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Asymmetric hydrolysis of enol esters with two esterases from *Marchantia polymorpha*

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Abstract

Two esterases participating in the asymmetric hydrolysis of α-alkylated enol acetates to α-chiral ketones were isolated from the cultured cells of *Marchantia polymorpha*. These two esterases had opposite enantioselectivities and both of them reversed the stereoselectivity of protonation into the enol intermediate in the hydrolysis when the chain length and the bulkiness of α-substituents were increased.

Optically active α-substituted ketone derivatives are widely employed as chiral synthons in asymmetric reactions[1] and there is a continuing interest in the development of efficient procedures to prepare them in enantiomerically pure form.[2] Recently, it has been found that yeast[3] and the cultured cells of *M. polymorpha*[4] were capable of performing the asymmetric hydrolysis of α-alkylated cyclohexanone enol esters to give α-substituted chiral ketones and that hydrolytic enzymes derived from yeast need an enantioselectivity-promoting factor to differentiate the enantiotopic face in the protonation of the enol intermediate.[5] We have now investigated the enzymes which are able to catalyze the asymmetric hydrolysis of enol acetates in the plant cell cultures of *M. polymorpha*.

Two hydrolytic enzymes named esterases I and II participating in the hydrolysis of enol esters were isolated from the cultured cells of *M. polymorpha*.[6] Several cyclohexanone enol acetates (**1**–**7**)[7,8] were subjected to enzymatic hydrolysis with these esterases to clarify the effect of various substituents at the β-position to the acetoxyl group on the enantiomeric ratio and the catalytic activity of enzymes.[9] The absolute configuration and enantiomeric excess of the resulting ketone were determined by the circular dichroism (CD) spectra of the products[10–14] and the peak area of the corresponding enantiomers in GLC analyses on CP cyclodextrin β 236M-19.[15] Hydrolysis of enol acetates, **1**–**3**, by esterase I gave the corresponding optically active ketones (**8**–**10**) whose CD

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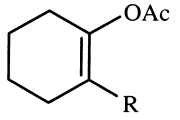
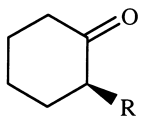
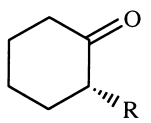
curves were positive (Table 1). The acetate **1** was the best substrate for esterase I, allowing us to achieve the highest enantiomeric excess (>99% *e.e.*) and yield (99%). In the case of **2**, the ethyl group at the α-position to the acetoxyl group markedly reduced the enantiomeric excess of the product (14% *e.e.*). Substrate **3** with isopropyl group as the α-substituent was hydrolyzed in 10% yield, and protonation at the α-position gave a poor enantioselectivity (17% *e.e.*). The chiral preference of esterase I was retained among these substrates: the protonation of the enol intermediates from **1**–**3** occurred preferentially from the same enantiotopic face of the C–C double bond. However, when *t*-butyl, *n*-propyl, *n*-pentyl and benzyl groups were introduced into the β-position to the acetoxyl group of the substrates (**4**–**7**), the CD curves of the corresponding products, **18**–**21**, were negative. This result indicates that the stereoselectivity of esterase I in the protonation of these enol intermediates is reversed by long chain (C≥3) and bulky substituents at the β-position to the acetoxyl group. It is noteworthy that esterase I converted **7** into optically active **21** in high enantiomeric excess (99%), although the hydrolysis of **7** with hydrolytic enzymes from yeast gave racemic ketone.



1: R=Me **2**: R=Et **3**: R=*i*-Pr **4**: R=*t*-Bu **5**: R=*n*-Pr **6**: R=*n*-Pnt **7**: R=Bnz



8: R=Me **9**: R=Et **10**: R=*i*-Pr **11**: R=*t*-Bu **12**: R=*n*-Pr **13**: R=*n*-Pnt **14**: R=Bnz



15: R=Me **16**: R=Et **17**: R=*i*-Pr **18**: R=*t*-Bu **19**: R=*n*-Pr **20**: R=*n*-Pnt **21**: R=Bnz

Table 1
Enantioselectivity in the hydrolysis of enol acetates by esterase I

Substrate	Product	Conv. / %	*e.e.*	Config.[a]
1	**8**	>99	>99	*S*
2	**9**	>99	14	*S*
3	**10**	10	17	*R*
4	**18**	21	5	*S*
5	**19**	>99	>99	*R*
6	**20**	20	26	*R*
7	**21**	15	>99	*S*

[a]Preferred configuration at the α-position to the carbonyl group of the products.

On the other hand, the conversion yield and enantiomeric purity in the hydrolysis with esterase II are very low in comparison to the case of esterase I (Table 2). In the hydrolysis of **1**–**3**, the protonation of the enol intermediate following the hydrolysis of these substrates occurred stereoselectively from the same enantiotopic face of the C–C double bond. In the cases of the enol acetates **4**–**7**, the protonation of the enol intermediate occurred preferentially from the reverse side of the C–C double bond with respect to the hydrolysis of **1**–**3**. Interestingly, the stereoselectivity of esterase II was opposite to that of esterase I.

Table 2
Enantioselectivity in the hydrolysis of enol acetates by esterase II

Substrate	Product	Conv. / %	*e.e.*	Config.[a]
1	**15**	4	4	*R*
2	**16**	3	2	*R*
3	**17**	3	3	*S*
4	**11**	20	2	*R*
5	**12**	5	4	*S*
6	**13**	16	7	*S*
7	**14**	11	14	*R*

[a]Preferred configuration at the α-position to the carbonyl group of the products.

Thus, two hydrolytic enzymes were isolated from the cultured cells of *M. polymorpha* and were confirmed to be capable of discriminating the enantiotopic face of the C–C double bond of the enol intermediate in the hydrolysis. The enantioselectivities in the protonation of the enol intermediate were opposite between these enzymes. The enantioselectivity of both enzymes reversed in the hydrolysis of the substrates with long side chain, bulky *t*-butyl or benzyl group at the β-position to the acetoxyl group, compared with the substrates having short side chains. Such inversion of the enantioselectivity may be explained by the occurrence of a turnover of the substrate in the active site of the enzymes due to the steric hindrance offered by the α-substituents. Recently, Matsumoto et al. reported that the hydrolytic enzymes from yeast and commercially available lipases from microorganisms could exhibit enantioselectivity in the hydrolysis of enol acetate only in the presence of an enantioselectivity-promoting factor.[5] Hydrolytic enzymes from plant cell cultures of *M. polymorpha* appear to be different from those from microorganisms.

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6. Homogenates of the cultured cells of *M. polymorpha* in 100 mM phosphate buffer (pH 7.0) were centrifuged at 100 000 *g* to give a cell-free extract, which was treated with ammonium sulfate (60–80% satd) to give a crude enzyme preparation. Butyl-Toyopearl column chromatography of the crude enzyme preparation gave a good separation of the two different esterases. Further purification by chromatography on a diethylaminoethyl-Toyopearl column and then a Sephadex G-75 column gave homogeneous esterases as judged by SDS–PAGE: esterase I, molecular mass ca. 54 000, dimeric form composed of two identical 27 000 subunits; esterase II, molecular mass ca. 45 000, dimeric form composed of two identical 22 500 subunits.
7. Cyclohexanone enol acetates, **1**–**7**, were prepared by treatment of their corresponding ketones with perchloric acid and acetic anhydride.[8]
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9. In a typical experiment 2-methylcyclohexanone enol acetate **1** (5 mg) and Triton X-100 (5 mg) were dissolved in 2 ml of the sodium phosphate buffer containing enzyme (pH 7.0). The mixture was shaken at 300 rpm and 35°C. After 0.5 h the reaction mixture was extracted with *n*-pentane and the product was identified by direct comparison with the authentic sample by GLC and GC–MS analyses. The other substrates (**2**–**7**) were subjected to the enzymatic hydrolysis by the same procedure. It was confirmed that neither non-enzymatic hydrolysis nor racemization of the product occurred under the incubation conditions.
10. The CD data of the products obtained in the hydrolysis with esterase I are as follows; **8**: $[\theta]_{288}$ +990 (c 0.25, MeOH) {lit.[11] $[\theta]_{288}$ −987 for *R* enantiomer (**15**)}; **9**: $[\theta]_{288}$ +351 (c 0.25, MeOH) {lit.[12] $[\theta]_{288}$ +2200}; **10**: $[\theta]_{288}$ +361 (c 0.12, MeOH) {lit.[13] $[\theta]_{288}$ +2126}; **18**: $[\theta]_{288}$ −86 (c 0.15, MeOH) {lit.[14] $[\theta]_{288}$ +1690 for *R* enantiomer (**11**)}; **19**: $[\theta]_{288}$ −2485 (c 0.25, MeOH) {lit.[12] $[\theta]_{288}$ +2480 for *S* enantiomer (**12**)}; **20**: $[\theta]_{288}$ −669 (c 0.14, MeOH); **21**: $[\theta]_{288}$ −1990 (c 0.15, MeOH) {lit.[12] $[\theta]_{288}$ +1750 for *R* enantiomer (**14**)}. The CD data of the products obtained in the hydrolysis with esterase II are as follows; **12**: $[\theta]_{288}$ +99 (c 0.09, MeOH); **13**: $[\theta]_{288}$ +173 (c 0.17, MeOH); **14**: $[\theta]_{288}$ +278 (c 0.15, MeOH). The CD data of the products (**11**, **15**–**17**) could not be obtained due to the low transformation rate and the lack of the products.
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15. Conditions for capillary GLC analysis: column, CP cyclodextrin β 236M-19 (0.25 mm×25 m); injection, 180°C; detector, 180°C; oven, 100°C; carrier gas, N_2 (50 ml min^{-1}). Retention times for the products in the GLC were as follows: **8** and **15**, 11.8 and 12.8 min; **9** and **16**, 12.7 and 12.9 min; **10** and **17**, 23.8 and 24.0 min; **11** and **18**, 18.1 and 18.4 min; **12** and **19**, 27.7 and 27.9 min; **13** and **20**, 72.1 and 72.8 min; **14** and **21**, 60.1 and 61.2 min.