Effect of Coenzyme Analogues on Enantioselectivity of Alcohol Dehydrogenase

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Secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* catalyzes the reduction of butan-2-one with much higher enantioselectivity when NADP is replaced by APADP, SNADP or NAD; as expected, the enantiomeric ratios $[(k_{cat}/K_m)_R/(k_{cat}/K_m)_S]$ of the reaction of SADH with (*R*)- and (*S*)-butan-2-ol increase with the coenzyme analogues.

Although enzyme use in asymmetric organic synthesis is now widespread as a result of the high stereoselectivity and specificity which may be achieved, the relative inflexibility of enzyme stereoselectivity (*e.g.* high stereochemical purity with one substrate, but not with another) often limits wider synthetic exploitation. Recent, novel approaches to expand the range of useful enzyme stereoselectivity involve changes in reaction conditions. We have reported our studies on the temperature-dependent enantiospecificity of secondary alcohol dehydrogenase (SADH) from *Thermoanaerobacter ethanolicus*.[1] Klibanov and co-workers have found a solvent dependent enantioselectivity of the protease, subtilisin Carlsberg.[2] Here, we report that coenzyme analogues can enhance the stereoselectivity of a dehydrogenase.

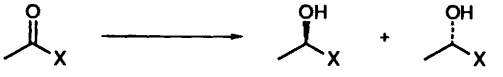
Asymmetric reduction of small aliphatic ketones using enzymes has enjoyed only limited success;[3] *e.g.* reduction of aliphatic ketones with yeast alcohol dehydrogenase gave secondary alcohols with only moderate optical purity,[4] and acyclic ketones are poor substrates for horse liver alcohol dehydrogenase (HLADH).[5] The secondary alcohol dehydrogenases from thermophilic bacteria, *Thermoanaerobium brockii* (TBADH)[6] and *T. ethanolicus* (SADH),[1,7] exhibit high activity and high stereoselectivity with a wide range of acyclic ketones. However, TBADH and SADH reduce butan-2-one and pentan-2-one with low enantioselectivity, using NADP as co-factor.[1,6] Since thionicotinamide adenine dinucleotide (SNAD) and 3-acetylpyridine adenine dinucleotide (APAD) showed significantly higher activity than NAD in HLADH oxidation reactions,[8] we decided to study the NADP analogues, SNADP and APADP, as well as NAD, to evaluate the effect of coenzyme structure on the stereoselectivity of butan-2-one and pentan-2-one reduction by SADH.

Table 1 summarizes the results obtained from these reactions.[9] For butan-2-one at 37 °C, when NADP was replaced by APADP, SNADP or NAD, the enantiomeric ratio, *E* (≡*R*/*S*), of the butan-2-ol product increased from 1.3 to 3.7, 2.9 or 4.6, respectively; at 47 °C, *E* increased from 1.9 to 9.4, 6.6 or 7.0. As the temperature of the reaction was increased, *E* also increased, *e.g.* with APADP, from 3.7 to 9.4 when the temperature increased from 37 to 47 °C. Whereas the reduction of butan-2-one by SADH with NADP is not sufficiently stereoselective (<30% e.e.) to be of preparative value, the (*R*)-butan-2-ol obtained under these latter conditions has stereochemical purity >80% e.e. In the reduction of pentan-2-one, we also observed a small but significant *increase* in *E* with the cofactor analogues (Table 1); the (*S*)-pentan-2-ol obtained is thus of somewhat lower enantiomeric purity. These results suggest that these cofactor analogues favour the hydride transfer on the *pro*-(*S*) face of the ketone, relative to NADP. In contrast to the oxidations reported with HLADH,[8] the relative rates of pentan-2-one reduction by SADH with these analogues are considerably slower than NADP (Table 1).

We have also studied the steady-state kinetics of the reaction of SADH and these coenzyme analogues with (*R*)- and (*S*)-butan-2-ol. Based on the results presented in Table 1, we predicted that $(k_{cat}/K_m)_R/(k_{cat}/K_m)_S$ for butan-2-ol would increase when NADP is replaced by APADP or SNADP. The kinetic parameters were measured with purified SADH as previously described.[1] The results of these kinetics studies agree with those observed from preparative reactions, since APADP and SNADP showed higher *E* values than NADP at each temperature. A plot of $-RT\ln E$ against absolute temperature (Fig. 1) shows that APADP and SNADP possess linear temperature dependencies of stereospecificity similar to NADP.[1]

By changing from NADP to coenzyme analogues, the enantioselectivity of the reduction of butan-2-one catalysed by SADH from *Thermoanaerobacter ethanolicus* increased significantly. We believe that in other NAD or NADP-dependent oxidoreductase reactions with poor stereoselectivity, choice of the appropriate coenzyme analogue could be a powerful factor in improving stereoselectivity and extending the utility of available enzymes in asymmetric synthesis.

Table 1 Reduction of acyclic ketones with coenzyme analogues catalysed by SADH from *T. ethanolicus*.[1]



1; X = Et **2**; X = Pr

Substrate	Coenzyme	T/°C	Ratio (*R*/*S*)	Relative rate[a]
1	NADP	37	1.3	
1	APADP	37	3.7	
1	SNADP	37	2.9	
1	NAD	37	4.6	
1	NADP	47	1.9	
1	APADP	47	9.4	
1	SNADP	47	6.6	
1	NAD	47	7.0	
2	NADP	37	0.33	100
2	APADP	37	0.36	21.9
2	SNADP	37	0.38	32.1
2	NAD	37	0.42	22.6
2	APADP	47	0.32	
2	NAD	47	0.45	

[a] The relative rate of pentan-2-ol formation with NADPH at 37 °C was assigned as 100%.

Experimental

Reduction of Ketones with SADH.—Reaction mixtures (10 cm^3 portions) containing coenzyme (0.05 mmol dm^{-3}) substrate (0.1 cm^3), propan-2-ol (1 cm^3), (SADH) (12 units; as sonicated cell extract of *T. ethanolicus*[1]), in Tris buffer (pH 8; 50 mmol) were kept in a constant temperature bath for 10 h and then

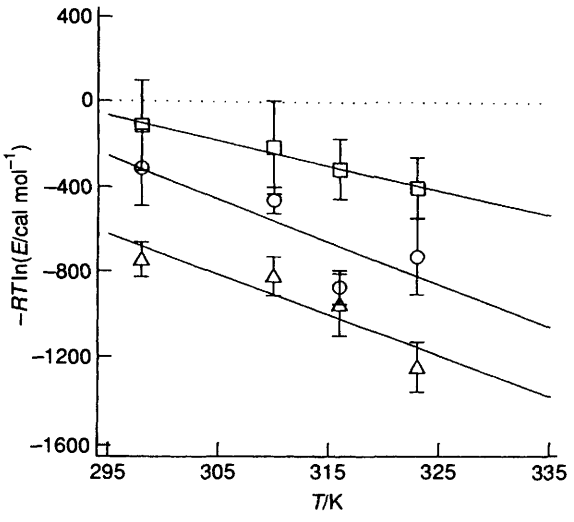


Fig. 1

worked up by saturation with $(NH_4)_2SO_4$ and ether extraction. After being dried (Na_2SO_4) and evaporated at reduced pressure, the crude mixtures were analysed for relative rate by gas chromatography.

Determination of Enantiomeric Ratio, R/S.—A sample of alcohol was mixed with (*S*)-*N*-trifluoroacetylprolyl chloride (2 equiv.) in CH_2Cl_2 at room temperature for 2 h. The resulting solution was then analysed by gas chromatography on a Chirasil-Vall capillary column.[1]

Kinetics Experiments.—Cuvettes contained the following components: coenzyme (0.1 mmol dm^{-3}), alcohol (0.2 mol dm^{-3}; 10–160 mm^3) and Tris-HCl buffer (pH = 8.9; 100 mmol dm^{-3}), in a final volume of 0.6 cm^3. Because of the high temperature coefficient of Tris, the pH of the Tris-HCl buffer was adjusted to 8.9 at each temperature. The cuvettes were preincubated at the appropriate temperature from 25–50 °C before the reaction was started by addition of enzyme solution. The rates were measured spectrophotometrically by the increase in absorbance of NADPH, APADPH and SNADPH at 340, 363, and 396 nm, respectively, on a Gilford Response UV–VIS spectrophotometer equipped with a six-cell holder and electronic temperature control. The values of k_{cat}/K_m for each enantiomer of the alcohol were calculated at each temperature.

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