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Practical tethering of vitamin B_1 on a silica surface *via* its phosphate group and evaluation of its activity

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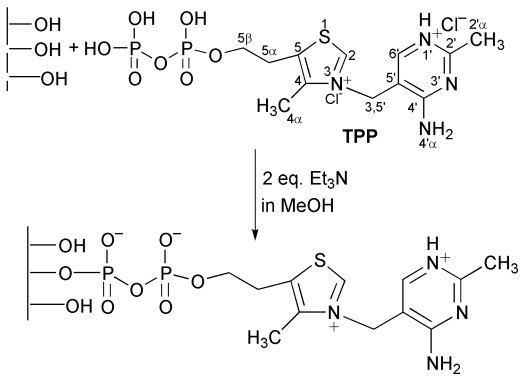
A convenient immobilization of thiamine pyrophosphate molecules on a silica surface through the phosphate group is developed, leading to a very active heterogenised biocatalyst for pyruvate decarboxylation.

Integration of bio- and chemo- catalysis together with materials science provides the opportunity to design and develop new materials for innovative applications. Hybrid organic–inorganic composite materials are one of the most attractive targets achievable by this co-operative process. Many of them have been developed by chemically modified silica gels with organic functionalities.[1] The fixation of active biomolecules *via* covalent attachment to a silica surface for biotechnological processes is a remarkable aspect of this research area.[2] Functionalised alkoxysilanes such as 3-aminopropyltriethoxysilane,[3] 3-sulfanylpropyltrimethoxysilane,[4] monoethoxydimethylsilylbutanal,[5] cyanoethyltriethoxysilane,[6] 3-(triethoxysilyl)propyl isocyanate,[7] (3-glycidyloxypropyl)trimethoxysilane[8] or iodopropyltrimethoxysilane[9] have been used as connecting links. To overcome the limited range of available silanes, post-modification of the grafted functions on the silica surface is often a necessary synthetic step.[10] However, the usually adopted multi-stage post-modification process for linking the silica surface with the active group of the biomolecule, in general, is problematic because it occludes the preparation of these materials. Here we report a one-step synthesis for the tethering of vitamin B_1 on a silica surface *via* the phosphate group of the biomolecule. Furthermore, in the novel process, no additional functional spacer is required. Evaluation of the catalytic activity of the novel material for pyruvate decarboxylation showed that it is a more efficient catalyst than the homogeneous system.

A solution of thiamin pyrophosphate chloride hydrochloride, TPP (0.5 g in 10 ml of ethanol 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 and dried at 60 °C under vacuum (Scheme 1). The achieved loading is *ca.* 0.3 mmol TPP per gram of modified silica, determined by elemental analysis.

Diffuse reflectance FTIR ('DRIFTS') data of the material, showed absorption bands of the thiamine molecule at *ca.* 1682 and 1538 cm^{-1} which are attributed to coupling of the pyrimidine ring (8a) with the $\delta(NH_2)$ group and to a pure pyrimidine ring vibration (8b) respectively.[11] ^{13}C CP MAS NMR spectrum of the tethered TPP displayed signals which characterize the immobilized biomolecule.† The ^{31}P CP MAS NMR spectrum presents the isotropic chemical shift at −11 ppm (relative to 85% H_3PO_4) accompanied by two sidebands. This feature confirms the presence of two slightly non-equivalent ^{31}P nuclei,[12] as expected in the present case. A Herzfeld–Berger analysis[13] gave chemical shift anisotropy tensor components of 75.5, 8.5 and −117.1 ppm (average = −11.0 ppm). The CP MAS ^{29}Si NMR spectrum of untreated silica gave a typical spectrum for a silica sample showing two major resonance peaks at −101 and −111 ppm assigned to Q^3 ($Si(OSi)_3OH$) and Q^4($Si(OSi)_4$) groups and a third, quite weak signal, at −92 ppm attributed to Si with two OH groups (designated Q^2). In the CP MAS ^{29}Si NMR spectrum of modified silica an enhancement of the Q^4 peak and absence of the Q^2 signal were observed indicating a decrease in the number of Si–OH groups; this is consistent with the loss of protons on the OH groups of the silica upon phosphorylation. There was, however, no new signal arising from the Si–O–P moiety of the solid. Either this peak is too weak to be distinguished from the noise, or it is obscured by the strong Q^3 and Q^4 signals.[14] To prepare a complex of modified silica, $[Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_n{\cdot}x\text{-}SiO_2$, with Zn(II), an excess of $ZnCl_2$ is added in methanol. The resulting material, $[(ZnCl_2)_2{\cdot}Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_m{\cdot}ySiO_2$, had a ratio of zinc to thiamine molecule equal to 2:1. The amount of Zn(II) was determined by back-titration of the remaining amount of Zn(II) into the solution. The ^{13}C CP MAS NMR data for the metallated material clearly indicated metal coordination to the pyrimidine $N_{1'}$ atom of the thiamine molecule.† This is also supported by DRIFTS data where the coupled ν (8a) + $\delta(NH_2)$ vibration as well as the ν (8b) band of the pyrimidine ring were shifted at *ca.* 1667 and 1544 cm^{-1} respectively.[11] The ^{31}P CP MAS isotropic chemical shift was at −9.7 ppm, while a Herzfeld–Berger analysis[13] gave δ_{11}, δ_{22} and δ_{33} at 73.1, −10.3 and −92.7 ppm respectively, indicating some change in the chemical structure of the phosphate moiety due to the Zn(II) approach. Thus, the metal-binding properties of the functionalised surface were dominated by the chemistry of the TPP molecule.



Scheme 1 Preparation of the immobilized TPP biocatalyst.

Thiamin enzymes catalyze the decarboxylation of α-keto-acids and the transfer of aldehyde or acyl groups *in vivo*.[15] The holoenzymes depend on the cofactors thiamin pyrophosphate (TPP) and bivalent metal ions such as Mg^{2+}, or Ca^{2+}.[15] There was some evidence that thiamine itself, in protein-free model systems, catalyzes pyruvate decarboxylation.[16] Here, based on GC-MS data, we confirm this ability in a protein-free system. Furthermore, we show that a significant improvement of the catalytic process can be achieved in the case of a catalytic reaction instead of a stoichiometric one. Pyruvate decarboxylation catalyzed by TPP occurs *via* two different procedures, in presence (A) or not (B) of acetaldehyde (Scheme 2).

To examine the effectiveness of TPP, both procedures have been followed. Interestingly, the catalytic activity of the immobilized TPP remained intact after its mild anchoring procedure. The pyruvate:TPP molar ratio used was 10:1 and

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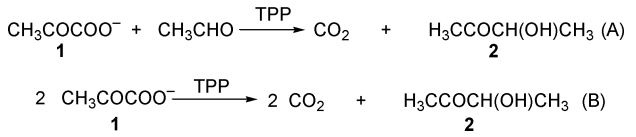

Scheme 2 Reaction of pyruvate decarboxylation catalyzed by TPP.

the reaction yield is related to the substrate conversion. Results are given in Table 1.

When testing the two assays, a relatively high activity was observed for all TPP samples. The heterogenised system fully converts the substrate at 110 or 60 min (in the presence or not of acetaldehyde respectively) instead of 330 min of the ungrafted TPP. Note that the immobilized TPP was able to fully convert two additional portions of pyruvate (2 × 200 μmol) without changes in its catalytic properties. The non-immobilized TPP showed zero conversion of the additional dose of the substrate. From these results, it is clear that the heterogenised system is much better than the homogeneous one. Based on the data of Table 1, we conclude that the metal coordination to the tethered biomolecule either does not affect the catalytic effectiveness of TPP or slightly increases the reaction time. These findings are important when contrasted with the fact that TPP metal complexes did not show any catalytic activity during enzymic studies.[11]

In summary, we have demonstrated a simple, mild and one-step procedure to tether TPP on a silica surface through its phosphate moiety providing the basis for a wide range of covalently heterogenised biomolecules. As far as TPP-immobilized activity is concerned, this method supplies a stable, very efficient, easily recovered and recyclable catalyst. It becomes obvious that further studies are required to relate thiamine catalysis in enzymic and non-enzymic systems.

Table 1 Catalytic activity of TPP for pyruvate decarboxylation

Catalyst	Reaction time/min	Yield (%)
TPP (homogeneous system)	330	100[a]
$[Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_n{\cdot}xSiO_2$	110	100[a]
$[(ZnCl_2)_2{\cdot}Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_m{\cdot}ySiO_2$	160	100[a]
TPP (homogeneous system)	330	88[b]
$[Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_n{\cdot}xSiO_2$	60	100[b]
$[(ZnCl_2)_2{\cdot}Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_m{\cdot}ySiO_2$	60	100[b]

[a] *Reaction conditions*: All reactions were carried out at 37 °C in MeOH (1 ml) with pyruvate (200 μmol), acetaldehyde (400 μmol), TPP (20 μmol) and NaOH (40 μmol). [b] *Reaction conditions*: The reactions were carried out at 37 °C in MeOH (1 ml) with pyruvate (200 μmol), TPP (20 μmol) and NaOH (40 μmol). In both cases, bromobenzene was used as internal standard. In the absence of TPP, there is no substrate conversion.

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Notes and references

† ^{13}C CP MAS NMR: (data for $[Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_n{\cdot}xSiO_2$) 162.6 ($C_{2'}$, $C_{4'}$), 149.1 ($C_{6'}$), 143.4 (C_4), 134.7 (C_5), 108.0 ($C_{5'}$), 65.4 (C_{5b}), 49.1 ($C_{5',3}$), 27.7 (C_{5a}), 22.4 (2′-CH_3), 12.1 (4-CH_3) (data for $[(ZnCl_2)_2{\cdot}Th\text{-}OP_2O_6\text{-}SiO_{3/2}]_m{\cdot}ySiO_2$) 163.5 ($C_{2'}$, $C_{4'}$), 144.0 ($C_{6'}$), 143.8 (C_4), 136.7 (C_5), 109.3 ($C_{5'}$), 65.9 (C_{5b}), 49.7 ($C_{5',3}$), 29.2 (C_{5a}), 22.9 (2'-CH_3), 13.2 (4-CH_3) (for the assignment, see *J. Biol. Inorg. Chem.*, 1998, **3**, 437 and *J. Biol. Inorg. Chem.*, 2000, **5**, 218).

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