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Communication

Selective Enzymatic Reduction of Aldehydes

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Abstract: Highly selective enzymatic reductions of aldehydes to the corresponding alcohols was performed using an *E. coli* JM109 whole cell biocatalyst. A selective enzymatic method for the reduction of aldehydes could provide an eco-compatible alternative to chemical methods. The simplicity, fairly wide scope and the very high observed chemoselectivity of this approach are its most unique features.

Keywords: Enzyme reaction, aldehydes, reduction, chemoselectivity.

Introduction

The chemical reduction of aldehydes to the corresponding alcohols is a well-known and easy reaction that can be performed using many different reagents. However, some other functional groups can interfere with the reaction, e.g. ketones, imines, epoxides, etc., making the reaction scarcely selective. Enzymatic reduction can be a valid alternative because the selectivity displayed by enzymes is usually greater. In addition, the possibility of using enzymes as catalysts adds an interesting environmental advantage to the preparation [1].

Several aldo-ketoreductases are known and their use is quite widespread, especially where the stereoselectivity is important, i.e. in the reduction of ketones [2-5]. In contrast, there are few examples where an enzymatic reaction has been applied to selectively reduce aldehydes. Indeed, we found only one example of such an application in the work of Grant *et al*. [6]. However, these authors were more

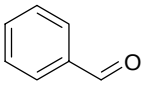
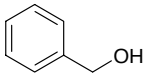
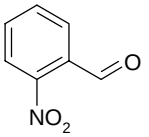
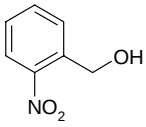
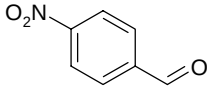
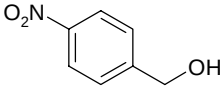
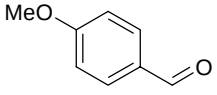
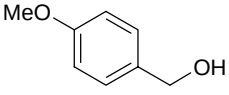
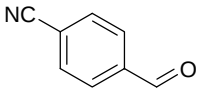
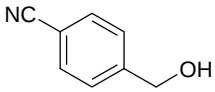
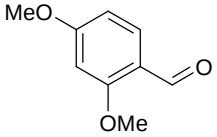
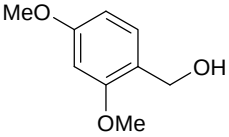
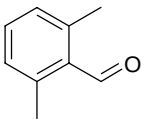
interested in the determination of the enzyme nature and action, and did not carry out a thorough analysis of its synthetic applications.

During the course of our studies on new enzymatic activities in whole cell biocatalysts we observed that the corresponding alcohol was often present a by-product when aldehydes were used as substrates. We have thus decided to examine this result in more depth. Because we commonly use an *E. coli* strain (JM109) as host in our enzymatic activity studies, we arrived at the conclusion that it should be responsible for the outcome. Consequently, we have used this strain to perform the reduction of several aldehydes in a whole cell preparation. To facilitate analysis of the reaction products the transformations were carried out in a two-phase system consisting of an aqueous buffer and an organic phase (9:1 isooctane/isopropyl ether) in a 7:3 ratio, although it is possible to add the organic substrate directly to the buffer solution.

Results and Discussion

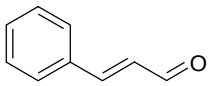
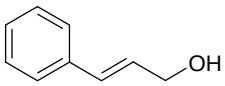
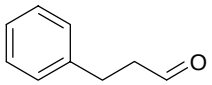
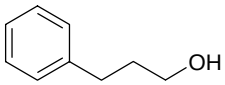
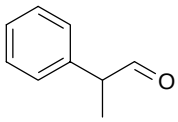
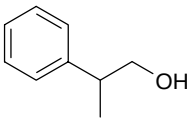
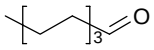
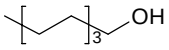
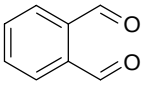
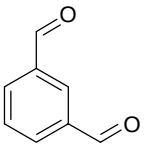
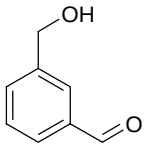
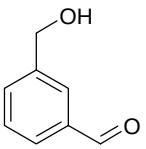
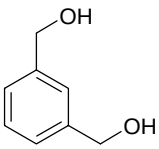
In Table 1 the aldehydes used are reported, together with the relative activity compared to benzaldehyde chosen as the reference substrate. In Table 2 the other substrates that we have tested are shown. All of them are completely unreactive and were recovered unchanged at the end of the transformations.

Table 1. Aldehydes reduced to alcohols by *E.coli* JM109.

Substrate	Product	Relative yield % [a]
		100
		12
		15
		25
		48
		49
	No reaction	Not determined

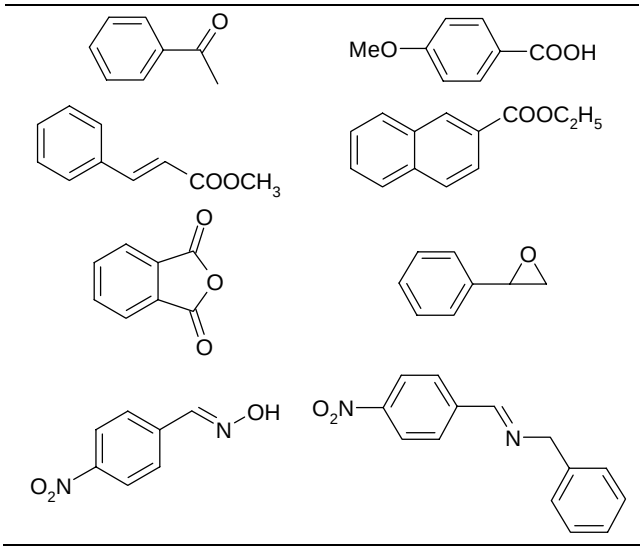
[a] Relative rates are calculated in comparison to benzaldehyde.

Table 1. (Contd.).

Substrate	Product	Relative yield % [a]
		8
		2
		48
		10
	Unidentifiable by-products	Not determined
		25
		20

[a] Relative rates are calculated in comparison to benzaldehyde.

Table 2. Unreactive compounds.



From the Tables it is clear that none of the potential interfering groups are affected by the enzymatic reaction; even methyl ketones and aldoximes are recovered unchanged. This is a remarkable characteristic of the enzymatic reaction, probably due to both electronic and geometric reasons. In

contrast, the recognition ability of the enzyme towards aldehydic groups is quite large, including aromatic and aliphatic aldehydes, unsaturated aldehydes and dialdehydes.

Some comments are in order. Benzaldehyde is the substrate that reacts faster than all the others, including activated and deactivated aromatic ones. Electronwithdrawing groups on the aromatic ring deactivate the reduction, whilst electron donating groups activate it, as it is nicely shown by the nearly twofold increase in the reduction rate resulting from the introduction of a second methoxy group on the ring. However, 4-cyano benzaldehyde is more reactive. In contrast, bis methyl ortho substitution inhibits the reaction. Aliphatic aldehydes react slower than aromatic ones, excluding 2-phenylpropionaldehyde that reacts as fast as 2,4-dimethoxybenzaldehyde.

It is also noteworthy that isophthalaldehyde reacts in two well separated stages; until the first group is not completely transformed the second is untouched. Consequently, the reduction of only one group is feasible. Phthalaldehyde is unfortunately unstable under the reaction conditions, producing unidentified side products even in the absence of cells. Finally, we tried a competitive reduction using 4-methoxybenzaldehyde and 3-phenylpropionaldehyde. In principle, the former should react 10 times faster than the second one. Surprisingly, when reacted in the same flask the two compounds react at the same rate. This is not due to the catalyst preparation, because comparative reactions have been performed using the two compounds, separately, and we could confirm that the rate ratio was ~10, as expected. It is clear that different factors affect the rate, e.g. transport and specific reactivity, affect each individual rate differently. A final series of experiments has been performed to verify if the reaction can be stereoselective. Using a racemic mixture of 2-phenylpropionaldehyde we could verify a partial selectivity at very low conversion. One of the enantiomers was transformed three times faster at 20% conversion. This reaction has not been optimized, but it demonstrates that the reaction is partially stereoselective, even if the reaction center is shifted one carbon with respect to the asymmetric carbon.

In summary, we have demonstrated that the use of *E. coli* JM109 permits the selective reduction of several types of aldehydes without any interference from the other tested functional groups. The mild reaction conditions and the possibility of exploiting the geometric selectivity of enzymes offer interesting synthetic alternatives to chemical reduction.

Experimental

Biocatalyst Preparation

The biocatalyst *E. coli* JM109 was prepared by adding 1 mL of an overnight LB culture to M9 medium (100 mL) containing glucose (10mM) and thiamine (0.05 mM); incubated overnight on a shaker at 30 °C.

Biotransformation Procedure

After growth to an OD of 1.2-2.0 (λ 600nm), the *E. coli* cells were separated by centrifugation (10,000 rpm, 4 °C) and added to M9 medium (70 mL) containing glucose (10 mM) and placed on a

shaker at 30 °C; the bioconversion was started by adding the substrate (at a concentration of 10 g/L in the organic phase) dissolved in a 9:1 mixture of isooctane/isopropyl ether (30 mL). The transformations were carried out at 30 °C.

Biotransformation Analysis

Bioconversion progress was followed by gas chromatography (DANI 86.10 gas chromatograph equipped with a FID) on a Chrompack Cp-Sil 8CB column (T = 100 ± 150 °C at 15 °C min, splitless injection) with the appropriate internal standard (dodecane or hexadecane). At intervals, 2 mL samples were taken from the reaction emulsion. The two phases were separated by filtration. The water phase was monitored by HPLC using a C18 Hibar Lichrosorb 50334, 5 μm, 25 cm column on a Merck/Hitachi L-6200 system connected to a UV-detector set at 230 nm eluting with 50:50 CH_3CN/water. Enantiomeric excesses of the unreacted 2-phenylpropionaldehyde and of the 2-phenyl-propanol produced were measured by comparison to a synthetic racemic mixture using a Chrompack ChiralDex-CB column. NMR spectra were recorded on a Bruker AC 200 (^{1}H-NMR at 200 MHz). All signals are expressed as ppm down field from tetramethylsilane. All the compounds showed spectra in agreement with the literature data.

Acknowledgements

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Sample availability: Contact the authors.