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1-(4-hydroxyphenyl)-ethanol dehydrogenases from A. aromaticum – mechanism of inactivation

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PURPOSE OF THE ABSTRACT

R- and S-specific 1-(4-hydroxyphenyl)-ethanol dehydrogenases (R-HPED and S-HPED) are enzymes coming from denitrifying bacterium Aromatoleum aromaticum (Azoarcus sp.). They belong to short-chain dehydrogenase/reductase (SDR) family and physiologically catalyze the NAD+-dependent stereospecific oxidation of (R)- or (S)-1-(4-hydroxyphenyl)-ethanol to 4-hydroxyacetophenone [1]. SDRs from A. aromaticum are also capable of catalyzing the reverse reaction (preferentially at slightly acidic pH) and are already used in industry for synthesizing various aromatic and heteroaromatic secondary alcohols with high enantiomeric purity [2, 3].

The presented study focuses on evaluating the enzyme activity and stability as well as investigating the relations between them, both under storage and process condition. The relationship between aggregation dynamics and activity loss under various pHs and in the presence of a glucose stabilizer was analyzed using spectrophotometric techniques and dynamic light scattering.

For R-HPED, we found out that at a pH of 8.5, the enzyme exhibits high stability and the highest productivity in the reduction of ketones, despite the relatively low activity shown at basic pH. Based on inactivation experiments, the mechanism of thermal inactivation at pH 8.5 was modelled for R-HPED. The irreversible first-order mechanism of R-HPED inactivation was verified by isothermal and multi-temperature data evaluation, confirming that at the pH of 8.5, aggregation of R-HPED is a secondary process occurring to already inactivated enzyme [4].

The mechanism of S-HPED inactivation was studied at pH 6.5 and 9.0 with and without the addition of glucose as a stabilizer. Analysis of the inactivation curves indicates that S-HPED, like R-HPED, is inactivated according to the "one step – two states" mechanism. The obtained results on the inactivation of HPEDs provide the basis for drawing first general conclusions on the inactivation mechanism of the SDR family.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

(R)/(S)-1-(4-hydroxyphenyl)-ethanol dehydrogenase | inactivation | aggregation | activity stabilization

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