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STEREOSELECTIVITY IN THE ENZYMIC OXIDATION OF RACEMIC ALKYL METHYLCARBINOLS - A NEW APPROACH TO THE S-ENANTIOMERS

H.-G. Müller*[a], H. Güntherberg[a], H. Drechsler[a], S. Mauersberger[a], K. Kortus[b], and G. Oehme[b]

[a]Central Institute of Molecular Biology, Robert-Rössle-Str. 10, O-1115 Berlin-Buch, GERMANY

[b]Central Institute of Organic Chemistry, Dept. Complex Catalysis Buchbinderstr. 6, O-2500 Rostock, GERMANY

Summary The R-enantiomers of racemic alkyl methylcarbinols are oxidized specifically by fatty alcohol oxidase (FAOD) from alkane-utilizing yeasts so that the S-enantiomers can be gained in pure state.

Alkane-utilizing yeasts like Candida maltosa, C. tropicalis, Pichia guilliermondii, or Yarrowia lipolytica oxidize n-alkanes terminally in three enzymic reactions to the fatty acids of the same chain length. The first step is catalyzed by a cytochrome P-450 monooxygenase[1] and the emerging primary alcohol is oxidized by a fatty alcohol oxidase (FAOD). The third reaction is catalyzed by a NAD-dependent fatty aldehyde dehydrogenase.[2-4] FAOD needs O_2 as electron acceptor, which is reduced in course of the enzymic reaction to H_2O_2. Its biological function is the oxidation of long chain primary alcohols.

We report now that the enzyme of alkane-utilizing yeasts catalyze also the oxidation of medium and long chain alkyl methylcarbinols even with high stereoselectivity. These compounds are no intermediates of the alkane catabolic pathway and, normally, they don't occur in alkane-utilizing yeast cells.

In our experiments P. guilliermondii was used. Yeasts were cultivated on a mineral salt medium with long chain n-alkanes as only carbon source, harvested and disrupted in a Dyno Mill, using Tris-buffer (100 mM, pH 8.8). After a low-speed centrifugation (6000 xg, 15 min) the pellet containing cell debris was discarded and $CaCl_2$ was added to the supernatant to a final concentration of 20 mM Ca^{2+}, which promote the

sedimenation of the membrane fragments. The pellet of a repeated centrifugation (6000 xg, 90 minutes) was collected, resuspended in the same buffer and centrifugated once more. The membrane fraction is able to oxidize both primary (C_5 - C_{16}, not shown here) and secondary alcohols (C_8 - C_{12}). The former are converted to the corresponding aldehydes, the latter to the methyl ketones as proved by comparison with authentic substances in glc. The homologous alkyl ethylcarbinols are not accepted as substrates. With decan-1-ol as substrate the specific activity of a freshly prepared membrane fraction is in the range of 800 - 1300 nmoles/mg protein/min, with decan-2-ol it varies between 280 and 400.

By means of a O_2 - or H_2O_2-electrode[3] the oxidation can be controlled on-line. As checked in analytical scale fatty alcohols in low concentration are completely oxidized in a short time. With alkyl methylcarbinols as substrates the reactions stop always near 50% of the supplied amount never exceeding this value. Such reaction curves let assume a stereospecific enzymic reaction.
To answer this question analysis of enantiomeric alcohols was carried out by glc after derivatization with isopropyl isocyanate on a fused silica capillary coated with an XE-60 bonded tert. butylvalinamide phase[5]. Peaks of the racemic mixture could be resolved to about 80%. However, the reproducibility was measured better than $\pm$ 2% and the accuracy was estimated better than $\pm$ 3% in the range of small amounts of the R (-)-enantiomer.
As shown in Fig. 1 the assumption was confirmed. With progress of the reaction a strong decrease of the R-enantiomer up to the complete oxidation proceeds, whereas the S-form is not transformed or only to a very low degree.

To confirm this result the oxidation of the racemic octan-2-ol and of both pure enantiomers was compared (Fig. 2). As expected the racemic form and the R(-)-enantiomer are rapidly converted. Whether a negligible oxidation of the S(+)-octan-2-ol takes place could not be decided on the basis of the electrochemical monitoring of the reaction. Therefore, after an incubation period of 15 minutes the mixture containing the S(+)-form was extracted by hexane and the concentrated sample was tested by glc for the ketone. In the limits of our methods no octan-2-one was detectable. From these results we can conclude that the oxidation of alkyl methylcarbinols by FAOD of *P. guilliermondii* is very stereospecific converting only the R(-)-form.

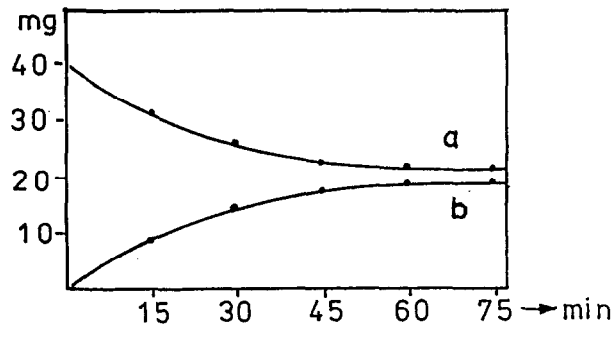

Fig. 1: Oxidation of racemic undecan-2-ol by FAOD - containing membranes of P. guilliermondii. Reaction conditions: 40 mg C_{11}-2-ol dissolved in 0.2 ml acetone 2 ml yeast membranes (39.6 mg protein/ml) 15 ml Tris buffer (1000 mM, pH 8.8), temperature: 30°C. The mixture was stirred. The oxidation was controlled by glc on a DEGS-10 column. a: undecan-2-ol, b; undecan-2-one. The relation of the enantiomers (R/S) was: 0′: 49.0/51.0 ; 15′: 25.4/74.6 ; 60′: 5.0/95.0 75′: not detectable/100. For larger amounts the reaction mixture was scaled up without further modifications.

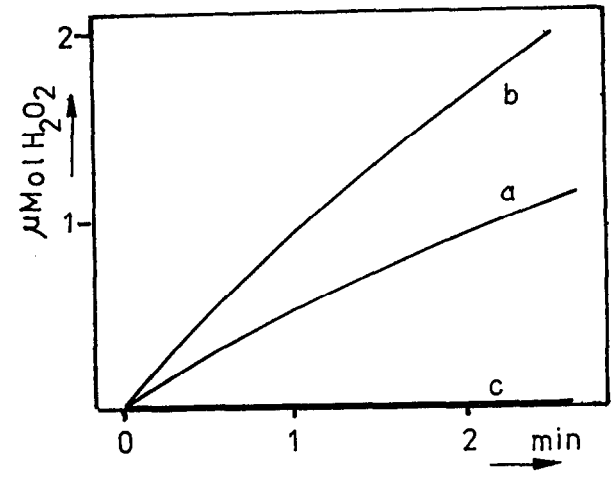

Fig. 2: Oxidation of racemic octan-2-ol /a/ and the pure enantiomers /b = R(-), c = S(+)/, both commercially available by Fluka, Switzerland, by FAOD of P. guilliermondii. Reaction mixture: 1.5 ml Tris-buffer (100 mM, pH 8.8) containing 20 mM Na-azide, 5 mg substrate in 0.025 ml acetone, 0.2 ml of the membrane fraction. The mixture was stirred at 30°C.

Without difficulties this highly stereospecific oxidation can be performed also in enhanced scale for obtaining the S-enantiomers of alkyl methylcarbinols in amounts between 0.1 and 1 g (see Fig. 1). For obtaining the remaining S-enantiomer the reaction mixture was extracted by hexane, followed by LC separation on a silica gel column (particle size 75 m, pore volume 1.2 ml g^{-1}) with CH_2Cl_2.

Alkyl methylcarbinols are clearly separated. Alkanes migrate with the front followed by methyl ketones, and after them the non-converted substrate is eluted. Its enantiomeric purity depends on the completeness of the preceded enzymic reaction.

Esters of these medium chain alkyl methylcarbinols with C-2, C-4, and C-6 carbonic acids are essential constituents of numerous fruit aromas. The pleasant sensory note correlates with the S(+)-enantiomers.[6] Therefore, they find interest for the stereospecific organic synthesis of these fragrant compounds.

The introduced method of selective enzymic oxidation of the R-enantiomers of alkyl methylcarbinols by FAOD for gaining the pure S-enantiomers from racemic forms has two advantages. It is highly stereospecific and it doesn't need any expensive cofactor, which has to be recycled, as necessary in dehydrogenase reactions.

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