ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 6 (1999) 549–553

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Polyaniline as a support for urease immobilization

J. Laska [a,*], J. Włodarczyk [a], W. Zaborska [b]

[a] *Department of Materials Science and Ceramics, University of Mining and Metallurgy, ul. Mickiewicza 30, 30-059 Kraków, Poland*
[b] *Department of Chemistry, Jagiellonian University, ul. Ingardena 3, 30-060 Kraków, Poland*

Received 20 October 1998; accepted 14 December 1998

Abstract

Polyaniline synthesized by chemical oxidative polymerization was used as an immobilization support for jack bean urease. Such immobilized enzyme has a good catalytic activity, storage stability, and reusability. Properties of free and immobilized urease were compared. Blends of polystyrene, cellulose acetate and poly(methyl methacrylate) with polyaniline were used for urease immobilization as well. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Urease; Immobilization; Polyaniline

1. Introduction

Enzyme immobilization has been the subject of attention for many years. Various methods including adsorption, covalent bonding and entrapment have been developed [1]. Although usually the immobilized enzyme shows lower catalytic activity than the free one, it is more stable, reusable, and in consequence less costly for many applications. Hence, still new immobilizing supports have been of great interest for many researchers [2–4].

The enzyme urease, widely distributed in nature [5] catalyzes the hydrolysis of urea to ammonia and carbon dioxide according to the reaction: $NH_2CONH_2 + H_2O \rightarrow CO_2 + 2NH_3$. The enzyme plays an important role in the determination of urea in blood, urine and in wastewater, in process of dialysis for removal of urea from blood in the treatment of uremia, etc. [6].

Many polymeric materials were used for enzyme immobilization, and during the last years, conjugated polymers, i.e., containing conjugated double bonds, were also successfully applied for this purpose [7]. The conjugated polymers are very promising supports for immobilization of enzymes due to a direct electron transfer between an enzyme and a polymer, and thus potential usability as sensing materials [8,9]. However, most of the published results are based on polypyrrole and oxidases [10,11]. In this work, we focused on the immobilization of urease on polyaniline films.

Among all conducting polymers, polyaniline (PANI) is the one of marked importance because of its environmental stability and unique

* Corresponding author. Tel.: +48-12-172-331; Fax: +48-12-331-593; E-mail: jlaska@uci.agh.edu.pl

1381-1177/99/$ - see front matter
PII: S1381-1177(99)00013-2

conduction mechanism. Polyaniline can be easily obtained by oxidative polymerization of aniline, and also, because of its solubility in selected organic solvents, can be processed into very stable, flexible free-standing films [12]. In addition, mechanical properties of polyaniline can be modified by mixing it with conventional polymers such as polystyrene, poly(methyl methacrylate), etc. [13]. On the other hand, polyaniline in the form of emeraldine base is known as the most untreatable conducting polymer. It is insoluble in water, most organic solvents, and it is stable in various chemical conditions. Possessing all these properties polyaniline seems to fulfill technological expectations as an immobilizing agent.

2. Experimental

2.1. Materials and methods

Aniline was purchased from POCh-Gliwice, Poland, and distilled twice before using. Jack bean urease was of Sigma type III, and was stored at 4°C. Its specific activity was 32 units per milligram of the protein. *N*-methylpyrrolidone (NMP) was purchased from Aldrich. Urea and all other chemicals were purchased from POCh-Gliwice, Poland.

Polyaniline was chemically synthesized by oxidative polymerization of aniline in the presence of sodium persulfate (Aldrich) in hydrochloric acid solution followed by deprotonation with ammonia [14]. It was characterized by FTIR and elemental analysis. FTIR (cm^{-1}): 3296, 3056, 1669, 1594, 1501, 1381, 1311, 1244, 1168, 1113, 981, 833, 746, 657, 507. Elemental analysis: calculated %C—79.56; %H—4.97; %N—15.47; experimental %C—73.90; %H—5.18; %N—14.13. The difference between calculated and experimental analyses is caused by water residues in the polymer sample.

Polyaniline films were prepared by casting from NMP, either as free-standing or glass-fiber fabric supported films. The NMP solution of PANI was prepared by dissolving of 750 mg of PANI in 25 ml of NMP, and stirring it for 12 h. Non-dissolved polyaniline was separated from the solution by centrifugation (10 min, 3000 rot min^{-1}). The solution was then cast on Petrie plates and kept at 30°C until solvent was completely evaporated.

2.2. Immobilization of urease

Urease was immobilized on both sides of the polyaniline films by adsorption in the following way: the films were immersed in 0.1% solution of the urease in phosphate buffer (pH = 7) for 1.5 h at room temperature, and then overnight at

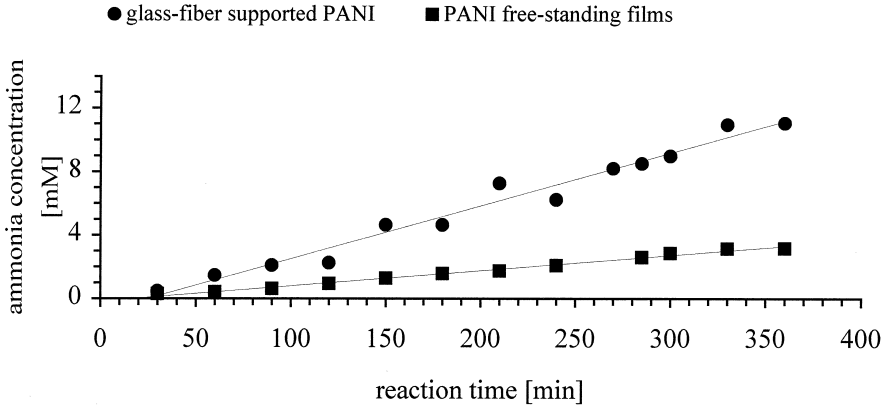

Fig. 1. Reaction rate of hydrolysis of urea catalyzed by urease immobilized on polyaniline films.

Table 1
Kinetic parameters of urease immobilized on PANI/glass-fiber fabric determined by different methods

Method	V_{max} [μM of urea min^{-1}]	K_m [mM]
Lineweaver–Burk	2.43	12.36
Eddie–Hofstee	2.31	10.71
Hanes	2.36	11.54
Hiperbolic fitting	2.42	12.36
Direct linear	2.35	11.79
Average value	2.38	11.74

4°C. The membranes were then washed with buffer until the washing were free of urease.

The reaction of hydrolysis of urea in the presence of urease was carried out in the phosphate buffer solution at pH = 7 containing 1 mM EDTA at room temperature. The concentration of ammonia was 10 g dm^{-3} to keep the reaction of zeroth order. The concentration of urea was determined by the phenol-hypochlorite method [15].

3. Results and discussion

3.1. Activity of immobilized urease

The activity of both, immobilized and free, urease was determined by measuring the amount of ammonia liberated in the urease-catalyzed reaction of hydrolysis of urea per unit time (Fig. 1). Urease immobilized on the glass-fiber supported polyaniline film has activity about three times higher than that immobilized on free-standing film (1.1×10^{-2} and 3.1×10^{-2} μM urea min^{-1} cm^{-2}, respectively). The most probable way the enzyme is bound to a film is adsorption, which strongly depends on the porosity of immobilizing material. The free-standing films of polyaniline are very smooth while glass-fiber fabric supported films are 'porous'. Therefore, higher activity of urease immobilized on the glass-fiber supported polyaniline films means more enzyme molecules trapped comparing to polyaniline free-standing films. The amounts of the protein immobilized on both PANI carriers were estimated from the determined activity assuming that the enzyme retained 100% of its original activity. The amounts are: 0.3 μg of protein cm^{-2} for PANI and 0.8 μg cm^{-2} for glass-fiber fabric supported PANI.

3.2. Kinetic parameters

In case of the immobilized enzyme the lag time in the reaction of hydrolysis of urea is observed. It is ~16 min for glass-fiber fabric

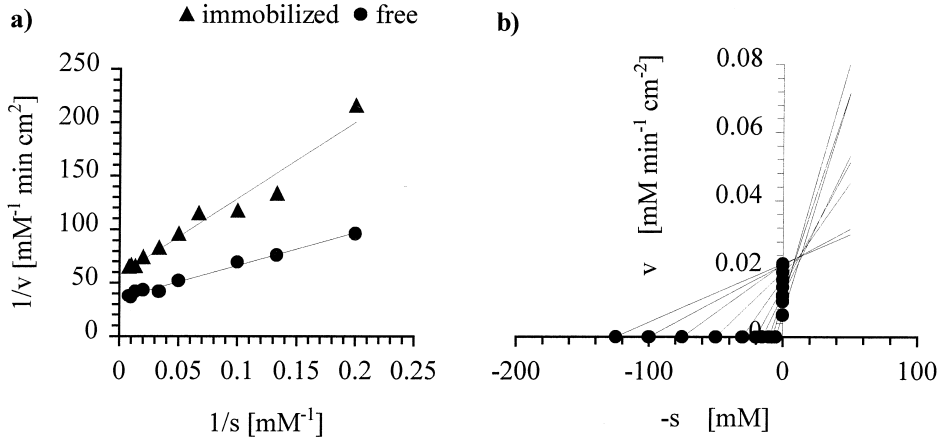

Fig. 2. (a) Lineweaver–Burk plots of free and polyaniline immobilized urease; (b) direct linear plots of polyaniline immobilized urease.

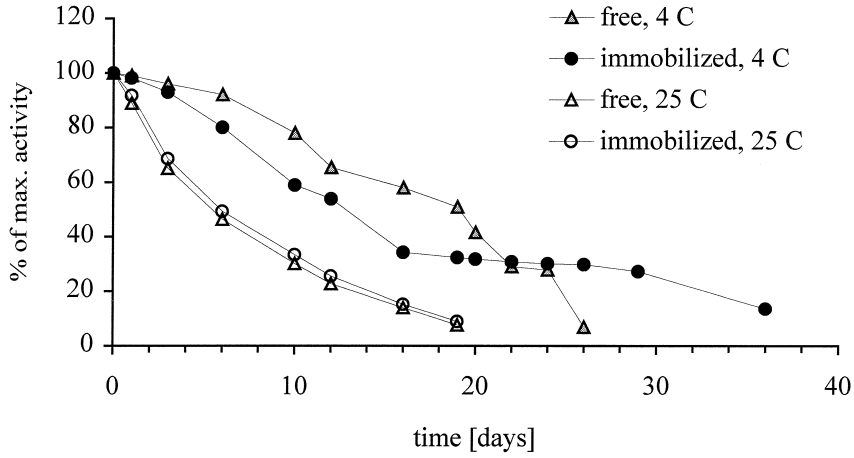

Fig. 3. Storage stability of free and polyaniline immobilized urease kept at 4 and 25°C.

supported PANI films and ~11 min for free-standing PANI films. Usually, it is caused either by occurrence of different enzymatic reactions at the phase boarder or difficulties in approaching the enzyme molecule by urea. The delay in starting the enzymatic reaction is very common in the case of immobilized enzymes.

Kinetic parameters, namely the Michealis–Menten constant and maximum rate, were calculated by different methods (Lineweaver–Burk, Eadie–Hofstee, Hanes, hyperbolic fitting, direct linear) and they are listed in Table 1. The Lineweaver–Burk and direct linear plots for free and immobilized urease are shown on Fig. 2. The average value of K_m is 11.7 mM for urease immobilized on glass-fiber supported film, and 8.7 for free urease. V_{max} is 2.4×10^{-3} and 2.8×10^{-3} mM min^{-1}, respectively.

3.3. Stability and reusability

Fig. 3 shows the stability of polyaniline immobilized urease stored at 4 and 25°C. As can be seen, the immobilization increases storage stability of the enzyme, i.e., it still remains about 10% of the initial activity after 36 days. At the temperature of 25°C the activity of both enzymes, free and immobilized, changes simi-

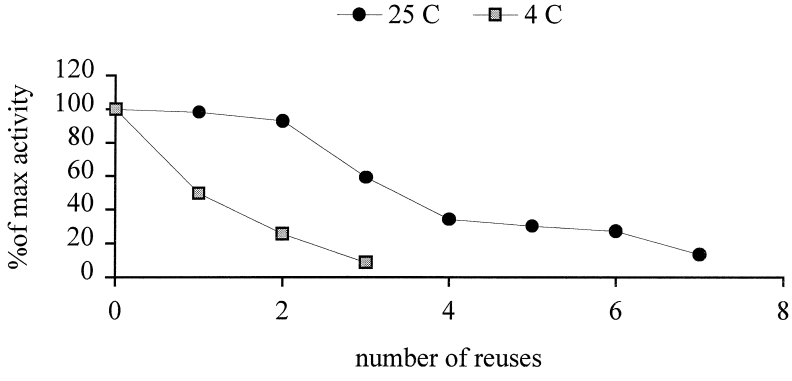

Fig. 4. Reusability of polyaniline immobilized urease at 4 and 25°C.

Table 2
Activity of urease immobilized on polyaniline blends with conventional polymers

Immobilizing support	Activity [μM of urea min^{-1} cm^{-2}]		
	Fresh samples	After 2 days	After 4 days
Polystyrene–PANI	1.68×10^{-2}	1.11×10^{-2}	3.37×10^{-3}
Cellulose acetate–PANI	2.39×10^{-2}	7.71×10^{-3}	3.51×10^{-3}
Poly(methyl methacrylate)–PANI	2.66×10^{-5}	–	–

larly, and it drops down to 8% of its initial value after 20 days. Similarly, the reusability of the enzyme is higher at 4°C (7 reuses) than at 25°C (4 reuses). Fig. 4 shows the reusability of polyaniline immobilized on polyaniline/glass-fiber fabric.

3.4. Immobilization of urease on polymeric blends containing polyaniline

Many organic polymers as, for example, nylon, polystyrene, etc., are used for enzymes immobilization, due to their excellent mechanical properties and the inertness in biological processes. However, rather complicated methods like, e.g., encapsulation must be used in these cases. We expected that the polyaniline addition to these polymers should facilitate the immobilization. Blends of the following polymers containing 10% of PANI have been tested: cellulose acetate, polystyrene, poly(methyl methacrylate). Activities of urease immobilized on these blends are given in Table 2. Activity of PANI–polystyrene and PANI–cellulose acetate immobilized urease was slightly higher than of pure polyaniline, although the storage stability was worse, i.e., after four days the activity decreased to 20% of its initial value.

4. Conclusions

This study shows that urease can be successfully immobilized onto polyaniline films without using any cross-linking agents. Polyaniline as a conjugated polymer has a good affinity to the enzyme, which can be immobilized on it by adsorption. Such immobilized urease remains active for about 40 days. Polyaniline mixed with conventional polymers facilitates immobilization of the enzyme on these polymers.

Acknowledgements

This work was supported by the Polish Committee for Scientific Research. Grant no 11.11.160.203.

References

[1] B. Krajewska, M. Leszko, W. Zaborska, Post. Fiz. Med. 23 (1988) 115.
[2] W. Kong, L.P. Wang, M.L. Gao, H. Zhu, X. Zhang, W. Li, J.C. Shen, J. Chem. Soc., Chem. Commun. (1994) 1297.
[3] Y. Liu, J. Qian, X. Fu, H. Liu, J. Deng, T. Yu, Enzyme Microbal Technol. 21 (1997) 154.
[4] A.-F. Hsu, T.H. Foglia, G.J. Piazza, Biotechnol. Lett. 19 (1997) 71.
[5] P.D. Boyer, H. Lardy, K. Myrbäck (Eds.), The Enzymes, Vol. 4, 2nd edn., Academic Press, New York, 1960, pp. 247–256.
[6] B. Krajewska, M. Leszko, W. Zaborska, J. Chem. Tech. Biotechnol. 48 (1990) 337.
[7] S.B. Adeloju, S.J. Shaw, G.G. Wallace, Anal. Chim. Acta 323 (1996) 107.
[8] P. Gros, A. Bergel, J. Electroanal. Chem. 386 (1995) 65.
[9] L. Boguslavsky, H. Kalash, Z. Xu, D. Beckles, L. Geng, T. Skotheim, V. Laurinavicius, H.S. Lee, Anal. Chim. Acta 311 (1995) 15.
[10] K. Kojima, H. Nasu, M. Shimomura, S. Miyauchi, Synth. Metals 71 (1995) 2245.
[11] L. Coche-Guerente, S. Cosnier, C. Innocent, P. Mailley, Anal. Chim. Acta 311 (1995) 23.
[12] Y. Cao, P. Smith, A.J. Heeger, Synth. Metals 48 (1992) 91.
[13] J. Laska, K. Zak, A. Proń, Synth. Metals 84 (1997) 117.
[14] J. Laska, A. Proń, S. Lefrant, J. Polym. Sci. A Polym. Chem. 33 (1995) 1437.
[15] M.W. Weatherburn, Anal. Chem. 39 (1967) 971.