see Table 1).

CONCLUSION

All the lanthanide ions are effective for the hydrolysis of alkyl esters of α-amino acids, whereas only the Ce(IV) ion is effective for the hydrolysis of the corresponding amide hydrolysis. The former reactions involve the rate-limiting formation of the tetrahedral intermediates, which is in contrast with the rate-limiting decomposition of the intermediates in the latter reactions. The departure of the very poor leaving groups in the amide hydrolysis requires strong acid catalysts. Consistently, only the Ce(IV) ion is active for the hydrolysis of DNA [4] and adenosine 3′,5′-cyclic monophosphate,[6] both of which have poor leaving groups. The present results provide basic information for the molecular design of catalysts containing lanthanide ions which hydrolyse various biomaterials.

Acknowledgements

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