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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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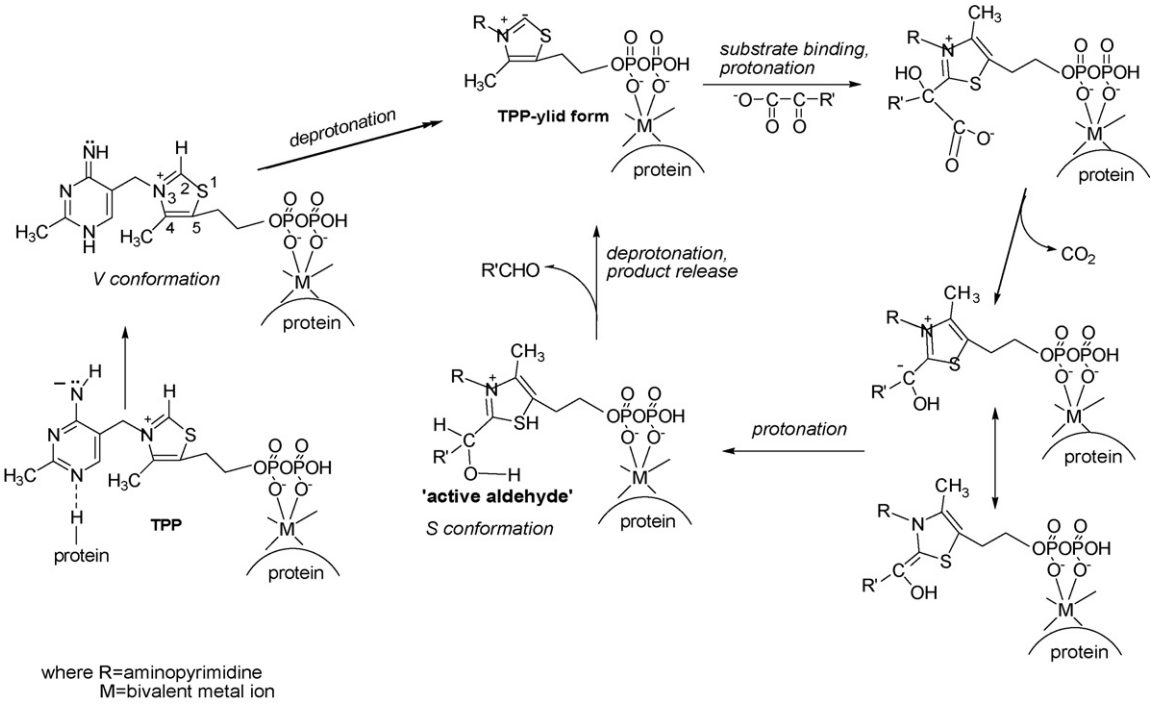


Scheme 1. Mechanism of action of α-ketoacid decarboxylases.

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

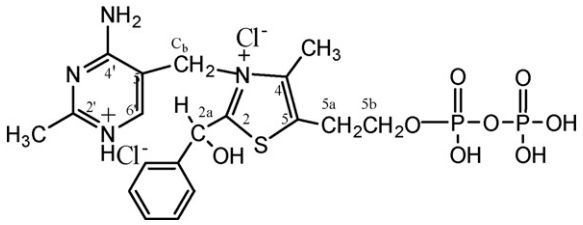


Fig. 1. 2-(α-Hydroxybenzyl) thiamine pyrophosphate chloride hydrochloride (HBTPP).

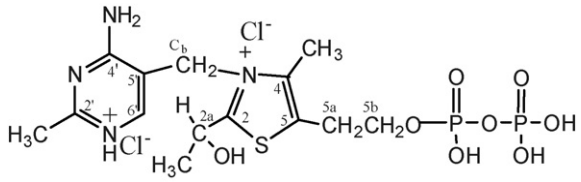


Fig. 2. 2-(α-Hydroxyethyl) thiamine pyrophosphate chloride hydrochloride (HETPP).

added to maintain a homogenous solution. After 3 h, concentrated HCl was added to adjust the solution to pH 2–2.5. This addition caused the reaction mixture to separate into to phases. The upper aqueous methanol layer was concentrated to apparent dryness and the residue was washed with acetone. The acetone-washed, dry residue was suspended in a small volume of water, forming a solution at 50–60 °C. This solution was filtered while hot, and was then allowed to cool, giving crystals of 2-(α-hydroxybenzyl) thiamine pyrophosphate chloride hydrochloride (Fig. 1). These are recrystallized from water, mp 178–181 °C with decomposition [25].

Anal. Calcd. for $C_{19}H_{26}N_4O_8P_2SCl{\cdot}CH_3OH$ (%): C:40.03, N:9.34, H:5.00, S:5.34. Found: C:40.54, N:8.72, H:5.02, S:5.12. IR (KBr, cm^{-1}, selected peaks) 3399: ν(OH), 3250: $\nu(NH_2)$, 1675, 1624: $\nu(8a) + \delta(NH_2)$, 1542: ν(8b), 1455: $\delta(CH_3)$, 1340: $\delta(CH_2)$, 1234: ν(P=O), 1086: ν(C–O), 933: ν(P–O), 482: δ(P–O). ^{1}H NMR(D_2O, δ) 7.4(s): $C_{2''}$–H, 7.3(s): $C_{3'',4''}$–H, 6.7(s): $C_{6'}$–H, 6.5(s): C_{2a}–H, 5.3–5.2(q): C_b–H_2, 4.2(s): C_{5b}–H_2, 3.3(s): C_{5a}–H_2, 2.5(s): 2′–CH_3, 2.4(s): 4–CH_3. ^{13}C NMR(D_2O, δ) 180.8: **C**(2), 164.3: **C**(2′), 163.6: **C**(4′), 147.8: **C**(4), 140.7: **C**(6′), 130.5–138.7: **C**(Phe), 136.6: **C**(5), 109.7: **C**(5′), 74.4: **C**(2a), 67.5: **C**(5b), 49.4: **C**(b), 30.2: **C**(5a), 23.1: –**C**H_3(2′), 13.8: –**C**H_3(4).

2.3. Immobilization of HBTPP on a silica support

A solution of 2-(α-hydroxybenzyl) thiamine pyrophosphate chloride hydrochloride (HBTPP) (0.5 g in 10 mL of methanol with 2 equiv. of Et_3N) containing suspended silica gel (1.25 g average pore diameter 60 Å) was refluxed for 2 h and then the recovered solid was washed with methanol, acetone, diethyl-ether and dried at 60 °C under vacuum. Anal. Found for $[HBTh–O–P_2O_6SiO_{3/2}]_n{\cdot}xSiO_2$ (%): C:2.82, N:0.65, H:0.81, S:0.50. Drift-IR (KBr, cm^{-1}, selected peaks) 3370: ν(OH), 3240: $\nu(NH_2)$, 1657: $\nu(8a) + \delta(NH_2)$, 1528: ν(8b), 1480: $\delta(CH_3)$, 490: δ(P–O). ^{13}C CP MAS NMR (δ) 180.6: **C**(2), 166.6: **C**(2′), 161.2: **C**(4′), 149.8: **C**(4), 145.6: **C**(6′), 130.0–137.0: **C**(Phe), 134.3: **C**(5), 107.9: **C**(5′), 72.2: **C**(2a), 65.0: **C**(5b), 49.4: **C**(b), 28.7: **C**(5a), 22.5: –**C**H_3(2′), 11.9: –**C**H_3(4).

2.4. Synthesis of the 2-(α-hydroxyethyl) thiamine pyrophosphate chloride hydrochloride (HETPP)

Freshly distilled acetaldehyde (120 mmol) was added to a 15-mL aqueous solution of TPP (6.0 mmol) in a 50-mL pear-shaped flask. The pH of the solution was adjusted to 8.0 with 5.0 M NaOH and then to 8.7 with 1 M NaOH. The flask was incubated for 2 h at 45 °C. The solution was cooled to room temperature and its pH readjusted to 8.7 with 1 M NaOH. It was then incubated for an additional 1 h at 45 °C. The pH of the product mixture was adjusted to 2.5 with 5N HCl and the solution concentrated by rotary evaporation in vacuum to approximately 1-mL total volume. The concentrated solution was desalted by passage through a 1.0 cm × 48 cm column of Sephadex G-10, pre-equilibrated and eluted with 1% formic acid. One-milliliter fractions were collected and the absorbance at 254 nm measured. The fractions having absorption at 254 nm were pooled and concentrated to about 2-mL total volume. The desalted product solution was then chromatographed through a 45 cm × 1.5 cm column of Sephadex SP-C25 cation-exchange resin, preequilibrated and eluted with 1% formic acid. Fractions 6 mL in volume were collected, and those containing HETPP (Fig. 2)were pooled and freed of water and formic acid by rotary evaporation. The colorless glassy product was dissolved in 2 mL of 0.10 M HC1 and the solvent removed as before [26].

Anal. Calcd. for $C_{14}H_{24}N_4O_8P_2SCl{\cdot}2H_2O$ (%): C:31.02, N:10.34, H:5.17, S:5.91. Found: C:31.60, N:9.70, H:5.40, S:4.98. IR (KBr, cm^{-1}, selected peaks). 3330: ν(OH), 3260: $\nu(NH_2)$, 1663, 1620: $\nu(8a) + \delta(NH_2)$, 1538: ν(8b), 1443: $\delta(CH_3)$, 1366: $\delta(CH_2)$, 1229: ν(P=O), 1099: ν(C–O), 1007: ν(P–O), 500: δ(P–O). ^{1}H NMR(D_2O, δ) 7.3(s): $C_{6'}$–H, 5.5 (s): C_b–H_2, 5.4(q): C_{2a}–H, 4.2(d): C_{5b}–H_2, 3.3(t): C_{5a}–H_2, 2.6(s): 2′–CH_3, 2.4(s): 4–CH_3, 1.7(d): 2a–CH_3. ^{13}C NMR(D_2O, δ) 181.5: **C**(2), 165.4: **C**(2′), 164.7: **C**(4′), 147.2: **C**(4), 141.9: **C**(6′), 136.7: **C**(5), 111.7: **C**(5′), 67.3: **C**(2a), 67.7: **C**(5b), 49.5: **C**(b), 30.4: **C**(5a), 23.6: –**C**H_3(2′), 14.1: –**C**H_3(2′,4), 24.6: –**C**H_3(2a).

2.5. Immobilization of HETPP on a silica support

A solution of 2-(α-hydroxyethyl) thiamine pyrophosphate (HETPP) (0.5 g in 10 mL of methanol with 2 equiv. of Et_3N) containing suspended silica gel (1.25 g average pore diameter 60 Å) was refluxed for 2 h and then the recovered solid was washed with methanol, acetone, diethyl-ether and dried at 60 °C under vacuum. Anal. Calcd. for $[HETh–O–P_2O_6SiO_{3/2}]_n{\cdot}xSiO_2$ (%):C:2.90, N:0.82, H:1.16, S:0.76. Drift-IR (KBr, cm^{-1}, selected peaks). 3364: ν(OH), 3251: $\nu(NH_2)$, 1655: $\nu(8a) + \delta(NH_2)$, 1512: ν(8b), 1471: $\delta(CH_3)$, 492: δ(P–O). ^{13}C CP MAS NMR (δ) 177.9: **C**(2), 163.1: **C**(2′), 163.1: **C**(4′), 144.5: **C**(4), 141.0: **C**(6′), 109.4: **C**(5′), 66.0: **C**(2a), 66.0: **C**(5b), 48.3: **C**(b), 31.6: **C**(5a), 22.4: –**C**H_3(2′), 12.5: –**C**H_3(4), 22.4: –**C**H_3(2a).

2.6. Catalytic reactions

All reactions in the (A) assay were carried out at 37 °C in methanol (1 mL) with substrate (200 μmol), aldehyde (400 μmol), catalyst (20 μmol) and NaOH (40 μmol). The ratio of [catalyst:aldehyde:base:substrate] was equal to [1:20:2:10]. Equal, all reactions in the (B) assay were carried out at 37 °C in methanol (1 mL) with substrate (200 μmol), catalyst (20 μmol) and NaOH (40 μmol). In this case, the ratio of [catalyst:base:substrate] was equal to [1:2:10]. In both cases, bromobenzene was used as internal standard.

The substrate conversion was monitored by GC–MS, by removing small samples of the reaction mixture. To establish the identity of the products unequivocally, the retention times and spectral data were compared to those of commercially available compounds. Blank experiments showed that without thiamine-based catalyst there is no substrate conversion.

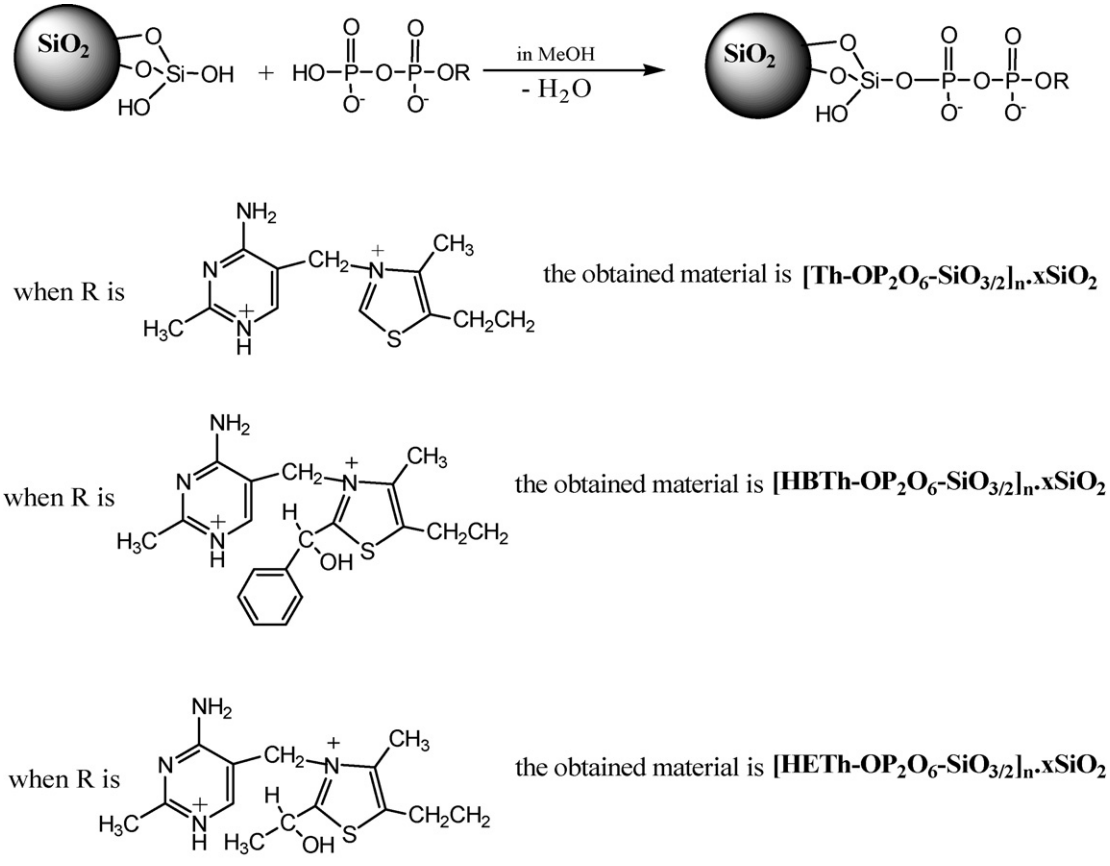
3. Results and discussion

3.1. Immobilization of "active aldehyde" thiamine derivatives on a silica surface

The synthesized "active aldehyde" derivatives of TPP, 2-α-hydroxybenzyl-thiamine pyrophosphate chloride hydrochloride (HBTPP) and 2-α-hydroxyethyl-thiamine pyrophosphate chloride hydrochloride (HETPP), were immobilized on a silica surface following the same procedure as for thiamine pyrophosphate immobilization reported recently by our group [24]. The synthetic procedures are described in detail, in Section 2. The obtained hybrid materials were the $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ and $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$, while for comparison purposes the $[Th–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ was also prepared (Scheme 2). The achieved loading was about 0.4 mmol of biomolecule per gram of modified silica, determined by thermogravimetric and elemental analysis.

The powder XRD spectra show that the biomimetically modified materials, $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ and $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$, are homogeneous and amorphous, like the untreated silica. This indicates that the biomolecules are covalently attached to the support and not co-crystallized with it (see Supplementary Material, Fig. 1).

Diffuse reflectance FTIR ('DRIFTS') data of the hybrid materials showed absorption bands assigned to vibrations of both the silica support and the attached biomolecules. In the spectrum of $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ the bands observed at 1657 and 1528 cm^{-1} and in the spectrum of $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ the corresponding bands at 1656 and 1525 cm^{-1} (see Supplementary Material, Figs. 2 and 3) are attributed to coupling of the pyrimidine ring (8a) with the $\delta(NH_2)$ group and to a pure pyrimidine ring vibration (8b), respectively [24,27–29]. In both spectra, the bands, centered



Scheme 2. Preparation of the immobilized TPP biocatalysts.

at 1200, 1080 and 806 cm^{-1} were assigned to vibrations of Si–O–Si and Si–O bonds from the support.

^{13}C CP MAS NMR spectra of the tethered thiamine derivatives have signals which characterize the immobilized biomolecules [27,28]. The assignments are based on the corresponding solution- and solid-phase spectra of the synthesized compounds [27–29]. In the spectrum of $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ the signals at 161.2, 145.6 and 107.9 ppm are assigned to the resonances of C(4′), C(6′) and C(5′) of the pyrimidine ring; the resonances of C(b) and the methyl carbons 2′–CH_3 and 4–CH_3 appear at 49.4, 22.5 and 11.9 ppm, respectively (see Supplementary Material, Fig. 4). The resonances of the corresponding carbon atoms C(4′), C(6′), C(5′), C(b), 2′–CH_3 and 4–CH_3, in the ^{13}C CP MAS NMR spectrum of $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$, are located at 163.1, 141.0, 109.4, 48.3, 22.4 and 12.5 ppm (see Supplementary Material, Fig. 5).

3.2. Catalytic reactions

Thiamine enzymes catalyse the decarboxylation of α-ketoacids *in vivo* [1–7]. There was some evidence that thiamine itself, e.g., in protein-free model systems, catalyses pyruvate decarboxylation [30]. We have previously confirmed this ability of thiamine in a protein-free system. Moreover, the immobilized TPP showed high catalytic activity for pyruvate decarboxylation demonstrating that it is a very active biocatalyst even more efficient than the homogeneous one [24].

Pyruvate and benzoyl-formate decarboxylation catalysed by thiamine adducts occurs *via* two procedures in presence (A) or not (B) of aldehyde additive (Scheme 3). When pyruvate was used as substrate the chosen aldehyde was acetaldehyde providing acetoine by both assays. In benzoyl-formate decarboxylation towards benzoin the additive was benzaldehyde (Scheme 3). To examine the effectiveness of homogeneous and heterogenised systems, both assays A and B have been followed. A [substrate:catalyst] molar ratio [10:1] was used. The reaction yield is related to both substrate conversion and product formation. Results are given in Tables 1 and 2.

For pyruvate decarboxylation, the homogeneous HBTPP catalyst showed higher activity than the homogeneous TPP and HETPP converting the substrate at 215 and 110 min in the pres-

Table 1
Pyruvate decarboxylation catalysed by thiamine-catalysts

Catalyst	Reaction time (min)	Conversion (%)
TPP (homogeneous system)	330	100[a]
HBTPP (homogeneous system)	215	90[a]
HETPP (homogeneous system)	330	78[a]
$[Th–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	110	100[a]
$[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	35	100[a]
$[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	160	95[a]
TPP (homogeneous system)	330	88[b]
HBTPP (homogeneous system)	110	100[b]
HETPP (homogeneous system)	330	72[b]
$[Th–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	60	100[b]
$[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	35	100[b]
$[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	115	98[b]

[a] *Reaction conditions*: All reactions were carried out at 37 °C in MeOH (1 ml) with pyruvate (200 μmol), acetaldehyde (400 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

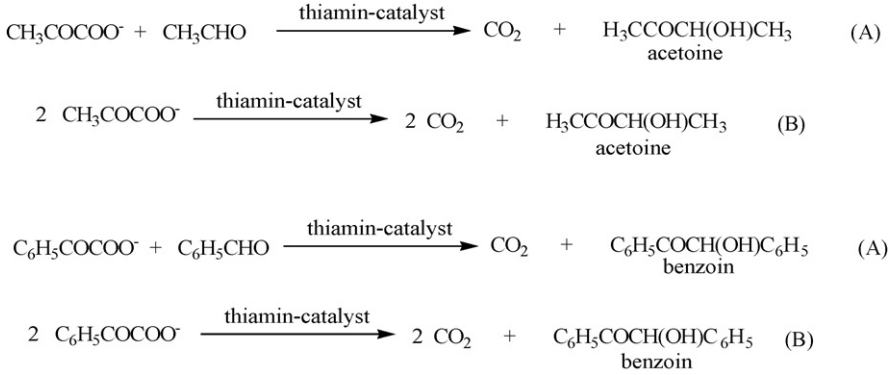
[b] *Reaction conditions*: All reactions were carried out at 37 °C in MeOH (1 ml) with pyruvate (200 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol). In both cases, bromobenzene was used as internal standard.

Table 2
Benzoyl-formate decarboxylation catalysed by thiamine-catalysts

Catalyst	Reaction time (min)	Conversion (%)
TPP (homogeneous system)	330	67[a]
HBTPP (homogeneous system)	250	74[a]
HETPP (homogeneous system)	330	72[a]
$[Th–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	5	100[a]
$[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	5	100[a]
$[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	5	100[a]
TPP (homogeneous system)	330	82[b]
HBTPP (homogeneous system)	215	90[b]
HETPP (homogeneous system)	330	85[b]
$[Th–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	5	100[b]
$[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	5	100[b]
$[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$	5	100[b]

[a] *Reaction conditions*: All reactions were carried out at 37 °C in MeOH (1 ml) with benzoyl-formate (200 μmol), benzaldehyde (400 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

[b] *Reaction conditions*: All reactions were carried out at 37 °C in MeOH (1 ml) with benzoyl-formate (200 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol). In both cases, bromobenzene was used as internal standard.



Scheme 3. Reactions of pyruvate and benzoyl-formate decarboxylation catalysed by thiamine catalysts.

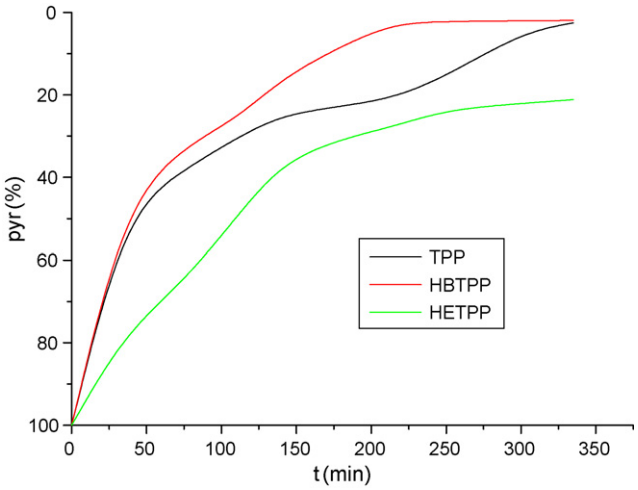


Fig. 3. Time-dependent reaction profile for pyruvate decarboxylation with acetaldehyde additive (assay A) catalysed by homogenous TPP, HETPP and HBTPP catalysts. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with pyruvate (200 μmol), acetaldehyde (400 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

ence or not of acetaldehyde, respectively (Table 1). The time course profiles for the pyruvate decarboxylation catalysed by homogeneous thiamine-systems are given at Figs. 3 and 4.

Immobilization of the catalysts clearly results in a remarkable improvement of their ability, given that the heterogenised TPP, HBTPP and HETPP catalysts fully converted the pyruvate with acetaldehyde additive at 110, 35 and 160 min instead of 330, 215 and 330 min of the ungrafted TPP, HBTPP and HETPP catalysts, respectively (Table 1, Fig. 5). By testing the second assay without acetaldehyde, the same behavior is observed, i.e., pyruvate decarboxylation catalysed by the immobilized TPP, HBTPP and HETPP biocatalysts is accomplished at 60, 35 and 115 min, respectively while the corresponding times of the homogeneous systems were found to be 330, 110 and 330 min

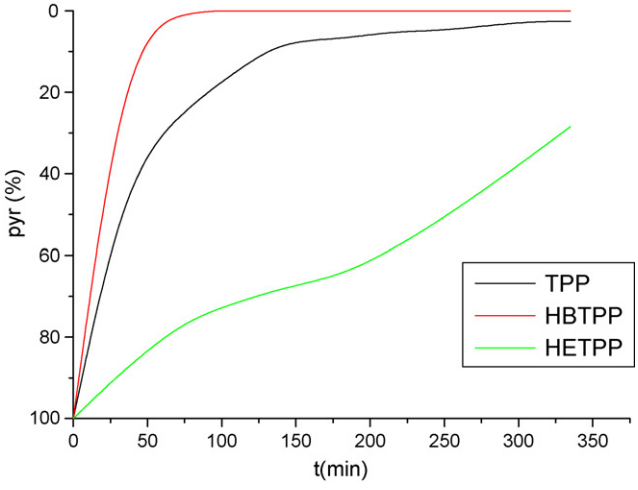


Fig. 4. Time-dependent reaction profile for pyruvate decarboxylation (assay B) catalysed by homogenous TPP, HETPP and HBTPP catalysts. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with pyruvate (200 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

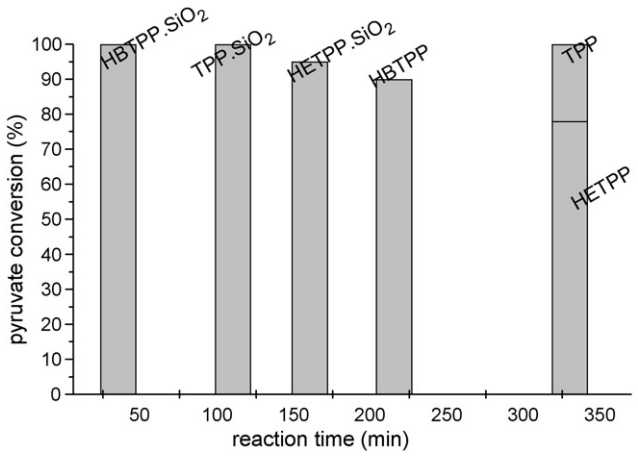


Fig. 5. Pyruvate decarboxylation catalysed by grafted or ungrafted TPP, HBTPP and HETPP with acetaldehyde additive. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with pyruvate (200 μmol), acetaldehyde (400 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

(Table 1, Fig. 6). From these data, it is clear that the immobilized HBTPP biomolecule is the most effective catalyst.

In homogenous benzoyl-formate decarboxylation, HBTPP converts 74% and 90% of the substrate at 250 and 215 min, respectively (in the presence or not of benzaldehyde) exhibiting better catalytic activities than the TPP and HETPP (Table 2). The time course profiles for the benzoyl-formate decarboxylation catalysed by homogeneous TPP, HETPP and HBTPP catalysts are presented in Figs. 7 and 8.

All the heterogenised catalysts presented outstanding effectiveness converting immediately ($t < 5$ min) the substrate in the presence of benzaldehyde towards benzoin (Table 2, Fig. 9). Without benzaldehyde (assay B), the immobilized TPP, HBTPP and HETPP biocatalysts remained very active, decarboxylating the substrate immediately (Table 2, Fig. 10).

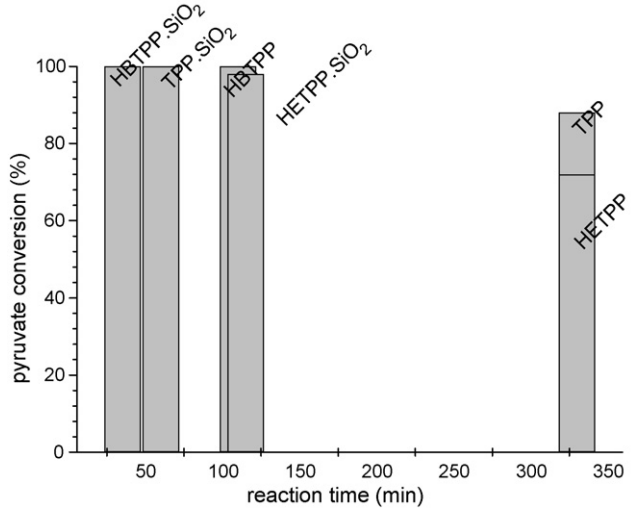


Fig. 6. Pyruvate decarboxylation catalysed by grafted or ungrafted TPP, HBTPP and HETPP. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with pyruvate (200 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

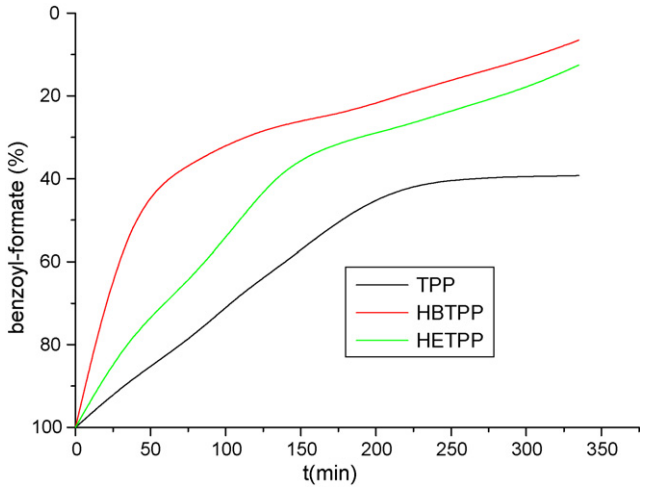


Fig. 7. Time-dependent reaction profile for benzoyl-formate decarboxylation with benzaldehyde additive (assay A) catalysed by homogenous TPP, HETPP and HBTPP catalysts. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with benzoyl formate (200 μmol), benzaldehyde (400 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

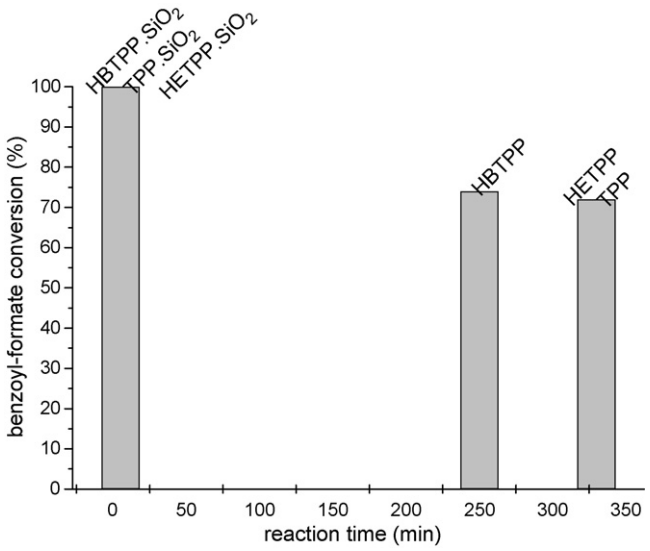


Fig. 9. Benzoyl-formate decarboxylation catalysed by grafted or ungrafted TPP, HBTPP and HETPP with benzaldehyde additive. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with benzoyl-formate (200 μmol), benzaldehyde (400 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

To clarify that the catalysis was performed by the heterogenised catalysts and not by leached thiamine molecules from the silica support, after the catalytic reactions were evolved, the solids were filtered and the filtrates allowed to further react in the same conditions. No additional products were detected, indicating that the catalytic activity is exclusively due to the heterogenised catalysts.

The ability of catalyst reuse was also evaluated. After the termination of the studied reaction, a new portion of substrate has been added. In the case of immobilized catalysts, it was observed that they can be reused at least 3 times, thus making them cost-effective for a putative application. On the other hand, the homogeneous systems showed zero conversion of the additional dose of the substrate. It is worth noticing, that the heterogenised catalysts recovered by filtration from the catalytic reactions exhibited (a) identical DRIFT-IR spectra with the 'unused' catalysts and (b) almost the same loading, e.g., determined by thermogravimetric analysis.

In summary, all the heterogenised systems prepared here were much better catalysts than the homogeneous ones. This fact could be due to the influence of the support polar surface which may favour the polar substrate approach and promote the 2-hydroxy-ketone release. Moreover, the solid surface does not seem to cause remarkable steric hindrance to the catalytic reaction, maintaining the active centres to an adequate distance. That is, the achieved immobilised mode through the phosphate group of the catalyst, which is analogous to

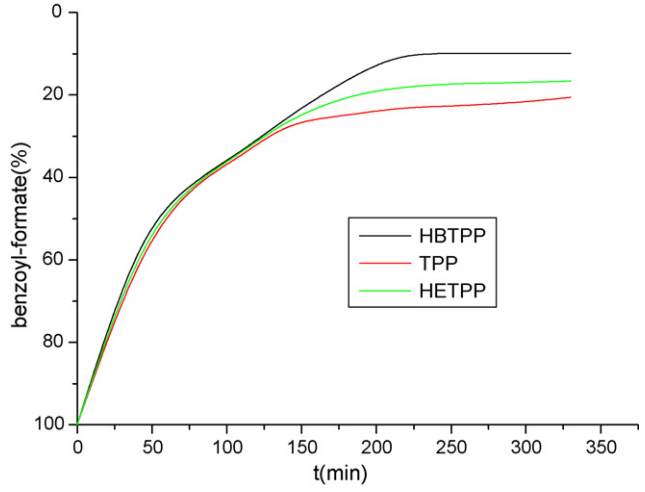


Fig. 8. Time-dependent reaction profile for benzoyl-formate decarboxylation (assay B) catalysed by homogenous TPP, HETPP and HBTPP catalysts. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with benzoyl formate (200 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

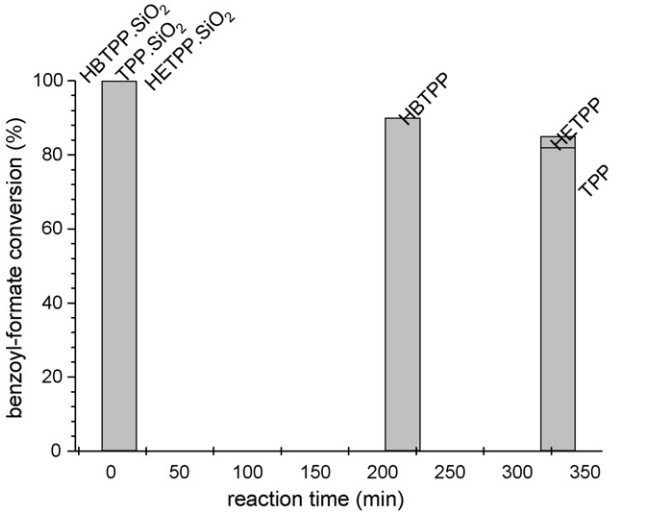
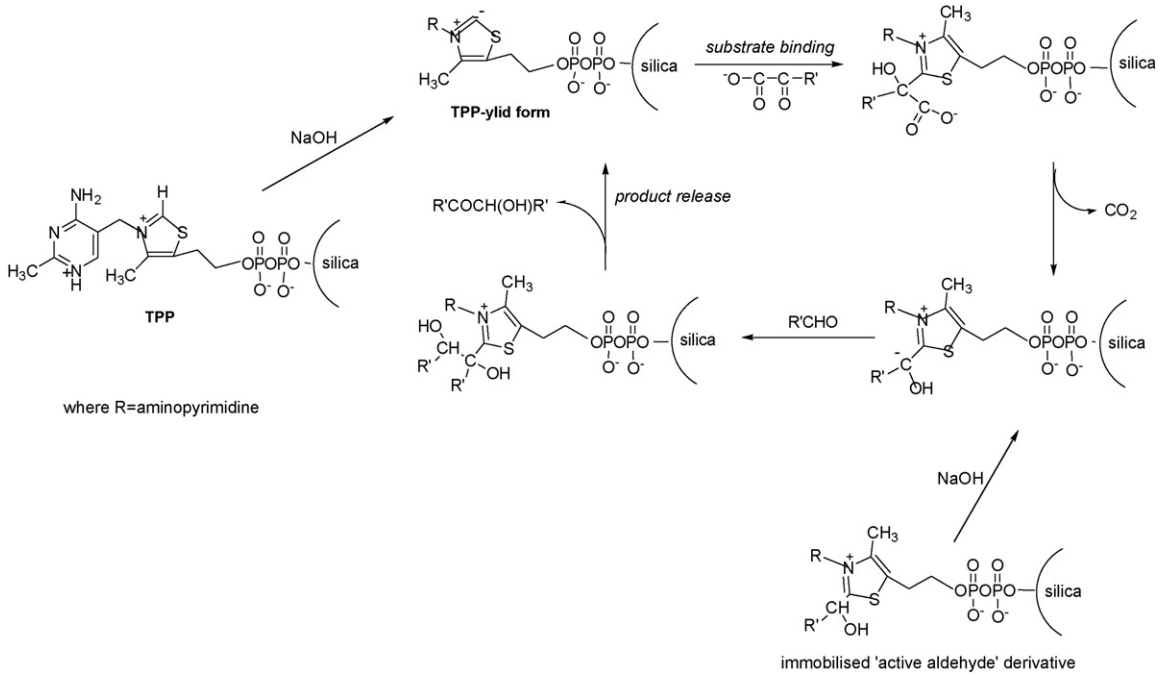


Fig. 10. Benzoyl-formate decarboxylation catalysed by grafted or ungrafted TPP, HBTPP and HETPP. *Conditions*: The catalytic reactions were carried out at 37 °C in MeOH (1 mL) with benzoyl formate (200 μmol), thiamine-catalyst (20 μmol) and NaOH (40 μmol).

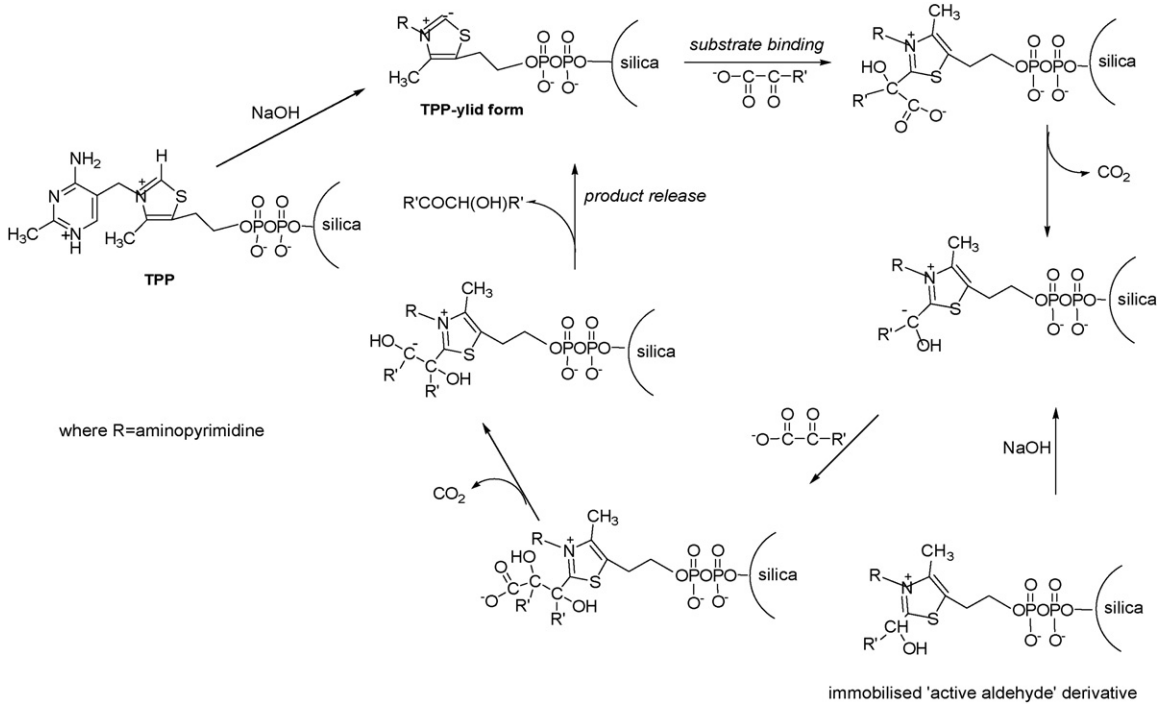


Scheme 4. Possible catalytic cycle of immobilized TPP and 'active aldehyde' derivatives in the presence of aldehyde as an acyl acceptor (assay A).

that of the biocatalyst binding to the protein, looks to be successful.

3.3. Mechanistic aspects

Possible mechanisms for α-ketoacid decarboxylation, in the presence (assay A) or not of an aldehyde additive (assay B), catalysed by immobilized TPP and 'active aldehyde derivatives' are presented in Schemes 4 and 5 related to that of thiamine-dependent enzymes (see Scheme 1). In the initial step, the deprotonation of the C(2) atom of thiazole moiety occurs into the basic medium resulting in the formation of the ylid. The C(2) carbanion of the catalyst once formed, is able to react with the carbonyl carbon of the substrate and to bind it (Schemes 4 and 5). Subsequent decarboxylation probably results in the C(2α)-carbanion intermediate, as in the enzymic catalysis



Scheme 5. Possible catalytic cycle of immobilized TPP and 'active aldehyde' derivatives without aldehyde additive (assay B).

(Scheme 1). When immobilized 'active aldehyde' derivatives are used as catalysts, their deprotonation generates immediately the active C(2α)-carbanion (Schemes 4 and 5). If the catalysed decarboxylation is performed in the presence of an aldehyde (assay A) (Scheme 4), the immobilized C(2α)-carbanion is ligated to an aldehyde molecule acting as an acyl acceptor. Finally, 2-hydroxy-ketone is eliminated regenerating the TPP-ylid form. When the assay B is followed, the immobilized C(2α)-carbanion is ligated to a second substrate molecule. Subsequently, (a) decarboxylation of the adduct formed and (b) product release lead to the TPP-ylid form initiating the catalytic cycle (Scheme 5).

4. Conclusions

We have immobilized on a silica surface two derivatives of thiamine pyrophosphate, 2-α-hydroxybenzyl-thiamine pyrophosphate (HBTPP) and 2-α-hydroxyethyl-thiamine pyrophosphate (HETPP) being the "active aldehyde" intermediates of the enzymic cycle of benzoyl-formate decarboxylase (BFD) and pyruvate decarboxylase (PDC), respectively. Their immobilization constitutes a convenient, mild and one-step procedure by using the phosphate moieties of the biomolecules. This mode presents structural similarities to that of the thiamine pyrophosphate binding on the protein support.

The obtained bio-composite materials, $[HBTh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$ and $[HETh–OP_2O_6–SiO_{3/2}]_n{\cdot}xSiO_2$, have been evaluated for pyruvate and benzoyl-formate decarboxylation. They are stable, very effective and recyclable catalysts for the production of 2-hydroxy-ketones, acetoine and benzoin. The silica polar surface clearly promotes their formation, since the heterogenised catalysts present much better properties than the corresponding homogeneous ones.

The reactions catalysed by the present biomimetically modified silicas seem to occur *via* mechanisms which are suggested here and are consistent to that of the enzymic systems.

With regards to future studies, it is important to evaluate the present non-enzymic catalysts for the enantioselective formation of various optically active 2-hydroxy-ketones.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcata.2006.11.049.

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