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The Effect of Dimethyl Sulfoxide on the Enantioselectivity in the Pig Liver Esterase Catalyzed Hydrolysis of Dialkylated Propanedioic Acid Dimethyl Esters

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The effect of dimethyl sulfoxide (DMSO) on the enantioselectivity in the pig liver esterase (EC 3.1.1.1) catalyzed hydrolysis of dialkylated propanedioic acid diesters was studied. Increasing concentrations of DMSO increased the ratio of the (*R*)-enantiomers of the produced monoesters. This effect was not due to the change in pH_{app} caused by the solvent. The reaction rate was lowered to one-tenth of the rate without DMSO when the DMSO concentration was increased to 50%.

INTRODUCTION

Enzyme catalyzed transformations *in vitro* have become an important tool for the synthesis of chiral compounds. The solubility of hydrophobic substrates will be low in aqueous solutions unless substantial amounts of organic solvents are added. The effect of cosolvent addition on the kinetic parameters k_{cat} and K_m has been extensively studied for different enzymes and solvent mixtures. However, little is known on the influence of organic cosolvents on the stereospecificity of enzyme catalyzed reactions. It has previously been shown that the enantioselectivity in the α-chymotrypsin catalyzed hydrolysis of methyl-2-acetamido-2-phenylacetate is decreased when the concentration of organic solvents is increased in the reaction medium (*1*). Recently we reported that the enantioselectivity obtained in the pig liver esterase catalyzed hydrolysis of benzylmethylpropanedioic acid dimethyl ester and derivatives thereof could be improved by addition of up to 50% (v/v) dimethyl sulfoxide (DMSO) (*2*). In this paper we report further investigations of this effect.

EXPERIMENTAL PROCEDURES

Enzyme and substrates. Pig liver esterase (EC 3.1.1.1) was purchased, as a suspension in aqueous $(NH_4)_2SO_4$, from Sigma. The suspension was centrifuged and the enzyme pellet was dissolved in the buffer used for the experiment. Dialky-

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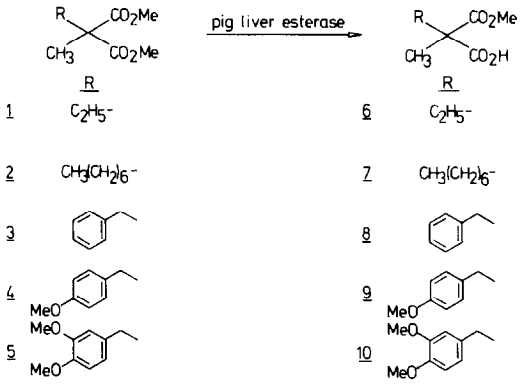

SCHEME 1

lated propanedioic acid dimethyl esters were prepared by alkylation of propanedioic acid dimethyl esters (*3*).

Analytical methods. Determinations of the enantiomeric excess (e.e.) of the monoesters were made by NMR studies in the presence of optically pure 1-phenylethylamine. The absolute configurations of **9** and **10** (Scheme 1) were determined by comparison with **8** of known configuration (*3*).

Incubations. Reactions were carried out in 20-ml buffered batches containing DMSO, pig liver esterase (1–2 mg) and substrate (15 mM). Reaction temperature was kept at 22–25°C. For substrates **1** and **2** the buffer was 0.375 M Tris–HCl, pH 7.5, and for substrates **3–5** the buffer was 0.095 M potassium phosphate, pH 7.0. The buffer was 0.2 M Mes–NaOH (2-(*N*-morpholino)ethanesulfonic acid) when the 50% (v/v) DMSO medium was pH_{app} adjusted to 7.19. This is the pH_{app} measured with the glass electrode for 0.095 M potassium phosphate, pH 7.0, 5% DMSO. When the 5% DMSO medium was pH_{app} adjusted to 7.9 and 8.7, 0.2 M Tris–HCl was used.

Rate determinations. The initial reaction velocities were determined in a Radiometer pH–stat under N_2 at 30°C and maintained at pH 7.5 with 0.010 M NaOH. The reaction mixture contained 12.7 mM substrate **5**, 0.04 mg pig liver esterase, 0.025 M $CaCl_2$, and DMSO in a total volume of 2.0 ml. One unit of enzyme activity was defined as the amount of enzyme catalyzing the hydrolysis of 1 μmol of substrate/min under the conditions described.

RESULTS

The ratios of the (*R*)-enantiomers in the monoester products of pig liver esterase hydrolysis of dialkylated propanedioic acid diesters (Scheme 1) were increased when the DMSO concentration in the incubation medium was increased.

The (*R*)-enantiomer of the monoester of dimethoxybenzyl methylpropanoic acid is one possible synthon for *L*-α-methyl Dopa (*S*-α-methyl-3,4-dihydroxyphenylalanine) (*2*). The optimization of the conditions for pig liver esterase hy-

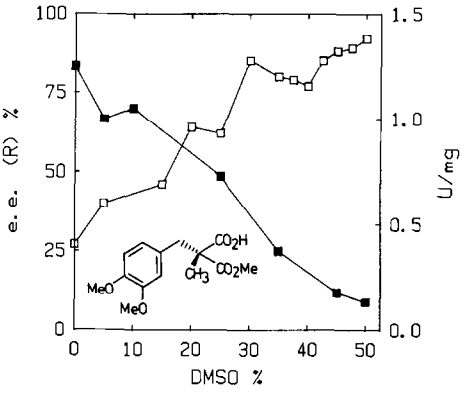

FIG. 1. Specific activity of the pig liver esterase catalyzed hydrolysis of **5** and enantiomeric excess of product **10** at various DMSO concentrations (■, U/mg (U = μmol/min); □, e.e. (*R*) % of **10**).

drolysis of the corresponding diester of this attractive synthon is shown in Fig. 1. The results in the figure show that the enantiomeric excess could be increased to a useful level (92%) at the expense of specific activity of the enzyme. A similar dependence of enantiomeric excess on increased DMSO concentration was observed also for the other monoesters produced by pig liver esterase hydrolysis (Fig. 2).

The pH_{app} as measured with a glass electrode of a 0.095 M potassium phosphate buffer, pH 7.0, was 7.2, 7.8, or 8.7 when the DMSO concentration was made 5, 25, or 50%, respectively. To distinguish pH from DMSO effects, 5% DMSO batches were adjusted to pH_{app} 7.9 and 8.7. Further 25 and 50% DMSO batches were set to pH_{app} 7.2. Using these batches in the pig liver esterase catalyzed hydrolysis of substrate we concluded that the effect on the enantiomeric excess imposed by the pH_{app} shift was negligible in comparison with the effect of DMSO.

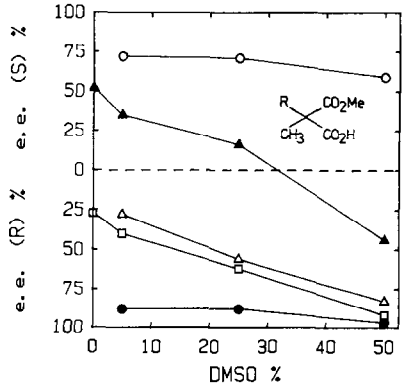

FIG. 2. Enantiomeric excess of the monoesters of dialkylated propanedioic acids obtained by hydrolysis of the corresponding diesters by pig liver esterase at various DMSO concentrations (○, **6**; ●, **7**; ▲, **8**; △, **9**; □, **10**).

DISCUSSION

In our recent investigation on the selectivity of pig liver esterase catalyzed hydrolysis of propanedioic acid diesters we studied substrate structural effects on the kinetics and the enantioselectivity. We reported on a change in enantioselectivity of the enzymatic hydrolysis, depending on the chain length of one of the alkyl groups in the dialkylated propanedioic diesters (*3*). Pig liver esterase is a serine hydrolase (*4*). The structure of the active site has not been very well investigated, but it is supposed to have one or more hydrophobic zones which interact with the acyl and alkyl group of the ester substrate (*5*).

According to our model a short side chain can interact with a hydrophobic pocket but for long alkyl groups this site is too small causing, in our case, an inversion of the enantioselectivity from (*S*) to (*R*). Using this model the observed change in enantioselectivity for the hydrolysis of dialkylated propanedioic diesters with increased concentration of DMSO can be interpreted as a hydrophobic effect. When the hydrophobicity of the solvent is increased the tendency for the more hydrophobic alkyl side chains to bind to the hydrophobic site becomes weakened and we observe an increase of the (*R*)-enantiomer. In a more polar medium a larger part of the long alkyl side chains will bind due to the increased hydrophobic interaction. Our results are in good accordance with the general rule proposed by Maurel: "The larger the contribution of the hydrophobic interactions in a binding process, the larger the organic solvent effect on $K_{m(app)}$" (*6*).

One important effect of cosolvent addition is the shift in pH_{app}. In mixed solvents the pH as measured with a glass electrode does not have the same meaning as in neat buffers (*7*).

Even though it is known that organic solvents affect the p*K* of buffer components and of amino acids (*8*) it is reported that the maximum acid phosphatase hydrolytic activity is obtained when the pH of the buffer used for making the hydroorganic mixture is the same as the pH of maximum activity without cosolvent (*9*). This result indicates that the glass electrode value of pH_{app} for the solvent mixture is not a good measure of the pH experienced on the enzymatic surface.

To investigate the possibility that the observed DMSO effect on enantioselectivity was coupled to the pH_{app} change, batches were adjusted to pH_{app} values corresponding to higher DMSO concentrations. When compared with batches with the same DMSO concentrations without prior pH adjustments no effect on the enantiomeric excess was obtained. This shows that pH changes are not responsible for the observed effect and supports the hypothesis that the major reason is the change in hydrophobicity caused by DMSO.

In organic synthesis enantiomeric excess is usually used as a measure of the optical purity. However, to evaluate the changes imposed by DMSO on the enzymatic level, we should compare the changes in rate constants for formation of the (*R*)- and (*S*)-enantiomers. For prochiral substrates, as those used in this study, the amounts of respective enantiomer produced are directly proportional to the rate constants for formation of each enantiomer independent of the percentage of substrate converted.

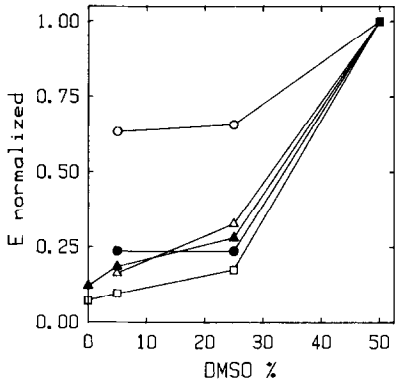

FIG. 3. Normalized enantiomeric ratios, E, at various DMSO concentrations. E was normalized to 1 at 50% DMSO for all substrates (○, **6**; ●, **7**; ▲, **8**; △, **9**; □, **10**).

The enantiomeric ratio, E, is in this case equal to the ratio of the rate constants (*10*). In the plot of normalized E values versus DMSO concentration (Fig. 3), it can be seen that in all cases the largest changes in relative rate constants occur at high DMSO concentrations. The effect of DMSO on the enantiomeric ratio is very similar for all substrates, except for that with two short alkyl substituents **1** and does not reflect the large differences in changes of enantiomeric excess. For example, the optical purity of the monoester **8** obtained upon hydrolysis of the benzyl side chain diester **3** changed from 52% e.e. (*S*) without DMSO to 44% e.e. (*R*) with 50% DMSO. With respect to the enantiomeric excess the change was less marked for product **7** which shifted from 88% e.e. (*R*) to 97% e.e. (*R*) with 5 and 50% DMSO, respectively. The effect on these two substrates is not distinguished, when the enantiomeric ratios are used instead. It is thus seen that DMSO has the same effect on all substrates used.

The reaction velocity of pig liver esterase shows an inverted relationship to the DMSO concentration. Assuming that the enzyme mechanism includes a deacylation step with water acting as a nucleophile the decreased water concentration should give a reduced rate of hydrolysis (*11*).

Another interpretation of the rate lowering effect is competitive inhibition or rate constant lowering imposed by DMSO. It has been shown that DMSO inhibits the hydrolysis of different substrates by α-chymotrypsin (*12*) and by subtilisin (*13*). Others have investigated the dioxane inhibition of α-chymotrypsin (*14*) and of trypsin (*15*). Besides the possibility that DMSO acts as a competitive inhibitor the solvent may also cause partial denaturation or aggregation of the enzyme. Furthermore, when the medium becomes less polar impaired binding of the hydrophobic substrate may lower the rate of hydrolysis.

A thorough investigation of the pig liver esterase active site is hampered by the difficulty of obtaining a homogenous enzyme preparation. In this investigation we have made the assumption that cosolvent addition is not causing a differentiated effect on possible isoenzymes having different enantiospecificities. A recent in-

vestigation of the isoenzymes separated by isoelectric focusing revealed that the stereospecificity of each isoenzyme was fundamentally the same (*16*).

To our knowledge the only investigation published so far on the influence on the stereospecificity of enzymes by organic solvents is α-chymotrypsin catalyzed hydrolysis of methyl-2-acetamido-2-phenylacetate (*1*). In this study the stereospecific ratio was reduced by the addition of organic solvents. Our results show that this is not a general phenomenon but for other enzymes and substrates organic solvents can instead improve the enantioselectivity.

ACKNOWLEDGMENTS

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