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Biomimetic oxidation of L-arginine with hydrogen peroxide catalyzed by the resin-supported iron (III) porphyrin

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Abstract

This work describes the supported iron (III) porphyrin catalyzed biomimetic oxidation of L-arginine with hydrogen peroxide. The result showed that the supported catalyst was efficient for the release of nitric oxide ($NO^{\bullet}$) and citrulline formation on the oxidation of L-arginine with hydrogen peroxide. Alkyl peroxide such as *tert*-butyl hydroperoxide and cumene hydroperoxide were not effective oxygen donors in this catalytic reaction. Unsupported iron (III) porphyrin complex was not stable in the presence of peroxides and the porphyrin groups were oxidized with the separation of iron ions. To overcome this decomposition, anionic porphyrin complex was immobilized on a counter ionic resin matrix. The immobilized catalyst was characterized by powder X-ray diffraction, FT-IR analysis and diffuse reflectance UV–vis spectroscopy. This resin-supported iron (III) porphyrin catalyst was easily recovered after the reaction and reused without loss of activity.

Keywords: Biomimetic; L-Arginine; Supported catalyst; Hydrogen peroxide; Iron porphyrin

1. Introduction

Enzymes catalyze a wide range of reactions under mild conditions and often with high selectivity. Understanding their mechanism and attempting to mimic their reaction with chemical systems has provided chemists with important challenges and research goals [1]. The development of catalysis has been devoted for a long time to the wide application of metal complexes and organometallic complexes as catalyst [2–6]. Among the studied complexes, metal porphyrins are known to serve as prosthetic groups for a vital category of proteins and enzymes, collectively known as haemoproteins and thereby exhibit the function of nitric oxide synthase, cytochrome P-450, catalase, peroxidase and cytochrome *C* [6]. Nitric oxide synthase, unusual members of the cytochrome P-450 family, catalyzes the five-electron oxidation of L-arginine to nitric oxide ($NO^{\bullet}$) and citrulline at the expense of NADPH and O_2 [7,8]. Metalloporphryrins are generally involved to prepare artificial mimics of heme proteins [1]. Organic peroxide or more rarely H_2O_2 are known to be a substitute for NADPH and O_2 in this catalytic cycle [9]. Unsupported iron (III) porphyrins undergo molecular aggregation and other destructive reactions under these oxidizing conditions [10–12]. One way to circumvent such problems is the immobilization of the iron porphyrins on the solid surface [13–18]. The aim has been to combine the advantages of heterogeneous catalysts with the activity of iron porphyrin for catalyzing oxidation in homogeneous solution [19,20]. Therefore, immobilization of the homogeneous catalyst system is an attractive challenge because it opens access to the preparation of new, environmentally friendly means of chemical synthesis [21].

In this paper, we report a simple and easy preparation of a supported iron (III) porphyrin catalyst (Fig. 1), showing the particular efficacy of the system in the oxidation of L-arginine with hydrogen peroxide. Attempts to model nitric oxide synthase using iron (III) porphyrins [22,23] suffer from some lacunae; most of them ignored L-arginine as the starting substrate for their models. There is inadequate information concerning the direct oxidation of L-arginine with hydrogen peroxide in the presence of supported iron porphyrin as catalyst. Therefore, the idea of the shunt mechanism was opted by using peroxide, to carry out this oxidation reaction. The introduction of the ferryl

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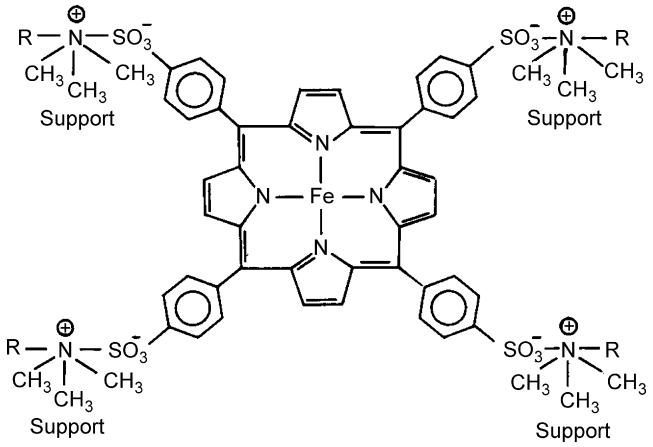

Fig. 1. Polymer supported porphyrin. R: styrene–divinyl benzene (gel), the matrix.

group from Fe^{+3} in this complex requires the treatment of the catalyst with hydrogen peroxide. Alkyl peroxide such as *tert*-butyl hydroperoxide and cumene hydroperoxide are unable to carry out the oxidation of L-arginine in the presence of the supported porphyrin catalyst.

2. Experimental

2.1. Materials

All chemicals and reagents used in this study were of analytical grade and procured from Sigma Aldrich, Fluka and Spectrochem Pvt. Ltd. Organic solvents were freshly distilled before use. Porphyrin and water-soluble iron porphyrin were synthesized with modified literature procedure [24,25] to obtain them in higher purity and yields. Highly pure iron porphyrin was anchored on the resin. HPLC solvents were of HPLC grade. Deuterated solvents such as $CDCl_3$ and D_2O were purchased from Aldrich and used without purification. Distilled water was obtained from the Milli-Q water purification system (Millipore). Griess reagent used in the reactivity study was prepared freshly by mixing aliquots of 1% sulphanilamide in 4N HCl and 0.2 ml of 0.1% *N*-(1-naphthyl) ethylenediamine in 0.4N HCl and their absorption was measured at 543 nm.

2.2. Physical measurements

IR spectra were recorded on a (5-Dx) Nicolet FT-IR instrument with KBr pellets. NMR spectral characterization was carried out with a Bruker DPX 300 MHz instrument with tetramethylsilane as internal standard for chemical shift measurements. UV–vis spectral measurements were taken with a Perkin-Elmer λ_{2S} spectrophotometer interfaced with a 486 digital computer. The cell holder of the spectrophotometer was connected to a Julabo F-30 temperature regulator. Absorbances against time were recorded by using PECSS software developed by Perkin-Elmer. Diffuse reflectance spectra were recorded on a Shimadzu U-265 instrument using optical grade $BaSO_4$ as the reference. X-ray diffraction patterns of powdered samples were obtained, using a Bruker D8 advanced diffractometer equipped with a rotating anode and Cu Kα radiation. Chromatographic identification of amino acid was done by reverse phase HPLC method using *o*-phthalaldehyde (OPA) as a precolumn derivatizing reagent with an SF 970 fluorescence detector equipped with a deuterium lamp. The OPA derivatives were detected with a monochromator set at around 330 nm (excitation wavelength) and around 418 nm (emission wavelength) cut-off filter. The column used was Ultrasphere ODS 25 cm × 4.6 mm i.d., 5 μm particle size.

2.3. Catalyst preparation

2.3.1. Synthesis of water-soluble iron porphyrin ($Fe^{III}TPPS$)

Pure $TPPH_2$ was prepared following the literature procedure [24]. It was used as starting ligand for the synthesis of tetrasodium tetrakis (*p*-sulphophenyl) porphyrin (Na_4TPPS) with some modification of the literature procedure [25]. The filtration step, as given in [25], was avoided and the sulphonated porphyrin was precipitated as a sodium salt instead of an ammonium salt of the diacid. This was done to remove the complicacy of the earlier method and also to increase the yield and purity of the sulphonated free base porphyrin. Trisodium dodecaaquatetrakis (*p*-sulphophenyl) porphyrinato iron (III) i.e. $Fe^{III}(TPPS)^{3-}$, $3Na^{+}\cdot 12H_2O$ (or $Fe^{III}TPPS$) was prepared by using the literature procedure [25] and a modification was made during the purification of the final product from excess unreacted ferrous sulphate and/or its hydrolyzed/oxidized products. Instead of following the purification by the cation exchange method that led to the demetalation and loss of yield, the classical method of removal of iron using phosphate salt was adopted. Thus the time-consuming method using cation exchange resin can be avoided and the product was obtained in a much better yield and in the pure form. Excess sodium chloride and sulphate present as impurities were removed by dissolving the crude product in methanol and precipitating $Fe^{III}TPPS$ from the solution of acetone. $TPPH_2$, Na_4TPPS, and $Fe^{III}TPPS$ were characterized by UV–vis and NMR spectroscopy, as given in the supplementary data sheet.

2.3.2. Method of immobilization of $Fe^{III}TPPS$ on Dowex resin (DR)

In a typical experiment a suspension of 200 mg of Dowex resin in distill water (10 ml) containing 4 mg of iron porphyrin was stirred with continuous change in surface for 2–3 h at room temperature. The carrier material (Dowex resin) turned greenish indicating that the homogeneous catalyst was loaded onto the support. After filtration and washing with distill water, the solid obtained (DR $Fe^{III}TPPS$) was found to retain completely the adsorbed iron porphyrin, when put in suspension of distilled water, even after several days.

2.4. Catalysis

2.4.1. Loading of the sulphonated iron porphyrin in the Dowex resin

Two hundred milligrams of Dowex resin (Dowex 1 × 2–400 ion exchange resin, in the chloride form) was loaded in a small

burette and soaked with deionized water. It was then slowly washed with 5% NaOH solution to change it to the corresponding –OH form. Excess alkali was removed by repeated washing of the resin beads with deionized water till pH of the eluant became neutral. Immobilization of 4 mg of iron porphyrin on this treated Dowex resin was performed according to the method described earlier (Section 2.3.2). After completion of the reaction, the entire mixture was poured into a glass frit (G-2) and was washed repeatedly with deionized water. The total filtrate and washed liquid was collected in a measuring flask and volume was made up to 50 ml. One milliliter of this solution was diluted further in another measuring flask to 10 ml and its concentration was measured spectrophotometrically. An identical amount of the porphyrin was weighed separately and was dissolved in water to make the dilution identical to what described above and absorbance was measured. The concentration for the amount of porphyrin adsorbed into the resin was obtained from the differences in absorbance of the Soret band of these two solutions following Lambert Beer's law.

2.4.2. *Spectrophotometric experiments for the stability of supported complex under three different peroxides*

The stability of the supported system was checked by taking 100 mg of the loaded resin in six different test tubes. In each tube, 1 ml of hydrogen peroxide of different concentrations varying from 200 to 1.5 mM was separately added to the test tubes and shaken for some hours. In each case the filtrate was taken in a cuvette for spectrophotometric analysis, for checking the presence of any colored species specially in the region of the Soret band. The same experiment was repeated with *tert*-butyl hydroperoxide, with the concentration varying from 10 to 50 mM and also in the low concentration range 250 μM to1.5 mM. For experiments with cumene hydroperoxide the concentrations used were 100 μM, 500 μM, 1.5 mM, 10 mM and 20 mM. This experiment was further repeated with HCl and three peroxides.

2.4.3. *Non-enzymatic synthesis of nitric oxide using hydrogen peroxide*

In 1997, Nagase et al. [26] have demonstrated a novel non-enzymatic method for nitric oxide synthesis involving hydrogen peroxide and L-arginine as substrate. They have used 10 mM reagent and on standing for 5 days nitric oxide was identified. So this reaction was first checked with blank resin as controlled experiment.

2.4.4. *Hydrogen peroxide and alkyl hydroperoxide experiments with* L-*arginine*

2.4.4.1. Preliminary studies. Loaded resin (200 mg) was taken in a cuvette and 1 ml of L-arginine (50 mM) was added with a pipette. The reaction was initiated by adding 0.5 ml of H_2O_2 (100 mM) and continued for 20 min at 25 °C. NO_2^- formation was determined by an automated procedure based on the Griess reaction. Amino acid product was identified by using two different techniques.

(i) RP-HPLC of the OPA derivative. The derivatization procedure described by Umagat et al. [27] was followed. The product was analyzed with good selectivity and sensitivity by reversed-phase high-performance liquid chromatography. Derivatized sample (10 μl) was injected onto the column. The mobile phase was linear step gradient with a ternary solvent mixture, THF/(0.05 mol l sodium acetate), pH 6.6/methanol. The flow rate for this method was 1.5 ml/min. Amino acid standards were used to quantify samples. 2-aminoethanol was used as an internal standard. Detection was generally possible in picomole range.

(ii) The formation of amino acid was also identified using an NMR probe. NMR spectra of L-arginine and L-citrulline procured from the market were recorded in D_2O in the presence of loaded resin. The spectra are shown in Fig. 8a and b. Loaded resin (50 mg) was used for these experiments because of the limitation in the NMR tube. Loading of porphyrin into the resin was carried out in D_2O. Into this loaded resin, 5 mg of L-arginine dissolved in 0.6 ml D_2O was added. Finally 6.4 μl of aqueous 30% H_2O_2 was added to initiate the reaction. The contents of the tube were shaken for taking the NMR spectrum.

Tert-butyl hydroperoxides and cumene hydroperoxide were also used as oxygen donors in this catalytic oxidation reaction.

2.4.4.2. Time course of NO_2^- formation in the oxidation of L-*arginine with hydrogen peroxide.* In a typical experiment 4.2 μl of aqueous 30% H_2O_2 (38.7 mM) was added with the help of microliter syringe to a stirred suspension of 50 mg loaded resin (0.688 μmol of loaded Fe^{III}TPPS) in 1 ml deionized water containing 35 μg L-arginine (200 μM) to initiate the reaction. Finally 2 ml of Griess reagent (freshly prepared) was added to it. The contents were shaken for a minute and the absorbance at 543 nm was noted. The reaction has been repeated with the same amount of unloaded resin. The nitrite standard reference curve was prepared in water in a high concentration range as well as in a low concentration range. The average absorbance value of each concentration of the nitrite standard was plotted against the nitrite concentration. The amount of NO_2^- released was determined from the nitrite standard reference curve.

2.4.4.3. Formation of nitrite (NO_2^-) as a function of hydrogen peroxide concentration in the oxidation of L-*arginine with loaded resin.* Oxidation reactions of L-arginine were carried out at 25 °C by varying the H_2O_2 concentration using loaded resin (DRFe^{III}TPPS). L-Arginine (35 μg, 200 μM) in 1 ml deionized water and the supported catalyst (50 mg, 0.688 μmol loaded Fe^{III}TPPS) were taken in a vial pre-occupied with a magnetic stir-bar. In each experiment after the addition of aqueous 30% H_2O_2, the reaction mixture was stirred for 5–6 min. Finally the reaction mixture was transferred to the cuvette containing Griess reagent. The cuvette was shaken for a minute and the absorbance was measured at 543 nm. From the calibration plot of nitrite, the concentration of nitrite corresponding to the measured absorbance value was obtained.

2.4.4.4. Stoichiometry of L*-arginine consumption and product formation.* The reaction mixture contains 38.7 mM H_2O_2, 200 μM L-arginine in 1 ml deionized water and DRFe^{III}TPPS (30–60 mg). After the addition of aqueous 30% H_2O_2, the reaction was continued for 20 min at 25 °C. Nitrite formation was measured as described in Section 2.4.4.2. Amino acid product was analyzed as described in Section 2.4.4.1. Concentrations of L-arginine and citrulline were determined from their calibration curves (concentration versus peak area) using HPLC methodology.

2.4.4.5. Performance of the catalysts for the oxidation of L*-arginine.* The activity and stability of supported (DRFe^{III}TPPS) and unsupported catalysts were tested in the oxidation of L-arginine with H_2O_2 at 25 °C. In each experiment the amount of the catalyst was adjusted in such a way that the net content of the active FeTPPS phase corresponded to 0.9 mg. The substrate and oxidant concentration used for these experiments were identical to those described in Section 2.4.4.2. The reaction was stopped when the conversion of the substrate remained constant after two successive analyses. The total time for the completion of reaction was 20 min.

3. Result and discussion

3.1. Characterization of DRFe^{III}TPPS

The FT-IR spectrum of DRFe^{III}TPPS is in excellent agreement with that of Fe^{III}TPPS with additional signals due to Dowex resin. The spectrum of the supported catalyst (DR Fe^{III}TPPS) shows signals at 1208, 1191 (asymmetric S=O stretching of sulphonate group), 1121, 1035 (symmetric S=O stretching of sulphonate groups), 999, 738, and 636.96 cm^{-1}, which are assigned to the iron (III) porphyrin complex, whereas Dowex resin does not show any peak in this region (Fig. 2). Absorptions at 3023, 2923, 2852, 1618, 1484, 888, and 704 cm^{-1} are identical with those of Dowex resin. The diffuse reflectance UV–vis spectrum of DR Fe^{III}TPPS (v_2) resembles with that of Fe^{III}TPPS (v_3) (Fig. 3). From this result it can be suggested that iron porphyrin is structurally unchanged and distributed uniformly in the polymer matrix. The supported catalyst was better than the neat iron (III) porphyrin complex in terms of stability and activity. This is due to the site isolation of the iron (III) porphyrin in the polymer matrix. The X-ray powder diffraction (XRD) patterns of the Dowex resin (v_1), iron porphyrin complex (v_3) and supported catalyst (v_2) were recorded at 2θ value between 3° and 60°. The X-ray diffraction patterns of the Dowex resin (v_1) and the iron porphyrin complex (v_3) were retained in the supported catalyst (v_2). The diffractogram of the carrier material contained a hump at 2θ value between 10° and 30° which is characteristic of the amorphous polystyrene. The hump was retained in the supported catalyst, which indicates that immobilization procedure did not promote any structural modification in the matrix (Fig. 4).

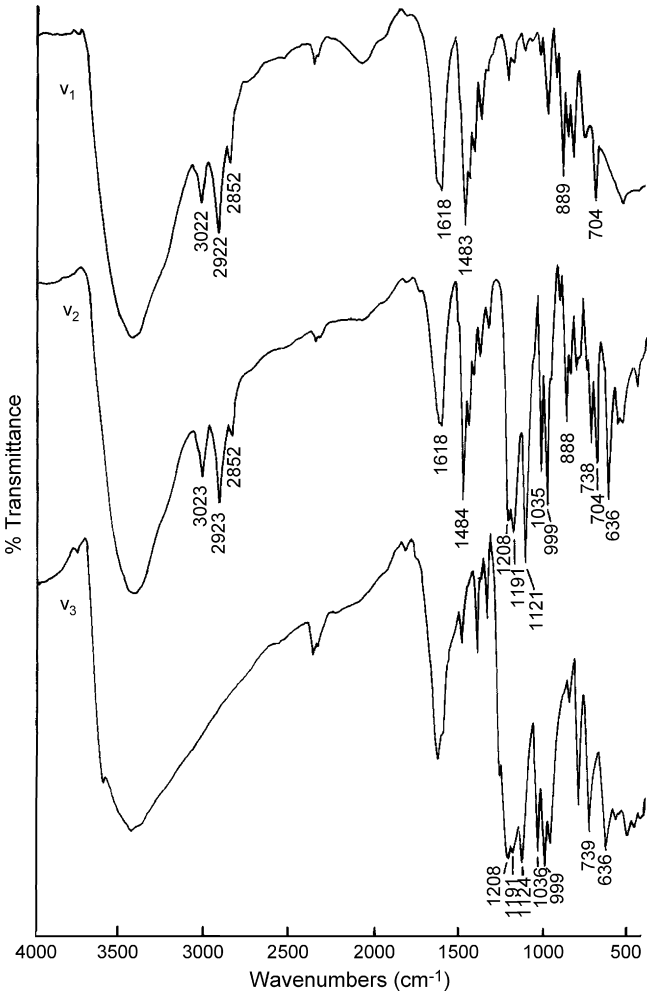

Fig. 2. FT-IR spectra of DR (v_1), DRFe^{III}TPPS (v_2) and Fe^{III}TPPS (v_3).

3.2. Catalysis

Dowex resin (1 × 2–400 anion exchange resin) has excellent mechanical and chemical stability, good kinetics and high regeneration efficiency. The matrix is styrene–divinylbenzene gel (microporous) and the active group is trimethylammonium. The iron (III) porphyrin complex (Fe^{III}TPPS) was immobilized on Dowex 1 × 2–400 anion exchange resin (chloride form) by

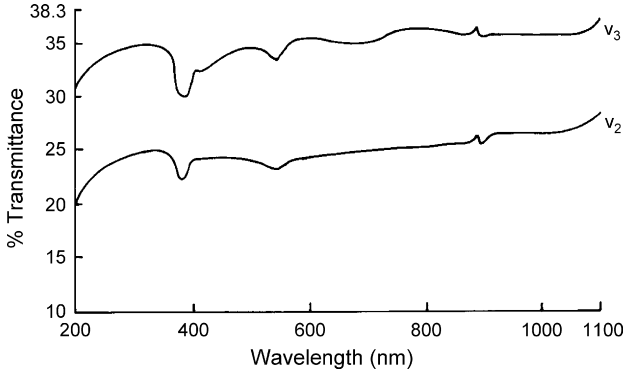

Fig. 3. Diffuse reflectance UV–vis spectra of Fe^{III}TPPS (v_3) and DRFe^{III}TPPS (v_2).

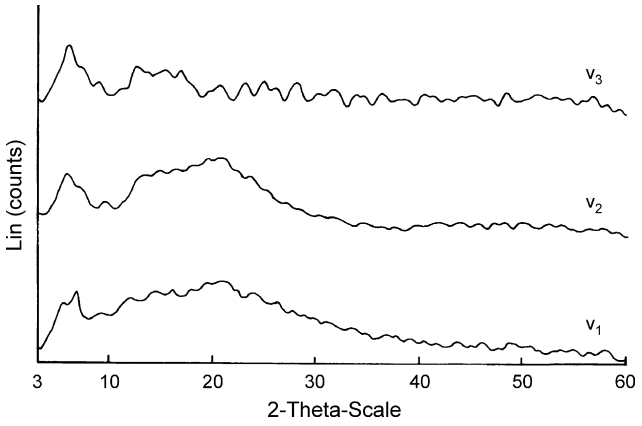

Fig. 4. XRD patterns of Fe^{III}TPPS (v_3), DRFe^{III}TPPS (v_2) and DR (v_1).

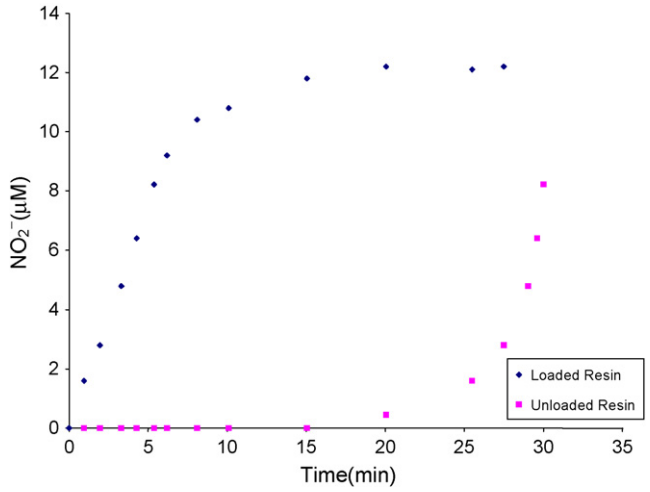

Fig. 5. Time course of (NO_2^-) formation from L-arginine with hydrogen peroxide in case of loaded and unloaded resin.

taking the advantage of a strong electronic interaction between sulphonate groups of ionic porphyrin and the active trimethylammonium groups on the surface of Dowex resin. The amount of porphyrin loaded was 3 mg. About 75% of iron porphyrin was loaded in the resin.

The supported catalyst was a very stable system for carrying out the oxidation reaction. There was no apparent release of porphyrin from the supported catalyst in all the experiments (Section 2.4.2). The experiment was further repeated with HCl and three peroxides in order to optimize the stability of the supported catalyst for subsequent use. Since Griess reagent contains HCl, the stability of the supported catalyst in the presence of Griess reagent was also checked from this experiment.

Three peroxides, H_2O_2, *tert*-butyl hydroperoxide and cumene hydroperoxide were examined with the supported iron (III) porphyrin catalyst to evaluate whether or not the peroxide shunt pathway was operative. Of the three hydroperoxide only H_2O_2 was able to promote the product formation from L-arginine. It has been reported that alkyl peroxide, which are usually better in the peroxide shunt than H_2O_2 in P450 catalyzed reaction, are not effective as oxygen donors in the nitric oxide synthase catalyzed reaction [9]. The active site of nitric oxide synthase is expected to be more hydrophilic than that of the typical P450 and it may be difficult for hydrophobic alkyl peroxide to gain access to the heme. So ferryl group introduction is inhibited when alkyl peroxide are used as oxygen donors, which is the reason for their inefficiency to carry out the oxidation reaction of L-arginine.

The formation of amino acid (citrulline) and inorganic product (NO_2^-) from L-arginine and H_2O_2 was determined as a function of the amount of the supported catalyst. The results from the experiments are shown in Table 1. The formation of citrulline and NO_2^- were stoichiometric to the amount of L-arginine consumed. Within experimental error, for every mol of arginine consumed, one mol each of citrulline and NO_2^- were formed.

3.2.1. *NO release from* L-*arginine oxidation using hydrogen peroxide*

Amount of NO released from L-arginine with H_2O_2 using loaded and unloaded resins was monitored spectrophotometrically at 543 nm. Griess reagent reacts with NO_2^- to produce a chromophore which absorbs at 543 nm. The formation of NO_2^- produced from L-arginine and H_2O_2 was observed with time at 25 °C shown in Fig. 5. From this figure, it is evident that the release of nitric oxide was instantaneous, when iron porphyrin immobilized on Dowex resin was used. The non-catalytic reaction with unloaded resin was very slow compared to the catalytic one. In the case of the loaded porphyrin system, hydrogen

Table 1
Stoichiometry of citrulline to NO_2^- formation from L-arginine[a] using supported catalyst

Experiment	DRFe^{III}TPPS (mg)	[Citrulline][b] formation (μM)	[NO_2^-][c] formation (μM)	[Arginine][d] consumption (μM)	[Citrulline]: [NO_2^-]
1	30[e]	7.6	7.2	7.7	1.06
2	40[f]	9.7	9.6	10.0	1.01
3	50[g]	12.4	12.3	12.6	1.01
4	60[h]	15.2	15.5	15.8	0.98

[a] All values are the average of duplicate measurements.
[b] Citrulline formation.
[c] [NO_2^-] was determined by quantitation of NO_2^- with Griess reagent as described in Section 2.
[d] Arginine consumption were determined by HPLC methodology as described in Section 2.
[e] 0.413 μmol of FeTPPS was loaded.
[f] 0.550 μmol of FeTPPS was loaded.
[g] 0.688 μmol of FeTPPS was loaded.
[h] 0.825 μmol of FeTPPS was loaded.

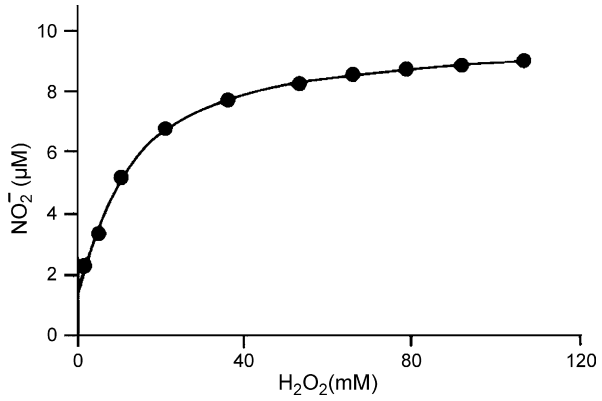

Fig. 6. Formation of nitrite (NO_2^-) as a function of hydrogen peroxide concentration in the oxidation of L-arginine with loaded resin.

peroxide reacts with the catalyst to form high valent iron oxo intermediate, which contains ferryl group [Fe=O] + 3, responsible for the oxidation of L-arginine. However, in the case of unloaded resin the autocatalytic Fenton's reaction takes place due to impurities of iron (0.001%) present in L-arginine. So there is an induction period which is necessary to built up sufficient hydroxyl radicals to initiate the reaction. When mannitol was added to the unloaded reaction system, the reaction was blocked and there was no nitrite formation. This proves that hydroxyl radicals are intermediates in this reaction, as mannitol is known as an effective quencher of hydroxyl radical. From this figure, it is can be seen that the reaction had a linear dependence on time for at least 6 min for the supported system. However, for the unsupported system (Fe^{III}TPPS) the linearity was kept only for 1–2 min (data not shown). The dependence of formation of NO_2^- on the concentration of hydrogen peroxide was determined for the loaded porphyrin catalyzed oxidation of L-arginine at 25 °C. All reactions were continued for 6 min in order to collect data for each concentration. The product formation was linear with time over this period and after that deviates from linearity (shown in Fig. 5). The results of these reactions are shown in Fig. 6. The data presented were average of duplicate set of experiments. This study was done to determine the kinetics (or kinetic parameters) of H_2O_2-dependent oxidation of L-arginine.

3.2.2. Amino acid identification in the reaction mixture

The amino acid product of the reaction was shown to be L-citrulline (Fig. 7). L-Arginine and L-citrulline were eluted at 23.7 and 21.4 min, respectively. 2-Aminoethanol (internal standard) was eluted at 32 min. No data was suggestive of the formation of

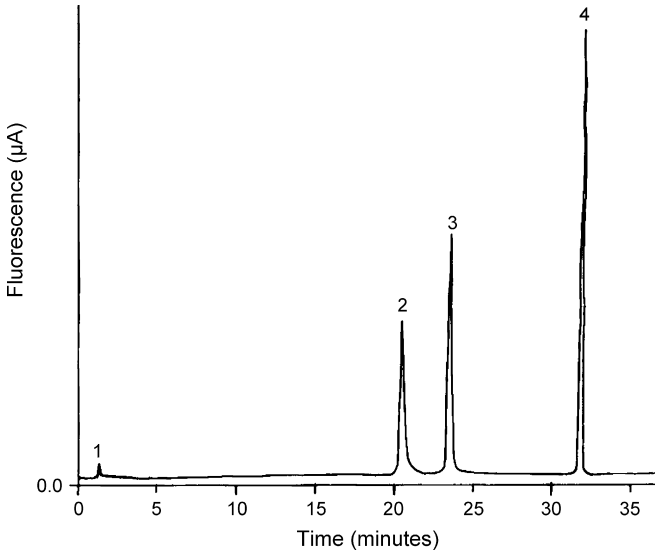

Fig. 7. Elution profile of OPA-derivatized amino acid product from oxidation of L-arginine of H_2O_2-dependent reaction catalyzed by DRFe^{III}TPPS. Peaks were identified as solvent (1) t_R = 1.3 min; L-citrulline (2) t_R = 21.4 min; L-arginine (3) t_R = 23.8 min; 2-aminoethanol (4) t_R = 32 min.

any other amino acid product. Chromatographic identification of citrulline was made by reversed-phase HPCL of the OPA derivative and agreed with the retention time of the identically treated citrulline standard. Unequivocal evidence of citrulline formation was also obtained from NMR spectroscopy. Identification of citrulline (Fig. 8b) can be readily made by monitoring its peak at 3.6(t), 2.9(t), 1.74–1.66(m) and 1.4–1.3(m), which clearly distinguishes its presence even in the presence of L-arginine (Fig. 8c). In the reaction with 50 mg of FeTPPS loaded resin (as described in Section 2.4.4.1), 0.75 mg of Fe^{III}TPPS was loaded. The NMR spectrum for the oxidation of L-arginine with H_2O_2 is shown in Fig. 8d. For carrying out all the operation 2–3 min of time is required. The NMR spectrum clearly showed the conversion of L-arginine to citrulline. The NMR peaks responsible for the formation of water (4.7δ) from hydrogen peroxide can also be seen from the spectra. Excess hydrogen peroxide peak was also seen around 11 ppm. No base line corrections were performed during spectral processing and rather correction to the base lines were made, if necessary when the peaks are integrated. Integration and any baseline modifications were performed using the origin 7.0.

No evidence supported the formation N^{δ}-hydroxy-L-arginine (L-NHA) in this oxidation reaction. This may be due to the insta-

Table 2
Catalytic performance of the supported catalyst (DRFe^{III}TPPS) and unsupported catalyst (Fe^{III}TPPS) in the oxidation of L-arginine with H_2O_2

Entry	Catalyst	Citrulline[a] yield (%)	NO_2^- [a] yield (%)	L-Arginine conversion (%)	Catalyst loss[b] (%)
1	Unsupported Fe^{III}TPPS	0.9	0.8	1.1	93–95
2	DRFe^{III}TPPS	7.6	7.7	7.9	<2
3	DRFe^{III}TPPS reused[c]	7.4	7.5	7.8	<3

Reaction conditions: 200 μM L-arginine, 38.7 mM H_2O_2, 1 ml deionized water, 25 °C. In each experiment content of active phase FeTPPS corresponded to 0.0009 g.
[a] Yield refer to the product isolated with respect to the substrate used.
[b] UV–vis spectrum of the reaction mixture indicated % of the catalyst destroyed.
[c] The reaction was performed after washing and drying of the catalyst.

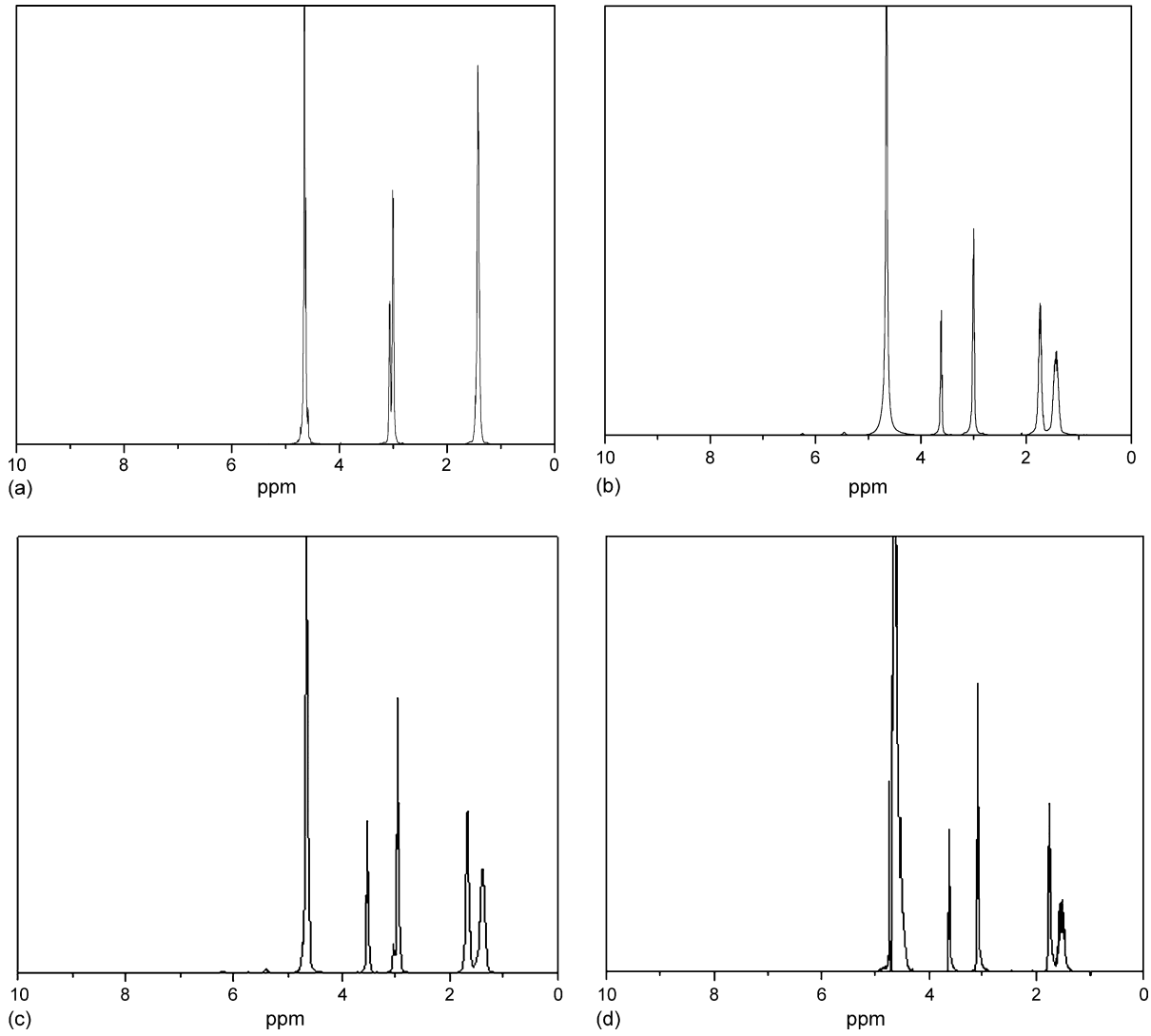

Fig. 8. (a) NMR spectra of L-arginine (5 mg) + $DRFe^{III}TPPS$ (50 mg) + D_2O (0.6 ml); (b) NMR spectra of L-citrulline (5 mg) + $DRFe^{III}TPPS$ (50 mg) + D_2O (0.6 ml); (c) NMR spectra L-arginine (5 mg) + L-citrulline (50 mg) + D_2O (0.6 ml) + $DRFe^{III}TPPS$ (50 mg). Small peak at 3.02 is due to L-arginine; (d) NMR spectra of L-arginine (5 mg) + 30% aqueous H_2O_2 (6.4 μl) + D_2O (0.6 ml) + $DRFe^{III}TPPS$ (50 mg).

bility of L-NHA in the presence of excess peroxide in the reaction mixture.

3.2.3. *Performance of the catalysts for the oxidation of* L-*arginine*

The reactivity and stability of the supported and unsupported catalysts in the oxidation of L-arginine by H_2O_2 at 25 °C are shown in Table 2. The activity of the catalyst was indicated by the degree of L-arginine conversion. The supported catalyst ($DRFe^{III}TPPS$) showed better performance than the unsupported iron porphyrin ($Fe^{III}TPPS$). The yields of L-citrulline and NO_2^- were relative to the substrate L-arginine used. The reaction systems containing the $Fe^{III}TPPS$ have proved to have little activity in this oxidation reaction. The supported catalyst gave higher activity than $Fe^{III}TPPS$ (entries 2, 3). The UV–vis spectrum of the reaction mixture indicated that most of the catalyst was destroyed (the intensity of the Soret band decreased to 5% of its original value) for the unsupported system. On the other hand the supported catalyst was still active after reuse and its diffuse reflectance spectrum was unaffected. The supported catalyst was separated from the reaction mixture after each experiment by filtration. It was washed with water and dried carefully before using it in subsequent runs. NO_2^- and citrulline formation in the second run did not vary much from the first run. The filtrates were used for determination of iron leaching. No iron was detected in the filtrate by atomic absorption spectroscopy. There was no change in the FT-IR spectra and XRD pattern of the recovered catalyst (data not shown). This suggests that in the homogeneous system, dimerization of iron porphyrin complexes rapidly occurred, resulting in bimolecular oxidative destruction of the catalyst. However, in the supported catalyst, the iron porphyrins were immobilized at the surface of the support and thus are held apart from each other, which makes the formation of the dimer species unlikely. So iron porphyrin when protected in a proper matrix such as ion exchange resin can efficiently catalyzed the oxidation of L-arginine to citrulline with the formation of nitric oxide.

4. Conclusion

A working model for nitric oxide synthase activity using the shunt mechanism has been developed, which showed the formation of nitrite and citrulline. The model consists of water-soluble iron (III) porphyrin immobilized on Dowex resin. This system is stable under the treatment of peroxide devoid of the degradation of the porphyrin moiety (active site). L-arginine instead of N^{δ}-hydroxy-L-arginine (L-NHA) was taken as the substrate for oxidation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcata.2006.11.012.

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