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Enantioselective ester hydrolysis by an achiral catalyst co-embedded with chiral amphiphiles into a vesicle membrane†

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Co-embedding of an amphiphilic non-chiral hydrolysis catalyst with amphiphilic chiral additives into the membrane of a phospholipid vesicle induces different rates of ester hydrolysis for enantiomeric amino acid esters.

The origin of chirality in nature,[1] why natural molecules are homochiral, and how such a rapid amplification of single enantiomers occurred, are still unanswered questions.[2,3] Synthetic chemistry uses chiral catalyst or ligands to convert achiral substrates enantio-selective.[4] The reactant and the source of chirality require typical close contact. The simple addition of chiral additives to the reaction mixture or using enantiopure chiral solvents[5,6] provide none or minute enantioselectivity. The intermolecular chirality transfer improves in 3D networks, such as chiral MOFs[7,8] or micellar solutions.[9–12]

The Raymond group reported an approach where chiral self-assembled capsules induce stereoselective reactions of achiral substrates by weak interactions.[13–15]

We report here the hydrolysis of enantiomeric amino acid esters on the modified surface of phospholipid vesicles (Fig. 1). A chiral, catalytically inactive membrane additive is co-embedded with a catalytically active achiral metal complex **Zn_2Cy** into the phospholipid membrane.[12,16] The membrane serves as two-dimensional platform with higher concentration of the amphiphilic membrane additives compared to the bulk solution.[17] This proximity of the chiral additive to the achiral metal complex affects its selectivity in ester hydrolysis and induces thereby different reaction rates for both enantiomers.

The bis-zinc-cyclen complex **Zn_2Cy** is anchored into the surface of the vesicle by its lipophilic alkyl chain and promotes the hydrolysis of activated carboxylic esters as previously reported (Fig. 2).[18] The Lewis acidic zinc ions coordinate one water molecule and the ester functionality. Enantiopure amphiphilic derivatives of L-proline **l-Pro**, (−)-sparteine **(−)-Spa**, L-glucose **l-Glu**, L-tartaric acid **l-Tar** and L-histidine **l-His-COOH** and the corresponding alcohol **l-His-OH** were used as chiral membrane additives. Enantiometrically pure 4-nitrophenol esters of phenylalanine, **PN-Phe**, serve as substrates for the catalysed hydrolysis. Upon cleavage of the ester bond, the coloured 4-nitrophenolate anion is released, which enables a facile determination of the reaction progress. However, derivatives **PN-Phe** with free amine group show fast spontaneous hydrolysis under the reaction conditions. Therefore compounds **PN-C12-Phe** and **PN-C2-Phe** with a protected amino-group were prepared, which are stable in the absence of the hydrolysis catalyst.

Results and discussion

All measurements were done in buffered solutions (HEPES buffer, 25 mM, 7.4 pH), at room temperature. Samples were prepared by sonication according to a previously reported procedure to form micellar solutions or 100 nm unilamellar

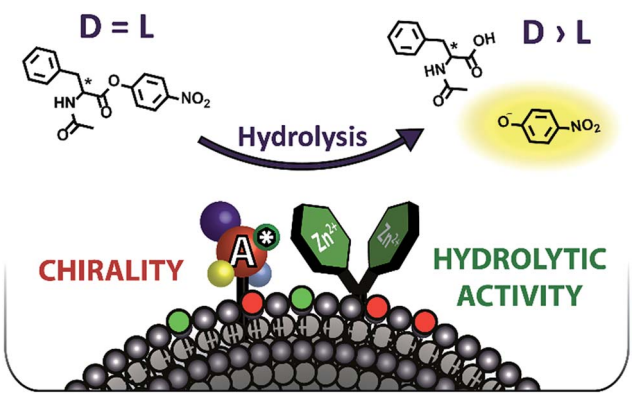

Fig. 1 Proposed concept of additive-induced enantioselectivity in a hydrolytic reaction.

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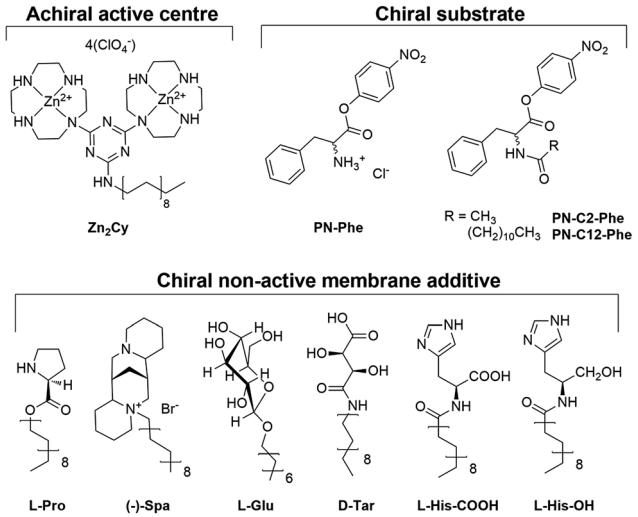

Fig. 2 Molecular structures of the achiral catalyst for hydrolysis, racemic substrates and chiral membrane additives.

functionalised vesicles.[18] The rate of substrate hydrolysis was determined colorimetrically as an increase in absorbance intensity at 400 nm (absorption maximum of 4-nitrophenol at pH 7.4; no other species absorb at this wavelength). The relative error of the measurement was estimated to be below 10% (Fig. 3).

Pseudo first order rate constants were calculated using the initial slope method. Every membrane additive was examined as a sole micellar solution (without lipid or **Zn_2Cy**), co-micellar solution (without lipid in the presence of **Zn_2Cy**), and in vesicular membranes with 1,2-dioleoyl-*sn-glycero*-3-phosphocholine (**DOPC**) and 1,2-distearoyl-*sn-glycero*-3-phosphocholine (**DSPC**) lipids (5 mol% **Zn_2Cy**, 10 mol% membrane additive, 85 mol% lipid). Lipids differ in their transition temperatures providing fluid (**DOPC**) and rigid (**DSPC**) membranes at room temperature. We measured the hydrolytic rate constants separately for the two enantiomers of the substrate **PN-C12-Phe**. This substrate is equipped with a long alkyl chain, which increases its lipophilicity and the adsorption to the surface of the membrane. The initial hydrolysis rates for different functionalized vesicles and co-micelles are summarised in Table 1.

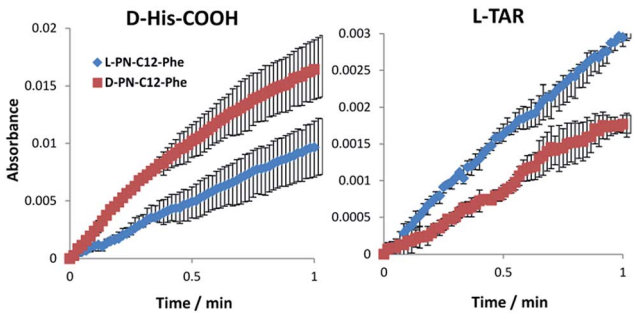

Fig. 3 Examples of the kinetic data for hydrolysis of the PN-C12-Phe substrate by vesicular solution (85% DOPC), Zn_2Cy (5%) and amphiphilic additive (10%).

Table 1 Pseudo first order kinetic rate constants for the hydrolysis of the L and D enantiomer of P-C12-Phe using different systems. All kinetic values are given in 10^{-3} s^{-1}

	DOPC		DSPC		Co-micelles		No cyclen, no lipid	
	k_L	k_D	k_L	k_D	k_L	k_D	k_L	k_D
$ZnCy_2$	31	30	65	50	201	198		
Pro	15	15	63	61	166	159	—[a]	—[a]
(−)-Spa	28	26	50	41	277	236	—[a]	—[a]
Glu	13	15	28	28	229	242	—[a]	—[a]
Tar	**24**	**14**	26	24	46	46	—[a]	—[a]
His-COOH	**212**	**113**	153	129	214	286	11	2
His-COOH	**127**	**233**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
His-OH	339	327	357	287	513	384	6	4

[a] No effect on hydrolysis observed.

Kinetic effects

Hydrolysis of the ester **PN-C12-Phe** is favoured in micellar solutions (Table 1). Confirming previous results,[18] membrane additives can affect the hydrolysis activity in vesicular and co-micellar solutions significant.

In **DOPC** membranes the hydrolytic rates are affected by two types of membrane additives: tartrate **l-Tar** decreases and histidine derivatives **l-His-COOH** and **l-His-OH** increase the initial rate of ester hydrolysis (Table 1). These observations are in accordance to effects on the previously studied hydrolysis of fluorescein diacetate by **Zn_2Cy**.[18]

Enantiodiscrimination in ester hydrolysis

The rates of ester hydrolysis were determined for both enantiomers as previously reported for micellar solutions.[10] The largest relative difference in hydrolysis rates for the enantiomeric esters is observed in **DOPC** vesicles with addition of amphiphilic tartrate **l-Tar** or histidine acid **l-His-COOH** as membrane additives (Fig. 4).

Both compounds contain free carboxy group. When the carboxyl group of histidine is reduced the effect in vesicles is lost. This indicates that the free acid might have a crucial role in this cooperative action. In buffered solution the acid is deprotonated and might interact with the Lewis acidic zinc complex. This interaction is weak in bulk solution, but may significantly increase due to the close proximity of the binding

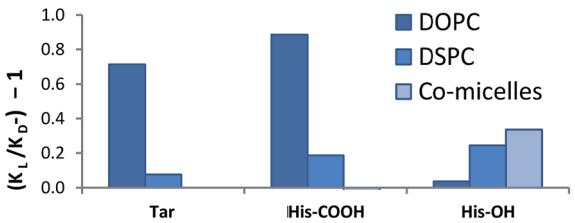

Fig. 4 Effect of membrane additives on the relative ratio of ester hydrolysis rates of enantiomeric amino acid esters represented by the fraction of pseudo first order kinetic constants.

Table 2 Pseudo first order rate constants of hydrolysis for both enantiomers by a vesicular solution of Zn_2Cy (5 mol%), l-Tar (10 mol%) and DOPC (85 mol%)

	k_L [10^{-3} s^{-1}]	k_D [10^{-3} s^{-1}]
PN-C2-Phe	35	17
PN-Phe	2059	1204

partners at the vesicular surface. In fluid **DOPC** membranes, embedded components diffuse and may thereby arrange optimal for the catalysis. In gel phase **DSPC** added amphiphiles form patches with restricted lateral movement decreasing the possibility for cooperative effects.[19–21] This was also previously observed by us.[18] Other membrane additives than tartrate or histidine do not show considerable effects on the relative hydrolysis rate of the enantiomeric esters. Substrates **PN-C2-Phe** and **Pn-Phe** exhibit similar enantio-selective enhancement (Table 2).

In order to confirm our observation, we prepared the enantiomeric amphiphilic histidine **d-His-COOH**. This compound was investigated under identical conditions as **l-His-COOH** and induced a similar hydrolysis rate enhancement of **d-PN-C12-Phe** (Fig. 5), but with opposite enantioselectivity.

In addition, the effect of the membrane additive loading on the enantioselectivity of the hydrolysis was investigated for **d-His-COOH** (Fig. 6). Addition of 10 mol% provided the highest relative difference between the hydrolysis rate constants.

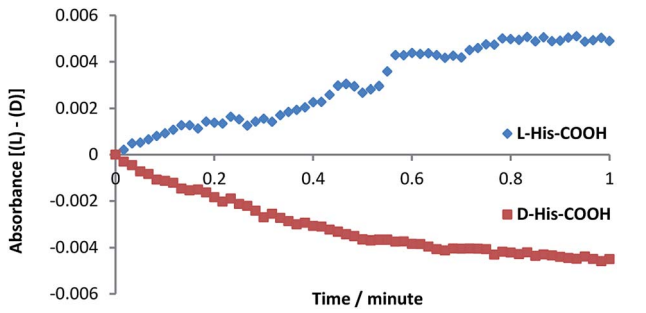

Fig. 5 Recorded difference in kinetics of L and D PN-C12-Phe substrate hydrolysis by vesicular solution (85% DOPC) with L or D His-COOH (10%) and $ZnCy_2$ (5%).

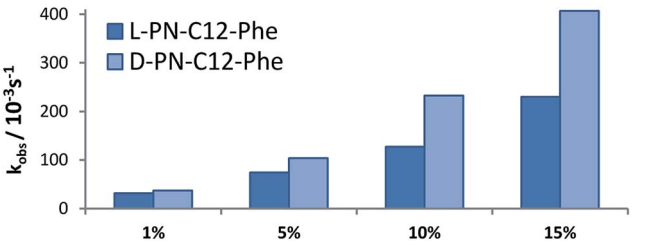

Fig. 6 Pseudo first order rate constants of hydrolysis for both enantiomers by a vesicular solution of Zn_2Cy (5 mol%) and DOPC with different loadings of d-His-COOH.

Conclusions

The relative catalytic hydrolysis rates of enantiomeric amino acid esters with a non-chiral catalyst become significantly different, if the catalyst is co-embedded into the surface of **DOPC** vesicles with chiral amphiphiles. The rates for enantiomeric phenylalanine nitrophenyl esters differ by a factor of two with co-embedded amphiphiles prepared from tartaric acid or histidine. This resembles an ee of approx. up to 40%, which may be expected for reactions performed from racemate. The fluidity of the membrane is essential to achieve the catalytic enantio-discrimination and therefore only observed in **DOPC** vesicles. Although the measured rate differences may not be useful for practical applications, the results prove that the intermolecular interaction between a Lewis acidic metal complex, chiral amphiphiles and activated amino acid esters co-embedded into a fluid membrane without covalent connection can affect reaction rates enantioselective.

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