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Intermediates of thiamine catalysis immobilized on silica surface as active biocatalysts for α-ketoacid decarboxylation

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Abstract

Thiamine-dependent enzymes catalyse the decarboxylation of α-ketoacids, by both non-oxidative and oxidative mechanisms. Based on the ability of thiamine-cofactor to catalyse itself the decarboxylation of pyruvate to some extent, we have immobilized on a silica surface two 'active aldehyde' intermediates of thiamine catalysis, 2-α-hydroxybenzyl-thiamine pyrophosphate (HBTPP) and 2-α-hydroxyethyl-thiamine pyrophosphate (HETPP). The two intermediates have been tethered by a convenient method on silica support *via* their phosphate groups providing the covalently heterogenised biomolecules $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ and $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$. These bio-composite materials have been evaluated as catalysts for pyruvate and benzoyl-formate decarboxylation in either the presence or not of an aldehyde additive. Our data show that they are stable, very effective and recyclable catalysts for the production of 2-hydroxy-ketones, acetoine and benzoin. Their catalytic behavior is much better than the corresponding behavior of the homogeneous thiamine-systems due to the selected immobilization mode which bears similarities to that of the thiamine-cofactor binding to the protein. Considering our results, possible catalytic pathways of the prepared bio-composite materials are suggested.

Keywords: Thiamine catalysis; Decarboxylation; Immobilised biocatalysts; Modified silica; 2-hydroxy-ketones

1. Introduction

Thiamine pyrophosphate (TPP) serves as a cofactor in a number of enzymic processes found in almost all major metabolic pathways. In living organisms, thiamine-dependent enzymes are mainly involved in the decarboxylation of α-ketoacids, by both non-oxidative and oxidative mechanisms [1–7].

Model and biochemical studies have provided a good insight into the elucidation of the mechanism of action of thiamine-dependent enzymes for which a very recent review is available [[8] and references wherein]. The key points of the catalytic mechanism are highlighted as follows: TPP binds to the apoenzyme by its pyrophosphate group and bivalent metal ions, and is forced by the protein to adopt the specific V conformation, bringing the 4′α-NH_2 group near C(2) of thiazole, attracting a proton, creating the "ylide" and initiating the catalytic cycle. This is followed by addition of the α-ketoacid substrate, decarboxylation of the formed adduct and formation of the "active aldehyde" intermediate which most probably adopts the S conformation. This conformation favors the release of the main aldehyde product regenerating, finally, the TPP-"ylid" form (Scheme 1) [8–13].

A unique feature of the TPP cofactor is its relative importance in catalysis, since TPP alone can perform the reaction, although over a million times less efficiently than the holoenzyme [14]. Given that the only conserved residues in the active site of thiamine-dependent enzymes are those directly bound to the cofactor or metal ion, it was suggested that it is the cofactor, its conformation and its environment that determine the catalytic efficiency [8,14].

The catalysis of C–C bond formation resulting in 2-hydroxy-ketone, which constitutes a side reaction of thiamine-dependent decarboxylases, is of great research interest. In

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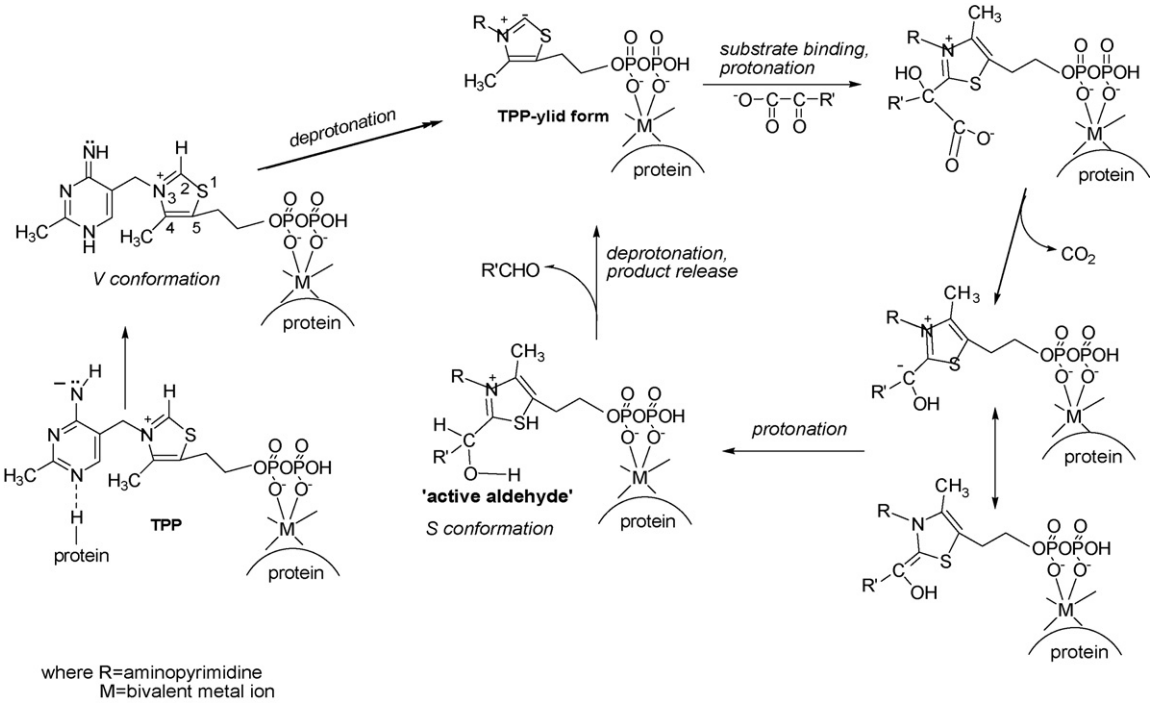

Scheme 1. Mechanism of action of α-ketoacid decarboxylases.

general 2-hydroxy-ketones are important structural subunits in many biologically active natural products and are also important reagents for stereo-selective syntheses. Several versatile methods chemical or enzymatic have been developed as alternatives to the classical benzoin condensation [12,15–19].

On the other hand, the fixation of active biomolecules *via* covalent attachment to a silica surface for biotechnological processes is a remarkable synthetic approach [20–23]. In a previous work, to take advantage of the catalytic ability of TPP, we have developed a one-step procedure to tether TPP on a silica surface through its phosphate moiety providing a hybrid organic-inorganic biocatalyst [24]. The TPP-immobilised catalytic performance for pyruvate decarboxylation producing a 2-hydroxy-ketone, acetoine, was clearly optimized when compared to those of the homogeneous system [24].

The main goal of this work is to immobilize on a silica surface active intermediate of the thiamine catalysis and to evaluate their efficiency for decarboxylation of α-ketoacids. To this end, we have synthesized two "active aldehyde" derivatives of TPP, 2-α-hydroxybenzyl-thiamine pyrophosphate (HBTPP) and 2-α-hydroxyethyl-thiamine pyrophosphate (HETPP) being the "active aldehyde" intermediates of benzoyl-formate decarboxylase (BFD) and pyruvate decarboxylase (PDC), respectively. Consequently they have been tethered on silica support *via* their phosphate groups providing the covalently heterogenised biomolecules $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ and $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$. Our results show that the novel bio-composite materials are very effective systems for pyruvate and benzoyl-formate decarboxylation resulting in acetoine and benzoin formation.

2. Materials and methods

2.1. Materials

All substrates were purchased in their highest commercial purity. Infrared spectra were recorded on a Spectrum GX Perkin-Elmer FT-IR System and solution NMR spectra were recorded with a Bruker AMX-400 MHz spectrometer with external TMS as reference. Solid-state ^{13}C NMR spectra were acquired by using cross-polarization (CP), magic-angle spinning (MAS), and high-power proton decoupling in a Chemagnetics CMX 300 apparatus with chemical shifts quoted relative to TMS. Diffuse reflectance UV–vis spectra were recorded at room temperature on a Shimadzu UV-2401PC with a $BaSO_4$ coated integration sphere. Thermogravimetric analyses were carried out using Shimadzu DTG-60 analyser. X-ray powder diffraction data were collected on a D8 Advance Bruker diffractometer by using Cu Kα (40 kV, 40 mA) radiation and a secondary beam graphite monochromator. GC analyses were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 mass spectrometer.

2.2. Synthesis of 2-(α-hydroxybenzyl) thiamine pyrophosphate chloride hydrochloride (HBTPP)

Five grams (∼15 mmol) of thiamine chloride hydrochloride were dissolved in water to a volume of approximately 10 mL, and the solution was adjusted to pH 8.0 with 3N NaOH. To this were added 23 mLof absolute methanol and 16 mL (∼150 mmol) of redistilled benzaldehyde. The mixture was stirred at room temperature and was constantly purged with nitrogen. Periodically, dilute NaOH was added to maintain the pH, and methanol was