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TEMPERATURE AND DMSO INCREASE THE ENANTIOSELECTIVITY OF HYDROLYSIS OF METHYL ALKYL DIMETHYLMALONATES CATALYZED BY PIG LIVER ESTERASE

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Abstract

Hydrolyses of methyl alkyl dimethylmalonates catalyzed by pig liver esterase were carried out at different temperatures. At 35°C, the stereochemical yields of the half-ester product are equal to or slightly greater than those obtained at 15°C. However, stereoselectivity is higher in the presence of 25% DMSO, and temperature has no effect on the stereoselectivity of the hydrolysis reaction.

One of the most striking properties of enzymes is their ability to discriminate enantiotopic groups in prochiral or *meso* compounds, resulting in high chemical and optical yields of products. This property of pig liver esterase has been widely exploited for the preparation of chiral intermediates for asymmetric synthesis.[1] The hydrolysis of dialkyl malonate diesters by pig liver esterase proceeds to give half-esters with good to excellent stereochemical purity (Equation 1).[2-4] Bjorkling and coworkers found that addition of 25% to 50% DMSO increased the stereoselectivity of the hydrolysis reaction.[4] However, the effects of temperature on the

Equation 1

reaction stereoselectivity were not previously determined. We have recently shown that temperature has a dramatic effect on the stereospecificity of oxidation of secondary alcohols by a secondary alcohol dehydrogenase (SADH) from *Thermoanaerobacter ethanolicus*.[5,6] We now report the effects of temperature and DMSO on the stereoselectivity of hydrolysis of methyl alkyl dimethylmalonates catalyzed by pig liver esterase.

Table I

Hydrolysis of Methyl Alkyl Dimethylmalonates by Pig Liver Esterase

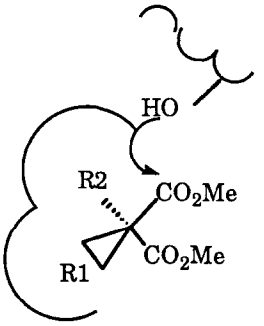
	DMSO: 0%				DMSO: 25%			
R	T°C	TIME hr	YIELD %	e.e.%	T°C	TIME hr	YIELD %	e.e.%
ETHYL (**1**)	15	4:45	96	60(S)	15	3:00	96	62(S)
	25	3:00	77	64(S)	25	2:00	96	62(S)
	35	1:30	71	65(S)	35	0:30	98	64(S)
PROPYL (**2**)	15	6:25	73	45(S)	15	6:20	87	50(S)
	25	4:35	77	45(S)	25	3:00	98	50(S)
	35	1:40	71	46(S)	35	1:40	88	51(S)
BUTYL (**3**)	15	5:20	70	46(S)	15	5:00	87	57(S)
	25	2:00	89	50(S)	25	4:00	86	57(S)
	35	0:30	89	51(S)	35	1:00	87	57(S)
HEPTYL (**4**)	15	23:00	77	80(R)	15	24:00	77	88(R)
	25	12:40	80	83(R)	25	13:45	87	88(R)
	35	8:00	80	84(R)	35	7:10	82	88(R)

The methyl alkyl dimethylmalonates were prepared by alkylation of dimethyl malonate.[7] Reaction with pig liver esterase[8] was performed as previously described by Bjorkling and coworkers[2-4], and the stereochemical purities of products were determined by 300 MHz ^{1}H NMR of the salts with (S)-1-phenylethylamine[9]. The results of these studies are presented in Table I[10]. Between 15° and 35°, there is either no change (**2**) or a modest increase (about 5%) (**1**,**3**,**4**) in the stereochemical purity of the half-ester products. However, in the presence of 25% DMSO, there is no significant change in the e.e's of the products obtained at different temperatures. Thus, the effects of changing temperature and DMSO on the stereoselectivity are not additive, suggesting a common mechanistic basis.

The intriguing feature of these reactions is the reversal of stereoselectivity as the alkyl chain increases in length from butyl to heptyl, as previously observed by Bjorkling et al.[3] A similar reversal is seen in the reduction of ketones and oxidation of alcohols by SADH.[11] We demonstrated that the temperature dependence of the stereospecificity of SADH is due to the activation entropy difference for the reaction of the two enantiomers[5,6]. The differences in stereoselectivity observed in the present work are too small for an accurate determination of the activation parameters. However, the increases in stereochemical purity observed at higher temperatures or in the presence of DMSO suggests that the activation entropy, possibly due to hydrophobic interactions, must also play a significant role in controlling the stereochemical

outcome of pig liver esterase reactions. These results are in contrast to those of Lam et al., who found that the hydrolysis of 3-methyl dimethyl glutarate gives optimal stereoselectivity at -10°C in 20% methanol.[12] Pig liver esterase is a serine esterase, and the enzyme must have two alkyl binding pockets, either of which can accomodate alkyl chains at least as large as n-butyl, that orient one of the ester groups for reaction (Scheme I).[12] The stereoselectivity of the hydrolysis reaction is determined by the preferential binding of the methyl and alkyl groups in these binding sites. When R1 is ethyl, n-propyl, or n-butyl and R2 is methyl, the (S)-malonate half-ester will be obtained, as we have observed (Table I). If R1 is methyl and R2 is a larger alkyl group, then the (R)-product will result. The stereochemical reversal which occurs between the n-butyl and n-heptyl compounds (Table I) may reflect steric congestion in binding of the larger n-heptyl group to the R1 pocket.

Scheme I



Although the effect of temperature which we have observed on the reaction stereoselectivity is modest, the results are of practical importance. Most preparative reactions of pig liver esterase have been performed at room temperature. Since optical purities of the malonate half-ester products are identical or slightly higher at higher temperatures, while reaction times are significantly reduced (Table I), the data presented herein suggest that pig liver esterase hydrolyses of methyl alkyl dimethylmalonates (and likely of other esters) should be performed in 25% DMSO at 35° for optimal results[13].

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8. The esterase was obtained from Sigma (Lot # 107F-8235) as a suspension in $(NH_4)_2SO_4$. The reactions were carried out in 0.375 M Tris-HCl, pH 7.5, using fixed amounts of enzyme (0.95 mg; 318 units) and malonate (100μL), and were monitored by G.C. of diethyl ether extracts on a Varian 3300 gas chromatograph using a Chirasil-Val 25-m capillary column (Alltech).
9. Solutions of the monoesters (ca. 10 mg) in $CDCl_3$ (0.6 mL) containing 20 μL pure (S)-1-phenylethylamine (Aldrich) were examined by 300 MHz ^1H NMR. The enantiomeric composition was determined by measurement of the height of the methyl singlet of each enantiomer.
10. The time given in Table I is that required for consumption of all reagent.
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13. At 45 °C, using 25 % DMSO, the reaction of *4* was completed in one hour and gave the same e.e. (88%) as at lower temperatures, but the isolated yield was reduced to 40%. The reason for the lower yield is not known, but may be due to competing non-enzymatic reactions of the ester at the higher temperature.