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Journal of Molecular Catalysis B: Enzymatic 16 (2002) 255–263

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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A cold-active esterase with a substrate preference for vinyl esters from a psychrotroph, *Acinetobacter* sp. strain no. 6: gene cloning, purification, and characterization☆

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Received 13 September 2001; received in revised form 4 October 2001; accepted 4 October 2001

Abstract

It has recently been shown that fatty acid vinyl esters serve as effective acylating agents for the synthesis of esters by enzymatic transesterification in high yields. To enhance the usefulness of this system at low temperatures, we have searched for the gene coding for a cold-active lipolytic enzyme with a substrate preference for fatty acid vinyl esters and obtained it from the genomic library of *Acinetobacter* sp. strain no. 6, a psychrotroph isolated from Siberian soil. The gene (termed *aelh*, 777 bp) encoded a protein of 258 amino acids, and sequence analysis revealed that the enzyme shows a high sequence similarity to β-ketoadipate enol-lactone hydrolase involved in the β-ketoadipate pathway for the bacterial catabolism of benzoic acid. The *aelh* gene was expressed in the *E. coli* C600 cells under the control of *lac* promoter and the expression product was purified to homogeneity and characterized. It was a monomeric esterase preferentially catalyzing the hydrolysis of enol esters, such as fatty acid vinyl esters with a short-chain acyl group. The enzyme was strongly inhibited by phenylmethylsulfonyl fluoride, a specific inhibitor for serine hydrolases. The enzyme could also catalyze transesterification, for example, between vinyl propionate and propanol yielding propyl propionate at 4 °C. These results indicate the usefulness of an esterase (termed AELH) for the enzymatic synthesis of esters by transesterification using vinyl esters as an acyl donor.

Keywords: Psychrotroph; *Acinetobacter*; Transesterification; Vinyl esters; β-Ketoadipate enol-lactone hydrolase

1. Introduction

Esterases and lipases are a class of hydrolases catalyzing hydrolysis and transesterification of fatty acid esters and are currently one of the most important groups of biocatalysts for synthetic purposes [1–3]. Recently, the usefulness of fatty acid vinyl esters as the acylating agent for the synthesis of esters by lipase-catalyzed transesterification was demonstrated [4,5]. Transesterification is a reversible equilibrium process and the yield of the transfer product cannot

☆ The nucleotide sequence reported in this paper have been submitted to the DDBJ with accession number AB071607.

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1381-1177/02/$ – see front matter
PII: S1381-1177(01)00070-4

exceed that at equilibrium. However, this problem can be circumvented by the use of the fatty acid vinyl ester as an acyl donor:

$$R\text{–}COOCH{=}CH_2 + R'OH \rightleftarrows R\text{–}COOR' + CH_2{=}CH\text{–}OH \quad \text{(Scheme I)}$$

Vinyl alcohol is subsequently tautomerized to acetaldehyde:

$$CH_2{=}CH\text{–}OH \rightarrow CH_3CHO \quad \text{(Scheme II)}$$

This results in a shift of equilibrium of the enzymatic transesterification in the forward direction, which allows high yields of the enzymatic ester synthesis.

In order to enhance the usefulness of the fatty acid vinyl esters in the enzymatic syntheses of unstable esters, we have attempted to clone the gene coding for a cold-active lipolytic enzyme, which is highly reactive to fatty acid vinyl esters from cold-adapted microorganisms. We have identified a clone expressing an enzyme with high vinyl ester-hydrolyzing activity in the genomic library of *Acinetobacter* sp. strain no. 6, which was a psychrotrophic bacterium isolated from Siberian tundra soil. Primary structure analysis of the cloned gene revealed that the enzyme was highly similar in primary structure to β-ketoadipate enol-lactone hydrolase (EC 3.1.1.24, Fig. 1), which plays a key role in the bacterial utilization of aromatic compounds via the β-ketoadipate pathway [6–9]. We here describe the gene cloning, expression, purification, and characterization of an esterase (termed AELH) from *Acinetobacter* sp. strain no. 6 to show its usefulness on the basis

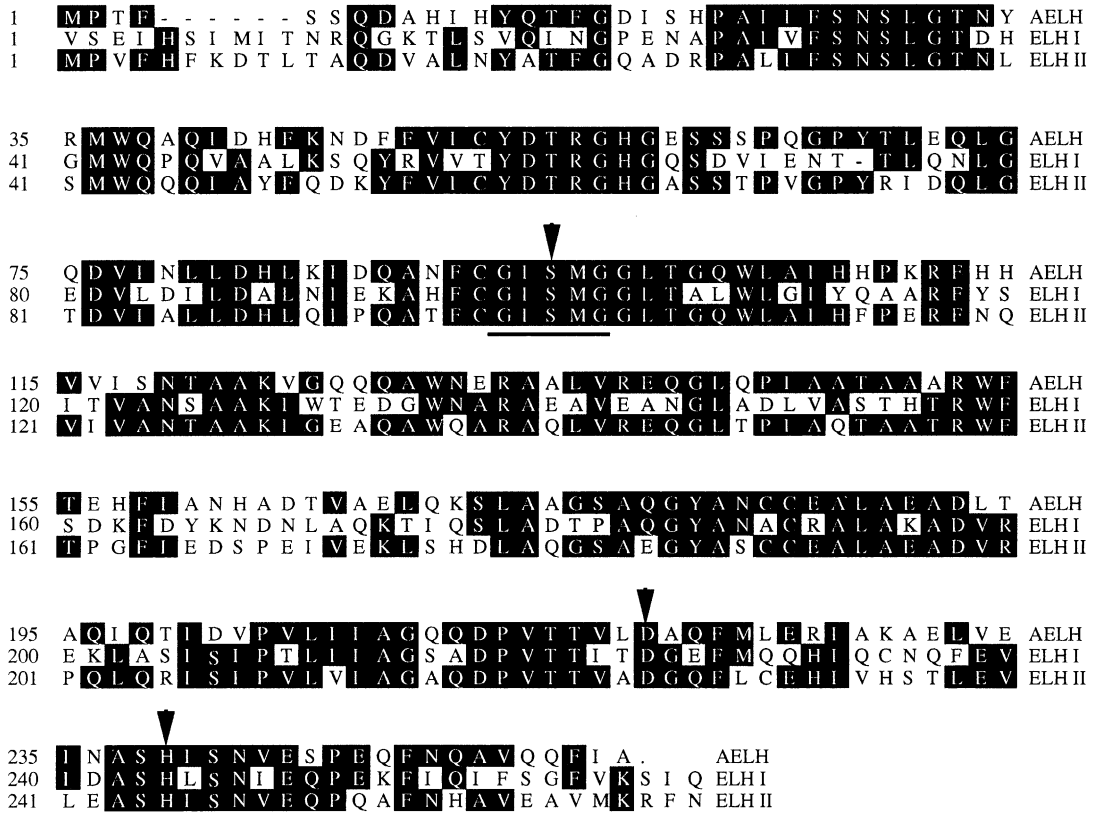

Fig. 1. Comparison of amino acid sequences between AELH (this study) and ELHs I and II [7,9] of the genus *Acinetobacter*. Numbers refer to the positions of the respective enzyme sequences from the *N*-termini. Identical residues among these three enzymes are shadowed and boxed. Putative catalytic triads (Ser95, Asp212, and His239) are labeled with black arrowheads. A consensus sequence, -Gly-X-Ser-X-Gly-, which is highly conserved among lipases, esterases, and other enzymes of the α/β hydrolase-fold superfamily of serine hydrolases is underlined.

of substrate specificity for enzymatic synthesis of esters by transesterification using vinyl esters as an acyl donor.

2. Experimental

2.1. Chemicals, bacterial strains and plasmid

Restriction enzymes, DNA polymerase, the ligation kit, and other enzymes for the DNA manipulation were purchased from Takara Shuzo (Kyoto, Japan) and were used according to the manufacturer's guide. All chemicals used in enzyme assays and buffers were of the highest purity available. The isolation and taxonomic characterization of *Acinetobacter* sp. no. 6 will be reported elsewhere [10]. *Escherichia coli* strain C600 and plasmid pUC118 were obtained from Takara Shuzo.

2.2. Cloning of the aelh gene

The *Acinetobacter* sp. no. 6 cells were grown in an LB medium (pH 7.2) at 28 °C with shaking for 16 h. The chromosomal DNA was isolated from the strain no. 6 cells by the procedure described by Saito and Miura [11]. The chromosomal DNA was partially digested with the restriction enzyme *Sau*3A1, and the partial digests were separated by 0.7% agarose gel electrophoresis. DNA fragments sized 1–10 kb were recovered from the agarose gels by electrophoresis and ligated with *Bam*HI-digested and dephosphorylated pUC118. *E. coli* C600 was transformed with the ligation mixture and grown at 20 °C after plating on an LB agar medium containing 1% (w/w) tributyrin, 50 μg/ml ampicillin, and 0.1 mM isopropyl β-D-thiogalactoside (IPTG). Immediately after the colonies were visible, the plates were incubated at 4 °C. Clear halos were formed at 4 °C around the colonies producing cold-active lipolytic enzymes. The other DNA manipulation procedures were performed by standard techniques [12]. DNA sequences were determined by the dideoxy-chain termination method [13] using an automated DNA sequencer, model 370A (Applied Biosystems), and analyzed and aligned using the DNASTAR software (DNASTAR Inc., USA). The entire sequence was confirmed in both orientations.

2.3. Enzyme and protein assays

2.3.1. Method I

The enzymatic hydrolysis of *p*-nitrophenyl butyrate was monitored by the amount of *p*-nitrophenol released at 25 °C. The standard assay mixture contained 1.0 μmol of *p*-nitrophenyl butyrate, 50 μmol of a potassium phosphate buffer (pH 7.5), 1 mg Triton X-100, and the enzyme in a final volume of 1.0 ml. The mixture without the enzyme was brought to 25 °C. The reaction was started by the addition of enzyme, and changes in absorbance at 400 nm were recorded with a spectrophotometer (Shimadzu UV-3000). The extinction coefficient for *p*-nitrophenol under these conditions was 14,630 $cm^{-1}\,M^{-1}$ [14].

2.3.2. Method II

Enzymatic hydrolysis of fatty acid ester was assayed by titration of the liberated fatty acid with NaOH as follows. The reaction mixture contained an appropriate amount (5–10 μmol) of a fatty acid ester, a 50 μmol potassium phosphate buffer, pH 7.5, and enzyme in a final volume of 1.0 ml. The reaction was started by the addition of enzyme. After incubation at 30 °C for 20 min, the reaction was stopped by the addition of 1 ml of in a 1:1 (v/v) mixture of acetone and ethanol and 10 μl of 1% (w/v) phenolphthalein in a 9:1 (v/v) mixture of ethanol and H_2O. The mixture was titrated with 0.05 M NaOH with gentle bubbling with N_2 gas until the mixture turned red, and the amount of fatty acid liberated was estimated from the amount of NaOH required. The blank did not contain the enzyme.

2.3.3. Method III

Enzymatic hydrolysis of fatty acid vinyl esters was spectrophotometrically monitored by a continuous assay coupled with aldehyde dehydrogenase [15]. The reaction mixture (final volume, 1.0 ml) consisted of 1–10 μmol of fatty acid vinyl ester, 50 μmol of a potassium phosphate buffer, pH 7.5, 50 μmol of NAD^+, and 0.02 unit of aldehyde dehydrogenase (Sigma) and was previously incubated at 25 °C. The reaction was started by the addition of enzyme, and changes in absorbance at 340 nm due to the formation of NADH were monitored. The extinction coefficient for NADH was 6220 $cm^{-1}\,M^{-1}$ [15].

The protein concentration was determined by the method of Bradford [16] using a kit (Bio-Rad) with bovine serum albumin as a standard.

2.4. Preparation of cell-free extract and enzyme purification

E. coli transformant cells were grown at 28 °C in 500 ml of L-broth containing 200 μg/ml ampicillin with shaking. At 3 h after the inoculation of the cells, IPTG was added to the culture broth to a final concentration of 2 mM, followed by further 15-h cultivation. The cells were collected by centrifugation, washed with 0.85% NaCl, resuspended in a 10 mM sodium phosphate buffer (pH 7.2), and disrupted by 30 cycles of ultrasonication (at 10 kHz for 30 s followed by an interval of 30 s). After removal of cell debris by centrifugation, the supernatant was used for the enzyme purification.

All operations described below were performed at 0–5 °C. Streptomycin sulfate was slowly added to the supernatant solution to a final concentration of 2% (w/v), and the mixture was stirred at 4 °C for 1 h, followed by centrifugation. The supernatant was dialyzed overnight against a 0.05 M Tris–HCl buffer, pH 8.0 (termed buffer A), containing 0.1 M NaCl, and the enzyme solution was subsequently applied to a column of DEAE-cellulose (2.7 cm × 35 cm, Nacalai Tesque) which was previously equilibrated with the dialysis buffer. The column was extensively washed with the equilibration buffer until no further protein was eluted. The enzyme activity was eluted with equilibration buffer A containing 0.15 M NaCl. Active fractions were pooled and concentrated with an Amicon PM10 ultrafiltration membrane. After the concentrate was dialyzed against a 0.8 M potassium phosphate buffer, pH 8.0, it was applied to a column of Butyl-Toyopearl (2.7 cm × 18 cm, TOSOH) equilibrated with the dialysis buffer, and the enzyme was eluted with the same buffer. Active fractions were pooled and concentrated. After dialysis against buffer A, the enzyme solution was subjected to rechromatography on DEAE-cellulose equilibrated with buffer A. After successive washing of the column with buffer A followed by buffer A containing 0.1 M NaCl (2 column volumes each), the enzyme was eluted with the buffer containing 0.15 M NaCl. Active fractions were pooled, concentrated, dialyzed against a 5 mM potassium phosphate buffer, pH 7.0, and then applied to a column of Gigapite (2.7 cm × 18 cm, Seikagaku Co.) equilibrated with the dialysis buffer. The enzyme was eluted with a 50 mM potassium phosphate buffer, pH 7.0. Active fractions were pooled and concentrated. The enzyme was then subjected to Fast Protein Liquid Chromatography (FPLC) with a superose 12 column (1.0 cm × 30 cm, AmershamPharmacia Biotech) equilibrated with a 0.01 M potassium phosphate buffer, pH 7.2, containing 0.15 M NaCl. The column was developed with the equilibration buffer at a flow rate of 0.5 ml/min by monitoring the absorbance at 280 nm.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 12.5% gels by the method of Laemmli [17]. Protein was stained with Coomassie brilliant blue R250 and destained in a 10:15:175 (v/v) mixture of ethanol, acetic acid, and water.

2.5. Determination of molecular weight

The molecular weight of the native enzyme was estimated by FPLC on a Superose 12 column (1.0 cm × 30 cm) under the same conditions as described above. For calibration, yeast glutamate dehydrogenase [molecular weight (M_r), 290,000], pig liver lactate dehydrogenase (M_r,142,000), yeast enolase (M_r, 67,000), yeast adenylate kinase (M_r, 32,000), and horse heart cytochrome c (M_r, 12,400) from Oriental Yeast Co., Osaka, Japan, were chromatographed under the same conditions. The subunit M_r was estimated by gel electrophoresis with a 12% gel in the presence of SDS [17]. For calibration, LMW calibration kit proteins (Amersham Pharmacia Biotech) were electrophoresed under the same conditions.

2.6. Effect of various reagents on enzyme activity

The enzyme was incubated at 25 °C for 1 h in a 0.05 M potassium phosphate buffer, pH 7.5, containing one of the following additives (final concentration, 1 mM): CH_3SO_3H, phenylmethylsulfonyl fluoride, sodium dodecyl sulfate, ethylenediaminetetraacetic acid, $CaCl_2$, $CuCl_2$, $MnSO_4$, $MgCl_2$, $FeCl_3$, and $ZnCl_2$. The remaining activity was then measured by assay method I. The activity of the enzyme treated as above without additives was taken to be 100%.

2.7. Stability studies

For the thermal stability studies, the enzyme was incubated in a 0.05 M potassium phosphate buffer, pH 7.5, at 30, 40, and 50 °C. At time intervals, aliquots were withdrawn and placed into tubes in ice and assayed for their remaining activity by assay method I. For the pH stability studies, the enzyme was incubated at 30 °C for 30 min in a 0.05 M potassium phosphate buffer, whose pH was adjusted at 3–9. After incubation, an aliquot was withdrawn and assayed for remaining activity by assay method I.

2.8. Immobilization of AELH and transesterification

The immobilized AELH was prepared as follows. The resin (Hyflo Super-Cel, Nacalai Tesque, 20 g) was suspended in 100 ml of a 0.05 M potassium phosphate buffer, pH 7.5, and allowed to stand at 4 °C for 1 h. After the buffer was removed by decantation, the enzyme solution (concentration, 8 mg protein/ml, 100 ml) was added, and the resultant mixture were left at 4 °C for 3 h with gentle stirring. The mixture was then lyophilized to obtain the immobilized AELH.

Enzymatic transesterification using vinyl propionate as an acyl donor was carried out as follows. The reaction mixture (final volume, 0.4 ml in *n*-hexane) consisted of 60 μmol of vinyl propionate, 120 μmol of propanol, 10 μg of the immobilized enzyme, and 2 μl of *n*-undecane as an internal standard. The reaction was started by the addition of the immobilized enzyme and the mixture was incubated at 4 °C for 24 h with gentle mixing. Product esters were analyzed by gas chromatography using a Shimadzu GC14A system with an Ulbon HR-101 capillary column equipped with a flame ionization detector. The column temperature was programmed from 50 to 120 °C at a rate of 5 °C/min, and the injector port and the detector oven temperatures were 250 and 300 °C, respectively.

3. Results and discussion

3.1. Isolation of a clone expressing vinyl propionate-hydrolyzing activity

The genomic gene libraries of several cold-adapted bacterial strains available from our laboratory [10,18,19] were screened for the expression of lipolytic activity on the basis of the formation of clear halos at 4 °C around the colonies grown at 20 °C on tributyrin plates. Crude extracts were prepared from the cells of the lipolytic transformants and then examined for the vinyl propionate-hydrolyzing activities by assay method III. The best producer of this activity was obtained from the genomic gene library of *Acinetobacter* sp. strain no. 6, a psychrotrophic bacterium isolated from Siberian tundra soil [10], which was selected for further analysis. Restriction enzyme analysis of this clone showed that it contained a 2-kb insert whose nucleotide sequence was determined as described in Section 2.2.

The insert contained a single open reading frame (ORF) consisting of 777 bp that encodes a protein of 258 amino acids. A putative Shine–Dalgarno sequence and an inverted repeat sequence were found upstream and downstream of the ORF, respectively (not shown). The predicted M_r of the enzyme was calculated to be 28,423. In the extensive search for sequence similarity in the databases, the amino acid sequence deduced from the ORF (termed *aelh* gene) showed the highest similarity to the sequences of β-ketoadipate enol lactone hydrolases (ELH) I (identity, 42%) and II (62%) of *A. calcoaceticus* [7,9] (Fig. 1). The genes encoding ELHs I and II, called *pcaD* and *catD* genes, respectively, are involved in the *ben-cat* super-operonic gene cluster, which encodes a series of enzymes required for the catabolism of benzoic acid via the β-ketoadipate pathway in some bacteria. However, molecular and catalytic properties of ELHs I and II are not known in detail. The nucleotide and deduced amino acid sequences of the *aelh* gene are more similar to the *catD* gene than to that of the *pcaD* gene and the upstream region of the *aelh* gene showed a high sequence similarity to the *catJ* region, which also lies upstream of *catD*, implying that AELH may be a homolog of β-ketoadipate enol lactone hydrolases corresponding to ELH II of *A. calcoaceticus*. Interestingly, *Acinetobacter* strain No. 6 could also grow on benzoic acid as a sole carbon source [10], suggesting that the β-ketoadipate pathway should thus also exist in strain No. 6. However, it remains to be determined whether AELH is indeed involved in the catabolism of benzoic acid in the *Acinetobacter* cells, and this point must be clarified in future studies.

The primary structure of AELH and ELHs contained a consensus sequence, -Gly-X-Ser-X-Gly- (Fig. 1), which is highly conserved among lipases, esterases, and other enzymes of the α/β hydrolase-fold superfamily of serine hydrolases [20]. All structurally characterized serine hydrolases contains an Asp(Glu)-His-Ser catalytic triad at their active centers [21], and, despite a low overall sequence similarity of AELH and ELHs to other serine hydrolases, multiple alignment of the amino acid sequences allowed us to identify a potential catalytic triad in AELH unambiguously (Ser95, Asp 212, and His239) and ELHs (see Fig. 1).

3.2. Expression, purification, and physico-chemical characterization of AELH

The recombinant *aelh* gene was effectively expressed in the *E. coli* C600 transformant cells under the control of a *lac* promoter, judging from the fact that cultivation in the presence of 2 mM IPTG gave higher levels of the gene expression than in the absence of IPTG. The highest expression was obtained when the transformant cells were grown at 28 °C for 15 h (see Section 2.4) and approximately 20% of the total proteins of the transformant cells were occupied by the expressed AELH, which could be purified to apparent homogeneity by a combination of ion exchange, hydrophobic interaction, hydroxyapatite, and gel filtration chromatographies in an activity yield of 6% (Fig. 2, Table 1).

When the purified enzyme was subjected to gel filtration chromatography on Superose 12, it was eluted at an elution volume corresponding to a native M_r of 27,000. Subunit M_r of the enzyme was estimated by SDS-PAGE to be 28200, which is consistent with the value (28423) predicted from the deduce amino acid sequence. These results indicate that AELH is monomeric.

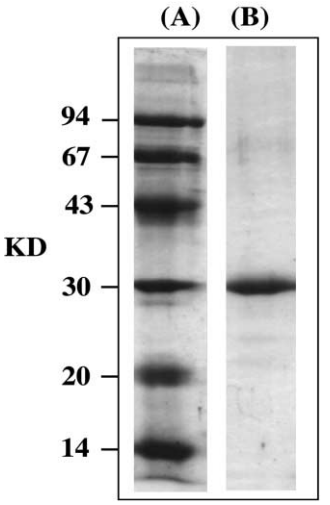

Fig. 2. Polyacrylamide gel electrophoresis of AELH. Lane (A): size markers (LMW electrophoresis calibration kit, Amersham Pharmacia Biotech). Lane (B): the preparation after Superose 12 column.

The enzyme retained more than 90% of its original activity after incubation at 40 °C for 30 min. Also, the activity was not lost when the enzyme was incubated at 30 °C for 30 min in 0.05 M potassium phosphate (pH 5.0–9.3). However, the enzyme was inactivated at higher temperatures (e.g. 50 °C) and under more acidic conditions.

3.3. Substrate specificity and catalytic properties

Table 2 shows the relative activities for hydrolysis of a wide variety of esters, lactones, and triglycerides

Table 1
Purification of recombinant AELH

	Total protein (mg)	Total activity (U)[a]	Specific activity (U/mg)	Fold	Yield (%)
Crude extract	110	410	3.6	1.0	100
DEAE-cellulose (1)	17	120	7.3	2.0	30
Butyl-toyopearl	11	93	8.9	2.5	23
DEAE-cellulose (2)	7.0	77	11	3.2	19
Gigapite	5.0	58	12	3.3	14
Superose 12	2.0	26	13	3.7	6.0

[a] One unit (U) of enzyme is defined as the amount that catalyzed the hydrolysis of 1 μmol of *p*-nitrophenyl butyrate under the conditions of assay method I.

Table 2
Substrate specificity of AELH

Substrate	Relative activity (%)
p-Nitrophenyl acetate[a,b]	100
p-Nitrophenyl propionate[a]	43
p-Nitrophenyl butyrate[a]	70
p-Nitrophenyl hexanoate[a]	17
p-Nitrophenyl octanoate[a]	2.0
Ethyl acetate[b]	21
Ethyl butyrate[b]	16
Ethyl octanoate[b]	33
2-Bromoethyl octanoate[b]	6.7
2-Chloroethyl propionate[b]	76
Vinyl acetate[b]	4700
Vinyl propionate[b]	5400
Vinyl butyrate[b]	490
Vinyl hexanoate[b]	140
Vinyl decanoate[b]	14
Vinyl laurate[b]	3.3
Isopropenyl acetate[b]	110
Triacetin[b]	270
Tributyrin[b]	5.6
Phenyl acetate[b]	93
p-Nitrophenyl laurate[c]	
p-Nitrophenyl palmitate[c]	
p-Nitrophenyl stearate[c]	
Ethyl decanoate[c]	
β-Butyrolactone[c]	
DL-1,4-Pantolactone[c]	
Tricaprylin[c]	
Benzyl acetate[c]	

[a] By assay method I.
[b] By assay method II.
[c] These were inert as substrates relative activity, less than 1%.

as determined by assay methods I and II. AELH is the most active on vinyl ester of the short-chain fatty acids (C_2–C_6). These results are consistent with the fact that this enzyme is a homolog of ELH participating in the β-ketoadipate pathway because β-ketoadipate enol-lactone, which is a physiological substrate for ELH, is also an enol ester. The enzyme could also hydrolyze the phenyl and *p*-nitrophenyl esters of short aliphatic fatty acids, which can also be regarded as enol esters. Fatty acid ethyl esters were poor substrates. Esters with longer acyl chain generally exhibited lower reactivity to the enzyme. Methyl esters of aromatic carboxylic acids and lactones were virtually inert as substrates for the enzyme. The K_m and V_{max} values of AELH for the hydrolysis of *p*-nitrophenyl butyrate and several vinyl esters were determined (Table 3). Judging from the V_{max} and V_{max}/K_m values, vinyl acetate was the best substrate for the enzyme among the substrates examined, although K_m value for vinyl esters was 6-times larger than that of a fatty acid aryl ester (i.e. *p*-nitrophenyl butyrate). Therefore, we conclude that AELH is an esterase with a high substrate preference for enol esters, especially for vinyl esters, of short acyl chain length.

With a continuous assay, it was possible to measure the activity of enzyme over temperature range from 5–50 °C (Fig. 3). An Arrherenius plot of the data yielded an activation energy of 11.2 kcal/mol. For comparison, an activation energy of 28.4 kcal/mol was obtained with the same reaction catalyzed by a lipase of mesophilic origin (Lipase PS(tm), Amano Enzyme Co., Nagoya, Japan). The optimal pH was found to be approximately 7.5–8.0, as was observed for ELH II [22].

The following (final concentration, 1 mM) did not affect the enzyme activity (remaining activity, more than 80%): CH_3SO_3H, ethylenediaminetetraacetic acid, $CaCl_2$, $MnSO_4$, $MgCl_2$, $FeCl_3$, and $ZnCl_2$. However, the enzyme was inhibited by $CuSO_4$ (remaining activity, 66%), sodium dodecyl sulfate (14%), and phenylmethylsulfonyl fluoride (28%). That the enzyme was inhibited by phenylmethylsulfonyl fluoride, a specific inhibitor of serine hydrolases, is

Table 3
Kinetic parameters

Substrate	V_{max} (μmol/(min mg)	K_m (mM)	V_{max}/K_m (μmol/(min mg mM)
p-Nitrophenyl butyrate[a]	8.7	1.0	8.7
Vinyl acetate[b]	700	22	32
Vinyl propionate[b]	270	14	19
Vinyl butyrate[b]	21	5.9	3.6

[a] By assay method I.
[b] By assay method III.

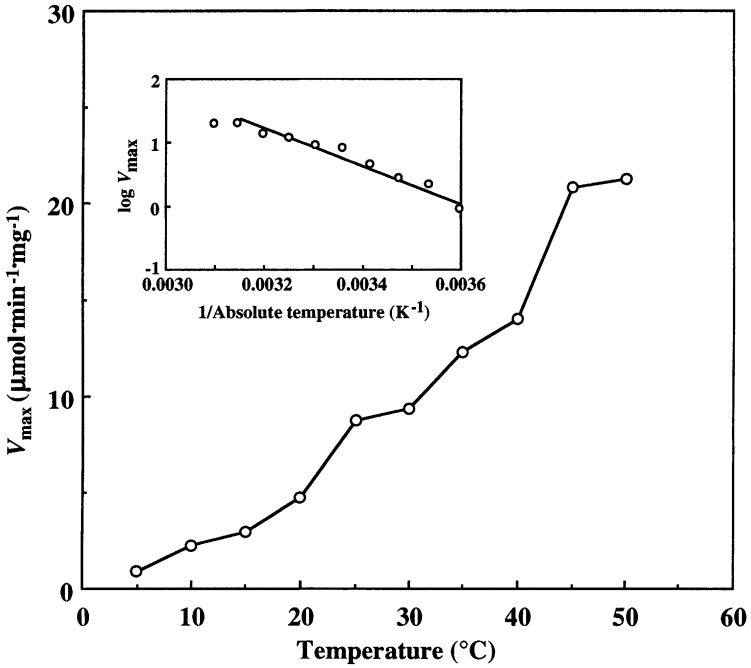

Fig. 3. Temperature-activity profile and its Arrhenius plot (inset) of *p*-nitrophenyl butyrate hydrolysis catalyzed by AELH. The effect of temperature on enzyme activity was examined essentially by assay method I, except that the reaction mixture (without enzyme) was preincubated for 5 min at a temperature of 5–50 °C. For the Arrhenius plots, the logarithm of V_{max} values is plotted as a function of the reciprocal of absolute temperature.

consistent with the fact that the AELH enzyme is structurally related to the "serine hydrolase super family" enzymes.

3.4. Transesterification

To demonstrate the AELH-catalyzed synthesis of esters by transesterification at low temperatures using vinyl esters as acyl donors, we first examined the immobilization of AELH on resins and found that the Hyflo Super-Cel resin gave the highest yields of immobilization (not shown). When the immobilized AELH was allowed to react with vinyl propionate and two equivalents of propanol in *n*-hexane at 4 °C for 24 h, 76% of vinyl propionate was converted to propyl propionate. The yield of the transfer product was higher than the yield (38%, determined experimentally) of the enzymatic transesterification between propanol and ethyl propionate used as a control acyl donor.

In conclusion, The AELH was very similar in primary structure to β-ketoadipate enol-lactone hydrolase involved in the β-ketoadipate pathway for bacterial benzoate catabolism, and was an esterase with substrate preference for enol esters. The enzyme's specificity warrants its usefulness in the synthesis of esters by enzymatic transesterification using vinyl esters.

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