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The influence of water activity on the enantioselectivity in the enzyme-catalyzed reduction of 2-pentanone

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Abstract

The stereoselective reduction of 2-pentanone by alcohol dehydrogenase from *Thermoanaerobium brockii* was studied at controlled water activity and at different reaction temperatures. The reaction rate increased when water activity was increased from 0.32 to 0.96 and when raising the temperature from 5°C to 40°C. The enantioselectivity, E, reached a plateau value at high water activities. The enantioselectivity increased with decreasing reaction temperature.

Keywords: Organic medium; Water activity; Temperature; Enantioselectivity; Alcohol dehydrogenase; *Thermoanaerobium brockii*

1. Introduction

The use of enzymes as catalysts for the preparation of stereochemically pure substances is an expanding research area [1]. The use of organic solvents as reaction media in enzymatic catalysis has opened up new possibilities. A synthetically useful enzymatic reaction is the stereoselective reduction of ketones catalyzed by alcohol dehydrogenases [2]. The reaction temperature, as well as the microenvironment of the enzyme influence the rate of catalysis. For instance, a low reaction temperature has been observed to increase the peptide yields in enzymatic peptide synthesis [3,4] and also to affect the stereoselectivity in alcohol dehydrogenase catalyzed reductions of ketones [5–7]. The water content in the organic solvent is of great importance for enzyme catalysis. The best way to control the amount of water associated with the enzyme molecules is by controlling the water activity. The hydration level, or amount of water bound to the enzyme increases with increasing water activity [8]. A high hydration level leads to a more flexible and usually more active enzyme. The effect of flexibility on enzyme selectivity is not clearly understood. In the literature, there are reports showing that when increasing the water activity the enantioselectivity is increased [9], decreased [10] or is unaffected [11]. The enzymes used in these reports are lipases from different sources and no

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known reports on alcohol dehydrogenase are available, to date.

In the present study, the reaction rate and enantioselectivity of a reduction of 2-pentanone catalyzed by an alcohol dehydrogenase from *Thermoanaerobium brockii* was studied at controlled water activity, and at different reaction temperatures.

2. Experimental

Alcohol dehydrogenase (E.C.1.1.1.2) from *T. brockii* (TBADH; specific activity of 7.3 U/mg solid) and $NADP^+$ (sodium salt) were immobilized together on celite, as previously described [7]. Immobilized enzyme (50 mg) and substrate solution (except for mercaptoethanol) were equilibrated with saturated salt solutions at 5°C, 25°C and 40°C in separate containers for at least 16 h. The salts used were $MgCl_2$ ($a_w = 0.32$, 40°C), $Mg(NO_3)_2$ ($a_w = 0.59$, 0.53, 0.48), NaCl ($a_w = 0.76$, 0.75, 0.75), KCl ($a_w = 0.88$, 0.84, 0.82) and K_2SO_4 ($a_w = 0.98$, 0.97, 0.96). The a_w values given are for 5°C, 25°C and 40°C, respectively [12]. Some reactions were carried out at controlled amount of water (1% and 2.5%, v/v) and they were incubated at the actual reaction temperature for at least 16 h.

The reactions were carried out in 4 ml stoppered glass bottles on a head-over-head incubator. The initial substrate concentration in hexane was 50 mM 2-pentanone and 300 mM of 2-butanol as co-substrate. Mercaptoethanol (3 mM) was added to each reaction vial after equilibration.

50 μl sample was used to determine the product yield and a 100 μl sample was used to determine the enantiomeric excess of the produced alcohol. The product yield was measured by packed gas chromatography using a Carbowax 20M on 80/100 Chromosorb WAW. For measuring the enantiomeric excess the alcohol was derivatized with *R*-(+)-1-Phenylethylisocyanate and the enantiomers were separated on a capillary (DB 210) gas chromatography column. The enantioselectivity, *E*, was determined as the ratio of the initial activity of *S* and *R* enantiomer.

3. Results and discussion

3.1. Effect of water activity on initial activity

The reduction of 2-pentanone was performed with TBADH and $NADP^+$ immobilized on celite and with 2-butanol as co-substrate for regeneration of the coenzyme. The reactions were carried out in hexane at controlled water activity. The initial activity was strongly influenced by the water activity (Fig. 1a). When increasing the water activity from 0.32 to 0.96 at 40°C the initial activity increased by a factor of 130. It has been shown previously for horse liver alcohol dehydrogenase (HLADH) that water increases the reaction rate by increasing the dielectric constant of the solvent and/or by facilitating the alcohol dissociation from the active-site zinc ion [13]. We can assume that TBADH works according to a mechanism similar to that of HLADH and the enhanced reaction rate at high water activity could be related to this. The water content in the substrate solution equilibrated with K_2SO_4 (highest a_w) were 0.020%, 0.016% and 0.011% at 40°C, 25°C and 5°C, respectively. When adding extra water to the hexane, 1% or 2.5%, the reaction rate slightly decreased (Fig. 1b). One visible difference between the reactions was that at high water con-

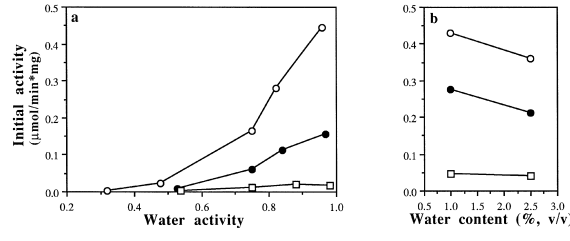

Fig. 1. The effect of water activity (a) and water content (b) on initial activity of the reduction of 2-pentanone catalyzed by TBADH. The reactions were performed in hexane and studied at three different temperatures, 40°C (○), 25°C (●) and 5°C (□).

tents, the enzyme preparation had the tendency to aggregate and adsorb on the glass wall of the reaction bottle. This may account for the observed decrease in reaction rate. The problem was more pronounced at 40°C which is the temperature giving the highest initial activity.

3.2. Effect of water activity on the enantioselectivity

The influence of water activity on the stereoselectivity of the enzyme was also investigated. The enantioselectivity (E) was determined as the ratio of initial rate of formation of the *S* and *R* enantiomer (Fig. 2). The formation rates of the enantiomers were calculated from the conversion yields and the enantiomeric excess of the product. In the water activity range from 0.32 to 0.96 the enantioselectivity reached a plateau value at high water activities (Fig. 3a). When increasing the water activity, the hydration level of the enzyme will be higher and leads to a more flexible enzyme. Whether this flexibility of the enzyme is in some way directly related to the enantioselectivity of the reaction, it is not clear. It has been observed in a previous report that for some proteases suspended in organic solvent the enzyme flexibility was directly related to a higher enantioselectivity [14]. High flexibility was essential for maximizing the favourable interactions with the substrate. This can be related to the selectivity between two substrate enantiomers. In the case of reduction of ketones, the substrate is the same for both pathways and we can assume that it can

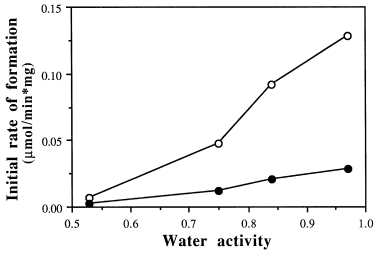

Fig. 2. The initial formation rate of the *S* (○) and *R* (●) enantiomers of 2-pentanol at different water activity. The reactions were carried out at 25°C.

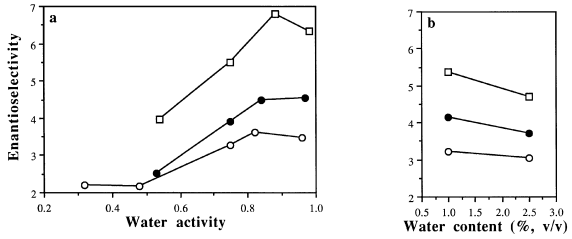

Fig. 3. The effect of water activity (a) and water content (b) on the enantioselectivity of the reduction of 2-pentanone catalyzed by TBADH. The reactions were performed in hexane and studied at three different temperatures, 40°C (○), 25°C (●) and 5°C (□).

bind in the active site of the enzyme in two different ways. Adding more water to the reaction system leads to a decrease in enantioselectivity with 19%, 22% and 44% for 40°C, 25°C and 5°C, respectively (Fig. 3b). The decrease in enantioselectivity at high water contents could be in some way related to diffusion limitations caused by the aggregation (described above) of the immobilized enzyme. This has been observed earlier for a lipase catalyzed resolution of suedenol [10].

3.3. Effect of temperature on initial activity

The initial activity was also affected by the reaction temperature. Changing the temperature from 5°C to 40°C at water activity of 0.84, the reaction rate increased with a factor of 15 (Fig. 4). A straight line was observed when fitting this data to an Arrhenius plot and the calculated activation energy was 55 kJ/mol. This is in good agreement with a previously published

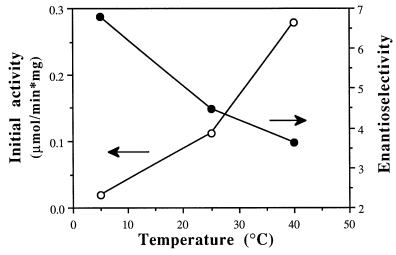

Fig. 4. The influence of the reaction temperature on the initial activity (○) and the enantioselectivity (●) of the TBADH catalyzed reduction of 2-pentanone. The enzyme preparation and the substrate solution were equilibrated with saturated KCl solutions at respective temperatures.

data [7]. The saturated salt solutions used for equilibration differ in water activity at the different temperatures (exact values under experimental part). The water activity at 5°C was higher (0.88) than the water activity at 40°C (0.82), equilibrated with the same salt solution (KCl). If it would have been possible to equilibrate at exactly the same a_w, the change in activity would have been even larger. The observed increase in initial activity with temperature is therefore, not attributed to a water activity change. The effect of water activity on the initial activity showed almost the same dependence at all temperatures studied.

3.4. Effect of temperature on enantioselectivity

The enantioselectivity was affected by the reaction temperature. This has been studied in detail for the oxidation of secondary alcohols [6] and for reduction of ketones [5,7]. These studies were performed with alcohol dehydrogenases from different sources and in different reaction media. In the present study, the influence of the reaction temperature was similar as in the reports mentioned, using 2-pentanon/pentanol as substrates. The enantioselectivity increased when the temperature was lowered. The highest enantioselectivity was observed at 5°C (Fig. 4). It has been shown in an earlier report that a further decrease in temperature will increase the enantioselectivity even more [7]. All investigated temperatures showed a similar water activity profile reaching a plateau at high water activities (Fig. 3a) and a decrease at water contents exceeding the water solubility in the reaction medium (Fig. 3b). The enantiomeric excess of the produced 2-pentanol increased from 59% at 40°C to 75% at 5°C.

4. Conclusions

In the present investigation, it has been shown that the reaction rate and the enantioselectivity are influenced by the water activity of the system. At high water activity, the reaction rate is high and the enantioselectivity is optimal. The reaction temperature has also a great influence on the reaction rate and enantioselectivity. The lower the temperature the higher the enantioselectivity. Even though the reaction rate is deceased when lowering the temperature, this could in some way be compensated with an increase in stereoselectivity.

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References

[1] G. Carrea, G. Ottolina, S. Riva, TIBTECH. 13 (1995) 63.
[2] W. Hummel, M.-R. Kula, Eur. J. Biochem. 184 (1989) 1.
[3] Å. Jönsson, P. Adlercreutz, B. Mattiasson, Biotechnol. Bioeng. 46 (1995) 429.
[4] Å. Jönsson, E. Wehtje, P. Adlercreutz, B. Mattiasson, J. Mol. Catal. B: Enzymatic 2 (1996) 43.
[5] E. Keinan, E.K. Hafeli, K.K. Seth, R. Lamed, J. Am. Chem. Soc. 108 (1986) 162.
[6] R.S. Phillips, TIBTECH. 14 (1996) 13.
[7] H. Yang, Å. Jönsson, E. Wehtje, P. Adlercreutz, B. Mattiasson, Biochim. Biophys. Acta 1336 (1997) 51.
[8] S.B. Lee, K.-J. Kim, J. Ferment. Bioeng. 79 (1995) 473.
[9] H.-E. Högberg, H. Edlund, P. Berglund, E. Hedenström, Tetrahedron Asymmetry 4 (1993) 2123.
[10] C. Orrenius, T. Norin, K. Hult, G. Carrea, Tetrahedron Asymmetry 6 (1995) 3023.
[11] E. Wehtje, D. Costes, P. Adlercreutz, J. Mol. Catal. B: Enzymatic 3 (1997) 221.
[12] L. Greenspan, J. Res. Nat. Bur. Stand. (Phys. and Chem.) A 81 (1977) 89.
[13] R.M. Guinn, P.S. Skerker, P. Kavanaugh, D.S. Clark, Biotechnol. Bioeng. 37 (1991) 303.
[14] J. Broos, A.J.W.G. Visser, J.F.J. Engbersen, W. Verboom, A. van Hoek, D.N. Reinhoudt, J. Am. Chem. Soc. 117 (1995) 12657.