Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 52–53 (2008) 67–73
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Biocatalyst expressing *cis*-naphthalene dihydrodiol dehydrogenase from *Pseudomonas fluorescens* N3 catalyzes alcohol and 1,2-diol dehydrogenase reactions

Guido Sello [a,*], Silvana Bernasconi [a], Fulvia Orsini [a], Paolo Mattavelli [a], Patrizia Di Gennaro [b], Giuseppina Bestetti [b]

[a] *Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, via Venezian 21, 20133 Milano, Italy*
[b] *Dipartimento di Scienze dell'Ambiente e del Territorio, Università di Milano-Bicocca, p.za della Scienza 1, 20126 Milano, Italy*

Available online 30 October 2007

Abstract

Alcohol and carbonyl groups are highly recurrent groups in organic compounds. Their redox equilibrium is often used by chemists to prepare several compounds. Carbonyl reactivity is often used to synthesize more complex structures; in contrast, alcohols are more usually found in the final products because their coordinative ability is fundamental both in biology and in chemistry. Dehydrogenase activities are an interesting alternative to chemical redox reactants because they are often chemo-, regio-, and stereo-selective. We prepared and used an *E. coli* recombinant strain expressing the naphthalene dihydrodiol dehydrogenase from *Pseudomonas fluorescens* N3. This biocatalyst showed satisfactory substrate recognition and good reactivity. It can transform primary and secondary alcohols and 1,2-diols. Besides, the geometry recognition is also significant. Finally, we will discuss some unexpected results that we obtained when using 1,2-diols.

Keywords: Chiral alcohols; Chiral 1,2-diols; Enzymatic methods; Dehydrogenase; Racemate resolution

1. Introduction

A large part of organic synthesis is based on the use of alcohols and carbonyls. Alcohols can be considered the source of several substitution reactions, in the same way as carbonyls are the prototype of addition reactions. Their reactivity has been thoroughly exploited by chemists in chemo- and stereo-selective mode. Besides, in the context of the everyday increasing demand for enantiopure compounds chiral alcohols represent an appealing source of chiral compounds. They can be prepared either by reduction of the corresponding prochiral carbonyls, or by resolution of racemic mixtures [1–4]. In this context, enzymatic procedures has become an appealing alternative to classical chemical synthesis.

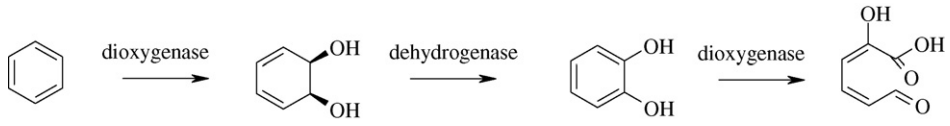
It is worth noting that catalysts for alcohol oxidation can be very infrequently used to perform the reverse reaction, i.e. carbonyl reduction, because the redox potential difference between products and reactants should be high enough to guarantee a complete conversion (the most well-known exception is the Meerwein–Pondorff–Verley and Oppenauer reaction pair). In contrast, enzymes are often reversible catalysts that can work both ways. As a consequence, it is possible to have access to both enantiomers using the same catalyst because if the reaction is stereo-selective the enzyme always recognizes the same geometry, thus producing and consuming the same stereoisomer.

Whilst the preparation of chiral alcohols through racemate resolution using alcohol dehydrogenases (ADH) has been thoroughly studied [5–13], 1,2-diol dehydrogenases (DDH) have been mainly used to catalyze the transformation of unsaturated 1,2-diols into their respective aromatic 1,2-dihydroxy derivatives. In fact, DDHs are enzymes that participate to the degradation of aromatic compounds via metabolic pathways that pass through a dioxygenation to give 1,2-dihydrodiols, followed by the dehydrogenation to give the 1,2-dihydroxy derivatives that are successively oxidized with simultaneous ring opening (Scheme 1).

The analysis of the native activity difference between DDHs and ADHs is worth to be extended because, if DDHs could also

* Corresponding author. Tel.: +39 0250314107; fax: +39 0250314106.
E-mail addresses: guido.sello@unimi.it (G. Sello), silvana.bernasconi@unimi.it (S. Bernasconi), fulvia.orsini@unimi.it (F. Orsini), patrizia.digennaro@unimib.it (P. Di Gennaro), giuseppina.bestetti@unimib.it (G. Bestetti).

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.10.014



Scheme 1. Initial steps of aromatic compound metabolism by microorganisms.

recognize and transform alcohols, their functioning could prove original, particularly concerning the 1,2-diols that are often poor substrates for ADHs. If the study of the activity of our dihydrodiol dehydrogenase will give interesting results, this enzyme can represent a new and original source of different chemoselectivity with respect to well-known alcohol dehydrogenases. As a consequence, the objective of this work is not the proposal of another alcohol dehydrogenase to prepare alcohols or carbonyls, but the analysis of the behaviour of our DDH in a chemical space that is not its native space. We should point that, as far as we know, this is the first study concerning the use of a DDH used as ADH.

2. Experimental

2.1. Biocatalyst growth conditions in flask

A single colony *E. coli* JM109 (pVL2028) [14a,b] was grown overnight at 30 °C in a 100 mL flask containing 20 mL of LB (Luria–Bertani) [15] medium, containing kanamycin (final concentrations: 50 μg/mL).

Then, 10 mL of the culture were centrifuged (10,000 rpm, 4 °C, 10 min), cell collected, and added to 100 mL of M9 [15] medium, in a 500 mL flask, containing kanamycin 50 μg/mL and IPTG (isopropyl-D-thiogalactopyranoside) 1 mM; glucose (0.2%, w/v) and thiamine (0.05 mM) were added; the culture was grown overnight at 30 °C.

2.2. Biocatalyst growth conditions in 3 L bioreactor

All the previous operations were also used to prepare the starting cells when the final growth was performed in a 3 L bioreactor. The final growth was performed using the cells coming from a standard flask growth. Hundred milliliters of the culture were added to 900 mL of M9, containing glucose (2 g/L), thiamine (1 mM), antibiotic and inducer at the usual concentration. The growth was performed overnight at 30 °C. The cells were harvested by centrifugation (10,000 rpm, 4 °C, 10 min) and stored at −20 °C.

2.3. Bioconversion cultures

When needed the required amount of cells was unfrozen by shaking at 30 °C for 1 h in M9 medium containing glucose (0.2%, w/v). Then, the culture cell density was adjusted at the required value by addition of M9 medium; finally, glucose (initial concentration 0.2% (w/v); similar amounts added when needed) and the substrate were added and the transformation was performed in a flask (usually 250 mL containing 50 mL of culture) at 30 °C on a horizontal shaker.

In both biocatalyst production and bioconversion the culture media were sterilized at 131 °C, 10 min; or by filtration on 45 μm Millipore filters; all equipments are autoclaved. Cell density was measured using a Shimadzu photometer at 600 nm. Glucose was monitored using enzyme sticks (Glukur Test) from Roche.

2.4. Bioconversion analysis

Substrate and product were monitored analysing the water phase by HPLC, Hitachi-Merck, UV–vis detector at 220 nm, reverse phase column C18 (Hibar LICHROSORB 50334, 10 μm, 25 cm), H_2O:CH_3CN 1:1 eluent, 1 mL/min flow, Hitachi D2500 integrator.

The substrate and product amount in the organic phases was measured using GLC analysis. GLC Dani 1000, capillary column Chrompack CP-sil 8CB or Chrompack ChiralDex-CB, FID detector (FID-861), using the conditions optimised for each compound. Data variance was mainly connected to the precision of the instruments used in the analysis; it is thus possible to assume a ±5% deviation.

^{1}H NMR spectra were obtained in $CDCl_3$ or CD_3OD (Merck) using Bruker AC-300 and Bruker AC-200 instruments. Thin-layer chromatography was carried out on silica gel plates (60 F_{254}, Merck): spots were detected visually by ultraviolet irradiation (254 nm) or using cerium-molybdic solution as stainer. All products were chromatographated over silica gel (*n*-hexane/ethyl acetate in different ratio as required).

2.5. Bioconversion comparison

To make a comparison between different experiments we prepared reference bioconversions for each series. A first standard bioconversion was performed using solid naphthalene dihydrodiol (3 g/L) as substrate in a reference flask containing exactly the same culture (same cells and same density) in M9 mineral medium, containing glucose (initial concentration 0.2% (w/v)). Both substrate and glucose were added when necessary to exclude their influence on the conversion rate. This value was used to roughly evaluate the biocatalyst activity. A second standard bioconversion was performed using benzyl alcohol (150 mg/L) as substrate in the same conditions; this last was used to evaluate the activity of the catalyst.

2.6. Chemicals

All the chemicals are from Acros, CarloErbaReagents, Sigma–Aldrich, Oxoid, or Fluka. TLC plates (Alugram SL G/UV254) are from Merck.

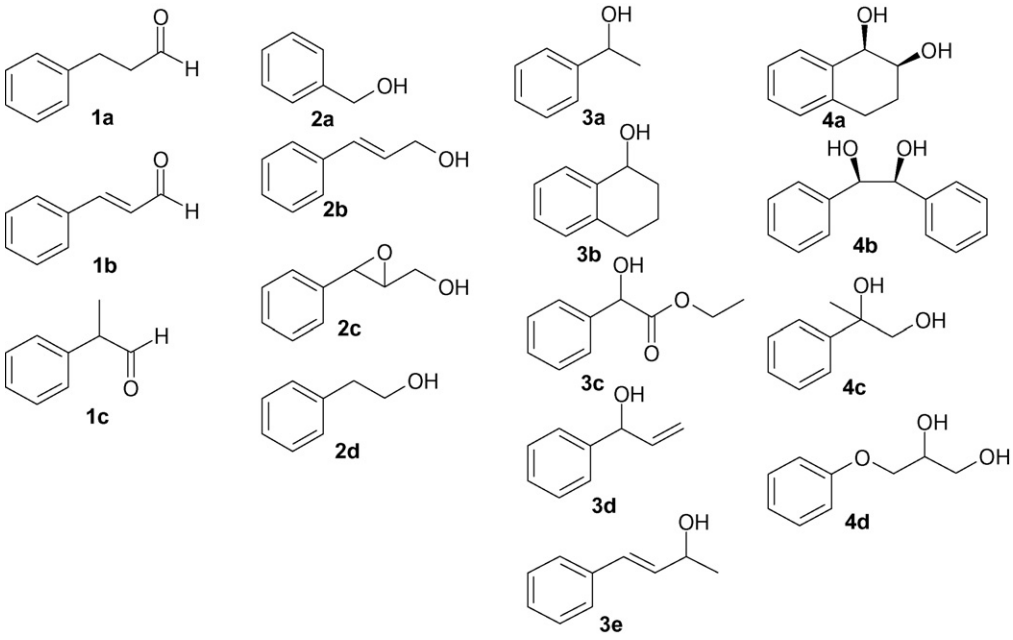


Fig. 1. Compounds used as substrates in NDDH catalyzed reactions.

3. Results and discussion

In the perspective of performing an analysis of the recognition potential of naphthalene dihydrodiol dehydrogenase (NDDH) we selected different compounds that can be assigned to different chemical classes. In particular, we used: aldehydes, primary alcohols, secondary alcohols, benzyl alcohols, allyl alcohols, 1,2-diols. In Fig. 1 the tested substrates are reported.

It is worth to raise some points before presenting the results. In our experiments with whole cell catalyst we encountered two perturbing activities of the *E. coli* host: an enoate reductase and a dehydrogenase. We are aware of their presence and we always carried control experiments with the wild host strain to control these interferences. Thus, we can present the results of NDDH keeping in mind the possible unexpected outcomes. It is clear that, in order to use this enzymatic activity as an efficient biocatalyst these interferences should be solved; nevertheless, this preliminary study gives enough hints on the enzyme performance as an ADH surrogate. In this line of reasoning we have also neglected the optimisation of the reactions in terms of yield, being our current aim different.

3.1. Aldehydes

Aldehydes are easily oxidized; sometimes, even the oxygen of air is sufficient to transform these sensitive compounds. However, in the used conditions the chosen compounds remain unchanged in the absence of the biocatalyst. We also knew, from previous experiments [16], that the host *E. coli* JM109 can easily reduce the aldehydes to their corresponding alcohols, whilst ketones are unaffected. The current experiments aim at proving the outcome of these compounds in the presence of the dehydrogenase activity. Considering the competition between the *E. coli* reductase [17] and the NDDH it is not surprising that the final result is a mixture of reduced (alcohols) and oxidized (acids) aldehydes. Compounds **1a–c** were quickly transformed into a 1:1 mixture of redox derivatives (see Table 1; Fig. 2). This result also gave another interesting hint: the alcohols will oxidize more slowly than the aldehydes as shown by their accumulation in the reaction medium. The transformation rates followed the expected order: alkyl > allyl > hindered alkyl. The isolated products were the corresponding alcohols and acids. In the case of

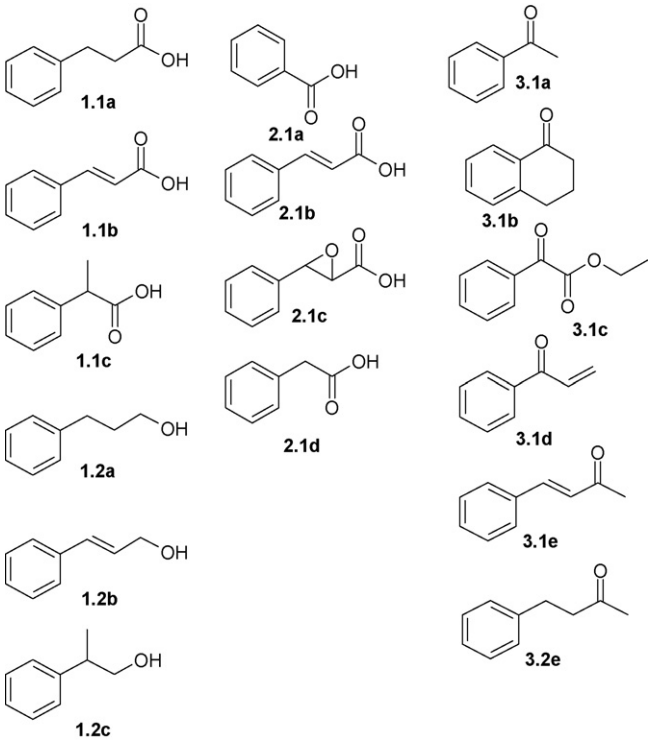


Fig. 2. Compounds obtained as products in NDDH catalyzed reactions.

Table 1
Redox reactions using *E. coli* JM109 (pVL2028) catalyst

Substrate[a]	OD[b]	mmoli L^{-1} (mg L^{-1})[c]	Special features[d]	Product[e]	*T*/2 (h)[f]	ee[g]
1a	1.3	2.15 (288)		**1.1a** (50%), **1.2a** (50%)	1	
1b	1.3	2.08 (274)		**1.1b** (50%), **1.2b** (50%)	0.6	
1c	2.6	1.12 (150)		**1.1c** (50%), **1.2c** (50%)	1.5	17%
1c	1	1.12 (150)		**1.1c** (50%), **1.2c** (50%)	1.5	
1c	1.3	2.09 (280)		**1.1c** (50%), **1.2c** (50%)	2.5	
2a	1.8	1.39 (150)	Glucose in phosphate buffer	**2.1a**	1	
2a	1.8	1.39 (150)	Pyruvate in phosphate buffer	**2.1a**	1.2	
2a	1.8	1.39 (150)	Acetate in phosphate buffer	**2.1a**	1.5	
2a	2	3.7 (400)		**2.1a**	2	
2a	2	3.7 (400)		**2.1a**	1.5	
2b	1.75	1.87 (250)	Pyruvate	**2.1b**	0.75	
2b	1.7	2.99 (400)	Pyruvate in tris HCl buffer	**2.1b**	1.25	
2b	1.75	1.87 (250)		**2.1b**	0.5	
2b	1.7	2.99 (400)	Glucose in tris HCl buffer	**2.1b**	1	
2c	2	1.07 (150)		**2.1c**	45	
2c	1.8	1.07 (150)		**2.1c**	40	
2d	2	1.64 (200)		**2.1d**	55	
3a	2	1.64 (200)		**3.1a**	45	97%
3a	1	1.23 (150)		**3.1a**	15	
3a	1.7	1.64 (200)		**3.1a**	15	
3b	1	1.01 (150)		**3.1b**	23	98%
3b	2	1.01 (150)		**3.1b**	29	
3b	2	1.01 (150)	Pyruvate	**3.1b**	29	
3b	2	1.01 (150)	Glucose + acetone	**3.1b**	29	
3b	2	1.01 (150)	Acetate	**3.1b**	29	
3c	1.5	0.83 (150)		**3.1c**	69 (35%)	10%
3c	2	0.56 (100)		**3.1c**	60	
3c	1.7	2.78 (500)		**3.1c**	65	
3d	1.5	1.12 (150)		**3.1d**	23	89%
3e	2	1.01 (150)		**3.1e**	4	60%
3e	1.8	1.01 (150)		**3.1e**	3	
3e	1.8	3.38 (500)		**3.1e**	6.5	
3.1e	2.1	1.37 (200)	*E. coli* JM109 w.t.	**3.2e**	12	
4a	1	1.34 (220)		See text	50 (20%)	See text
4a	1.7	0.61 (100)		See text	7 (20%)	
4a	1.7	0.73 (120)		See text	26 (30%)	
4a	1.8	0.91 (150)	Two phases	See text	24 (35%)	
4a	2.25	0.91 (150)	Acetylated	No product	n.d.	
4b	2.2	0.75 (160)		See text	n.d.	See text
4c	1.9	1.05 (160)		**4.1c**	60	n.d.
4d	1.9	1.19 (200)		**4.1d**, **4.2d**	50	n.d.

[a] See Fig. 1.
[b] Cell density measured at 600 nm.
[c] Substrate molar concentration, in parentheses the corresponding weight concentration.
[d] Special cases: experiences performed using different co-oxidant, or different broth composition, or different biocatalyst, or substrate derivative.
[e] See Fig. 2.
[f] Transformation half time.
[g] Enantiomeric excess, measured by chiral GLC or HPLC.

1c the experiment was performed using the racemic mixture. At the end of the reaction the produced alcohol was partially enantio-enriched.

3.2. Primary alcohols

The second group contains four primary alcohols in different molecular environment. Benzyl and cinnamyl alcohols were rapidly oxidized to their corresponding acids. There was no trace of the intermediate aldehydes. Compounds **2c** and **2d**, that are not π-conjugated, were oxidized much more slowly. This is the first signal that the redox potential is fundamental for this enzyme. The products were the corresponding acids (Fig. 2); but, in the case of compound **2c**, the obtained product was quite unstable and readily hydrolysed to the vicinal diol derivative. Starting from the racemic mixture the recovered substrate was partially enantio-enriched. Considering that both **2c** and **1c** carry the asymmetric carbon one position away from the reaction centre the geometry selection is interesting.

Concerning the benzyl alcohol we performed several transformations because we chose this compound as our reference. In fact, the transformation rate of **2a** is appropriate to be routinely used and correctly analysed. Using this substrate several experiments were made to determine the best reaction conditions. We selected some variables: temperature, pH, reaction medium, co-oxidation substrate. The results did not indicate a set of conditions that can be considered the most favourable; in fact, our biocatalyst was working similarly in many of the tested conditions of temperature, pH, reaction medium, co-oxidation substrate. Thus, we could only confirm that our standard procedure (pH 7, $T = 30\,^{\circ}C$, M9 medium, glucose) is still one of the best.

3.3. Secondary alcohols

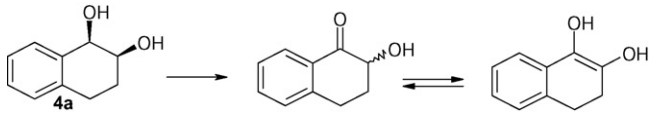
Secondary alcohols are more interesting substrates because they are intrinsically chiral compounds. In fact, excluding alcohols that have two identical substituents, the alcoholic carbon is a stereogenic centre. This explains the great interest that they stimulated in chemists. Considering the results observed using primary alcohols we decided to initially study activated alcohols, i.e. benzylic and allylic ones. Nevertheless, the transformation rates were very diverse showing half-life time from some hours to some days. This result was clearly related to the substrate structure. 1-Phenyl ethanol (**3a**) is the simplest secondary benzylic alcohol; it required 12 h to halve its amount that is six times slower than benzyl alcohol. This result was expected because the accessibility to secondary carbon is known to be more difficult. When the alcoholic carbon can be activated from the two sides (compound **3d**) the rate increased roughly twice. Using 1,2,3,4-tetrahydro-naphthalen-1-ol the introduction of a second cycle made the recognition more difficult, decreasing twice the rate with respect to **3a**. These three compounds are very similar in the accessibility to the reaction centre, because they share the vicinal position of the phenyl ring. The situation was different when we reacted 4-phenyl-but-3-en-2-ol that carries the alcoholic group two carbons away from the phenyl ring. This was reflected by a rate increase of four times with respect to compound **3b**. Compound **3e** is the secondary alcohol corresponding to compound **2b** and its transformation rate was four times slower. This is very similar to the difference between **2a** and **3a**. From the comparison of all the results we can see that there is a good response of the enzyme to structural variations. The last secondary alcohol in our list is **3c**, ethyl mandelate. It is very similar to compound **3d**, but the presence of an electron withdrawing group slowed the reaction and this substrate was by far the least reactive.

All products were the expected ketone derivatives (Fig. 2). There was one notable exception that we would like to deepen somehow. When using substrate **3e** the isolated product depends on the time of reaction work-up. In particular, if the product was isolated after 4–6 h it was recognized as the expected 4-phenyl-but-3-en-2-one; however, if the transformation was allowed to proceed for 24 h the isolated product was 4-phenyl-butan-2-one, i.e. the compound derived from the previous product by reduction of the double bond. On the base of this finding we accurately analysed the reaction course. First, we performed a reaction using 4-phenyl-but-3-en-2-one as substrate, and, not surprisingly, we recovered the same final reduced product. Then, suspecting an activity in the host cell, we performed the same reaction using *E. coli* JM109 wild type again obtaining the same result. This clearly points to an activity of the host; the next step was the control of the structural requirements of the enzyme. Either using 4-phenyl-but-3-en-2-ol or its methyl ether derivative there was no reaction. Besides, using this last compound in the presence of our biocatalyst we had no reaction even. Thus, we can suggest that the C=C double bond reduction is possible only in the presence of an activating group. A similar activity was found also in a different host: *P. putida* PAW 340. We think, in agreement with the literature, that this activity is connected to the metabolism of fatty acids [18].

3.4. 1,2-Diols

This last class of compounds is particularly interesting because vicinal diols are not easily oxidized by alcohol dehydrogenases. However, our enzyme is active on 1,2-dihydro-1,2-dihydroxy naphthalene, thus we expect a special activity towards other 1,2-diols. Compounds **4a–d** have different structures that include alcohol groups of diverse nature: benzylic, alkylic, secondary, primary. In a sense they include all the possible variants of the other analysed compounds.

Compound **4a** is very similar to the native substrate for this enzyme, the sole difference being the absence of the double bond in the second ring. We expected a reactivity slightly lower than the unsaturated derivative. First experiments were unclear; the reaction, followed by HPLC, showed an initial slow decrease of the diol concentration, followed by the alternation of positive and negative variations. The analysis of the reaction after 24 h revealed a 10% decrease of the substrate concentration. This result was surprising. All the attempts performed to increase the transformation efficiency failed. After several experiments performed using the 1*R*,2*S*-diol prepared by bioconversion, thus enantiopure, we prepared the racemic mixture of the two enantiomers to check if, at least, the reaction showed some selectivity. To follow this new experiment we used, besides the HPLC, a chiral GLC. The reaction was, in fact, partly enantioselective preferentially transforming the native 1*R*,2*S*-enantiomer. However, the real surprise came from the control experiment carried using only the native enantiomer: after some time we noticed the presence of the second enantiomer (1*S*,2*R*) and, in due time, the complete transformation of the starting enantiomer into its counterpart. Since we found the predicted oxidation product (1,2,3,4-tetrahydro-1-oxo-2-hydroxy naphthalene) we can only presume that this compound can equilibrate with its symmetric enolic tautomer (see Scheme 2).



Scheme 2. Oxidation of 1,2-naphthalene diol and tautomeric equilibrium of its reaction derivative.

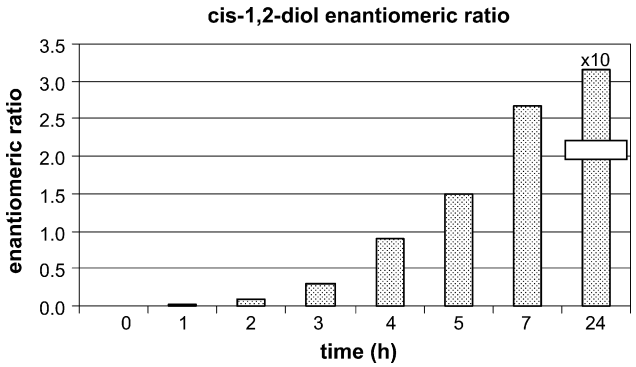


Fig. 3. Time change of the enantiomeric ratio of *cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene (1*S*,2*R*/1*R*,2*S*). At time = 0 1*R*,2*S*-diol is enantiopure.

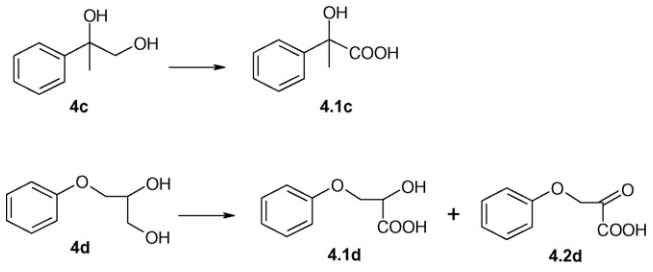
In a successive series of experiments we could observe: the initial oxidation of the 1*R*,2*S*-enantiomer, followed by the production of the 1*S*,2*R* enantiomer and by its slow consumption (Fig. 3). At the end of the transformation (after 28 h) we had no *cis*-diol remaining and the main product was the hydroxy ketone derivative. This last compound is not stable in oxygen and it is slowly oxidized to polymeric by-products. After two reaction days the only remaining compounds was the *trans* isomer in traces, in racemic form, that is apparently not oxidized by the catalyst. Because we performed the corresponding experiment using the wild type *E. coli* without observing any visible transformation, we can be confident that the transformations are managed by NDDH and not by the contaminating *E. coli* dehydrogenase.

The second analysed substrate was **4b**, the meso form of dihydrobenzoin. In this case we could characterize two oxidation products: benzoin (**4.1b**) and traces of benzyl, i.e. the mono and diketo derivative. Also here we found a small amount of the *trans* isomer, in enantio-enriched form.

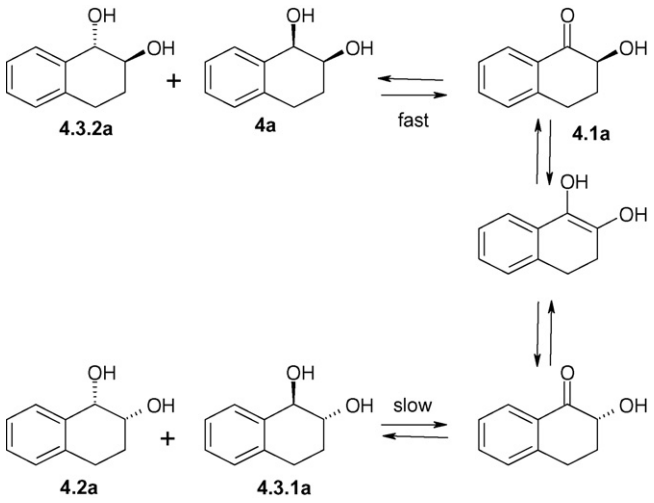
Compounds **4c** and **4d** are different with respect to **4a**–**b** because they carry a primary alcoholic group; thus, we expected their transformation into the acid derivative. This was exactly the result; in addition, the oxidation rate was lower for **4c** and both were transformed more slowly than **4a**. Some traces of the di-oxidized derivative was found for **4d** (see Scheme 3).

3.5. Geometry selectivity

In Table 1 we report the results of the analysis performed using the chiral GLC. The results concern the composition of the



Scheme 3. Products of 1,2-dihydroxy, 2-phenyl propane and of 1,2-dihydroxy, 3-phenoxy propane.



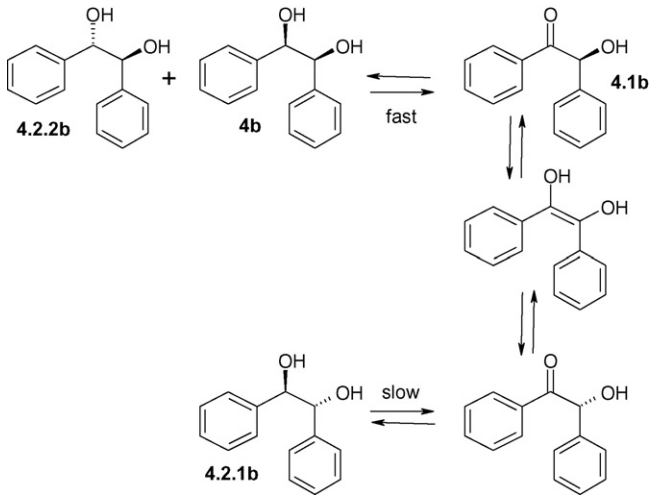
Scheme 4. Hypothetic equilibria in the redox reaction of naphthalene 1,2-diol.

remaining enantiomeric mixtures of the substrates because most of the products have lost their asymmetric centre. The selectivity is from good to high (90–100%) demonstrating that the enzyme preferentially recognize one of the stereoisomers. This result could be expected because alcohol dehydrogenase are usually as selective. However, in the perspective of the results obtained using compound **4a** we can add some more comments. At first sight the back reaction (i.e. the reduction of carbonyls) seems to be partly unselective; in fact, at the end of the reaction we get the enantiomer of **4a**, probably accumulated because its oxidation is slower (Scheme 4).

But this inference is not certain because it also implies that the reduction rates are different, being the production of the *S*,*R* isomer faster. Everything considered we cannot distinguish, in racemate resolution, between a high selectivity during the oxidation step and a high selectivity during the reduction step. Using the racemate as the starting point we note an initial fast decrease of the *R*,*S*-enantiomer followed by the development of an equilibrium where the two enantiomers are present in a constant ratio and, finally, by the disappearance of the *R*,*S*-enantiomer. The very final result is the vanishing of the *cis*-diol isomer that leaves traces of the *trans* racemate; this last seems to remain unchanged for long time. Further studies are required to clarify these results.

Compound **4b** contains alcohol groups in an environment similar to **4a**. In particular both the hydroxyl groups are secondary and benzylic alcohols. In this case we could note a transformation that is parallel to the previous one: the *cis*-meso form slowly equilibrates with the *trans*-chiral form, showing a preference for one of the two enantiomers (*R*,*R*:*S*,*S* ratio 2.5:1); in addition, the small amount of benzoin formed is mainly present in one configuration (Scheme 5).

Compounds **4c** and **4d** contain a primary alcohol that is oxidized to the corresponding acid; they both contain also an asymmetric carbon near to the reacting group, therefore the geometric recognition cannot be directly exercised. **4c** carries a tertiary alcohol whilst **4d** has a secondary alcohol. We expect a lower selectivity; however, we cannot determine the ee in these cases.



Scheme 5. Hypothetic equilibria in the redox reaction of hydrobenzoin.

4. Conclusion

Naphthalene dihydrodiol dehydrogenase usually transforms 1,2-dihydrodiols into their aromatic derivatives. In theory, this reaction has a redox potential that is lower than the potential required by alcohol oxidation; in addition, the reaction, from the chemical viewpoint, is irreversible because the product is aromatic and stable. Our results show that the enzyme redox power is greater than expected; the enzyme works well with different alcohols, both primary and secondary, and the reaction rates are, in some cases, comparable to other alcohol dehydrogenases. In addition, the enzyme shows a good geometry recognition that, in the case of secondary alcohol, is highly interesting.

In our opinion, it is more interesting the behaviour shown by the enzyme in the 1,2-diol case. Here, the reaction is still relatively fast, but the results in terms of the geometry outcome are the real surprise. Not only the reaction is stereo-selective, it is also reversible, as expected, and can show different recognition depending on the reaction direction, arriving at the complete inversion of configuration of compound **4a**.

As the NDDH structure is not available, we cannot currently rationalize the outcome. The only diol dehydrogenase structure available in the literature concerns biphenyl 1,2-diol dehydrogenase [19]. This protein is not highly dissimilar with respect to NDDH, but we are still working at the definition of NDDH and BDDH similarities at the level of catalytic site.

A different problem is represented by the efficiency of the reaction. The whole cell approach sometime experiences the limitation of the product yield. Consequently, the isolated and purified enzymes are often preferred. However, whole cells represent the most convenient and flexible way to perform enzymatic reactions and, in our opinion, they will be highly exploited in the near future. In this sense, there are more and more examples of cell reaction applications, where existing problems are solved [20].

In conclusion, we succeeded in using our enzyme in a domain that is not its native domain; more, we reached results that raised several indications for future developments.

Acknowledgements

Universita' degli Studi di Milano and MIUR (Ministero dell' Istruzione, dell'Universita' e della Ricerca) are acknowledged for financial support. The authors would like to acknowledge the fruitful suggestions made by reviewers.

References

[1] U.T. Strauss, U. Felfer, K. Faber, Tetrahedron: Asymmetry 10 (1999) 107–117.
[2] E. Schoffers, A. Golebiowski, C.R. Johnson, Tetrahedron 52 (1996) 3769–3826.
[3] H.B. Kagan, J.C. Fiaud, Top. Stereochem. 18 (1988) 249–330.
[4] (a) A. Horeau, Tetrahedron 31 (1975) 1307–1309;
(b) C.J. Sih, S.-H. Wu, Top. Stereochem. 19 (1989) 63–89.
[5] R. Csuk, B.I. Glänzer, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Dekker, New York, 2000, pp. 527–578.
[6] P. D'Arrigo, G. Pedrocchi-Fantoni, S. Servi, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Dekker, New York, 2000, pp. 365–396.
[7] J. Grunwald, B. Wirts, M.P. Scollar, A.M. Klibanov, J. Am. Chem. Soc. 108 (1985) 6732–6734.
[8] W. Hummel, Trends Biotechnol. 17 (1999) 487–492.
[9] M.R. Kula, U. Kragl, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Dekker, New York, 2000, pp. 839–866.
[10] K. Mori, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Dekker, New York, 2000, pp. 59–85.
[11] R.N. Patel, C.G. McNamee, A. Benerjee, J.M. Howell, R.S. Robinson, L.J. Szarka, Enzyme Microb. Technol. 14 (1992) 731–738.
[12] C.H. Wong, D.G. Drueckhammer, H.M. Sweers, J. Am. Chem. Soc. 107 (1985) 4028–4031.
[13] M. Kataoka, A. Kotaka, A. Hasegawa, M. Wada, A. Yoshizumi, S. Nakamori, S. Shimizu, Biosci. Biotechnol. Biochem. 66 (2002) 2649–2655.
[14] (a) C. Cavallotti, F. Orsini, G. Sello, P. Di Gennaro, E. Galli, G. Bestetti, Tetrahedron 55 (1999) 4467–4480;
(b) P. Di Gennaro, E. Galli, F. Orsini, F. Pelizzoni, G. Sello, G. Bestetti, Res. Microbiol. 151 (2000) 383–391.
[15] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY, 1982.
[16] G. Sello, F. Orsini, S. Bernasconi, P. Di Gennaro, Molecules 11 (2006) 365–369.
[17] A.W. Grant, G. Steel, H. Waugh, E.M. Ellis, FEMS Microbiol. Lett. 218 (2003) 93–99.
[18] (a) X. Liang, C. Thorpe, H. Schultz, Arch. Biochem. Biophys. 380 (2000) 373–379;
(b) S. Nakano, W. Sakane, H. Oinaka, Y. Fujimoto, Bioorg. Med. Chem. 14 (2006) 6404–6408.
[19] M. Hulsmeyer, H.-J. Hecht, K. Niefind, B. Hofer, L.D. Eltis, K.N. Timmis, D. Schomburg, Protein Sci. 7 (1998) 1286–1293.
[20] K. Goldberg, K. Edegger, W. Kroutil, A. Liese, Biotechnol. Bioeng. 95 (2006) 106–192.