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Hydrophilic ionic liquids as reaction media for the determination of guaiacol using horseradish and soybean peroxidases

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Experimental procedures

GQ oxidation by t-*BuOOH catalyzed by HRP or SBP in the presence of [BMIm][BF_4] or [BMPy][BF_4]*. First, a 0−290 μl portion of IL, a 5−9 μl portion of 7.3 M *t*-BuOOH, a 0−290 μl portion of the optimal buffer solution (Table 2), a 5 μl portion of GQ solution the concentration of which varied from 0.06 to 600 mM were placed successively into a cell of a polystyrene immunoassay //board. A solution in the cell was mixed by a thin glass stick. Then, a 5−15 μl portion of the enzyme solution with the concentration varying from 0.1 to 5 μM was added. The total volume of a reaction mixture was 0.3 ml. A timer was turned on at the moment when the enzyme solution was added to the reaction solution and mixed. The absorbance of the solution was measured at 15 s after reaction proceed with 15 s intervals for 3.5 min on the micro tablet photometer Multiskan EX (Thermo Labsystems) (λ_{eff} = 450 nm).

GQ oxidation by t-*BuOOH catalyzed by HRP or SBP in the presence of DMSO (acetonitrile)*. First, a necessary volume of a phosphate buffer solution (pH 6.0), a 0.10 −1.50 ml portion of DMSO or acetonitrile, a 0−0.70 ml portion of rectified alcohol, a 0.02−0.25 ml portion of the enzyme solution (0.45−6.2 μM), a 0.01−0.2 ml portion of GQ solution (0.045−0.9 mM) were placed successively into the graduated glass test-tube with a stopper. The solution was mixed and a 0.005−0.15 ml portion of 7.3 M *t*-BuOOH solution was added. The total volume of a reaction mixture was 5.00 ml. A timer was turned on at the moment when a *t*-BuOOH solution was added to the reaction solution and mixed. The reaction solution was poured into a cuvette and its absorbance was measured 30 s after the reaction start with 15 s intervals during 3 min on a UVmini-1240 spectrophotometer (λ_{max} = 470 nm).

The rate of the peroxidase GQ oxidation in the aqueous-organic and hydrophilic IL media was

controlled spectrophotometrically as an increase in absorbance of the colored product of the GQ oxidation, *o*-methoxyquinone, in time and characterized by the value of the slope of a kinetic curve (tgα) plotted in the coordinates the absorbance (A) *versus* time (t, s).

Experimental details

The stock solutions of HRP and SBP (2.4 and 2.7 μM) were obtained by dissolving their solid preparations (Sigma; RZ 2.7 and 0.5, respectively) in 0.05 M phosphate buffer solution (pH 7.0) and stored at +4°C. The concentration of the enzyme solutions were determined spectrophotometrically (UVmini-1240, Shimadzu; l = 1 cm, $\varepsilon_{403} = 9.46\times10^4$ M^{-1} cm^{-1} for both peroxidases). The commercial preparations of [BMIm][BF_4] and [BMPy][BF_4] (Merck) were dried on the Rotavapor R-210 rotary evaporator (Buchi) *in vacuo* (18 mm Hg) and 70 °C during 3 h.[1] Acetonitrile and DMSO (Khimmed) were used without further purification.

The GQ solutions were obtained daily in dark vessels by the dilution of initial liquid preparation (Sigma) in rectified alcohol (Khimmed). The 70% *t*-BuOOH water solution (7.3 M, Merck) was used. The preparations of K_2HPO_4, KH_2PO_4 (Merck); imidazole, tris(hydroxymethyl)-aminomethane (**Tris**), 2,4,6-collidine (all from Sigma); KOH and HCl solutions (Reakhim) were used for preparing the buffer solutions. All reagents were of analytical grade or higher purity. The buffer solutions were prepared according to the recommendations.[2] pH of aqueous solutions was measured using a potentiometer in the pH-meter mode (Econics-Expert-001). Micro-dosage devices produced by Biohit and Eppendorf were used for sampling. Doubly distilled water was used when preparing all aqueous solutions (Millipore, Ω = 18.2 MΩ×cm^{-1}, 25°C).

References

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