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Dioxane enhanced immobilization of urease on alkyl modified nano-porous silica using reversible denaturation approach

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ABSTRACT

Efficient immobilization of urease was achieved upon dioxane-induced unfolding/refolding strategy on alkyl modified porous silica with an average pore size of 66 nm. Structural exploration of the urease was carried out to find the optimum condition of solvent polarity which provides efficient adsorptive immobilization through emphasizing on dual approaches: creation of hydrophobic intermediates as molten globule like states and improved accessibility of substituted alkyl chains. The optimum percent volume of dioxane in phosphate buffer to fulfill such aim was achieved at 30% (v/v). Restoring of native-like secondary structure was observed using circular dichroism; moreover, improved exposure of hydrophobic surfaces of urease was confirmed using a set of UV-analysis, intrinsic fluorescence, and differential 8-anilino-1-naphthalene-sulfonate fluorescence spectroscopy, at 30% as an optimum concentration of dioxane. The yield of immobilization was doubled using reversible denaturation approach and storage stability of the immobilization product of urease was noticeably improved (half live of the multi-used immobilized urease was resulted to be 6.5 folds higher than the half live of the free enzyme). Moreover, 7–13.5 folds activation of the enzyme was resulted upon immobilization. Improved immobilization ensures the efficiency of this strategy, for applied approaches, along with providing further evidences for enhanced surface hydrophobicity of the multimeric urease at defined concentration of dioxane.

1. Introduction

Ureases (urea amidohydrolases, EC 3.5.1.5) are a group of widespread enzymes in nature, classifying as the most proficient enzymes (with proficiency more than 10^{14}) [1], and standing as protagonist in biochemistry for several reasons. Urease was the first ureolytic enzyme obtained and named in the late nineteenth century, with landmark significance in enzymology as the first enzyme crystallized (in 1926 by Sumner) to approve the proteinous nature of the enzymes. Also, urease was the first enzyme shown to possess nickel ions in its active site, essential for activity [2]. Since its substrate; urea is pervasively available in nature, urease was important to provide organisms with nitrogen in the form of ammonia for growth. In addition, urease encompasses an even broader role which should not be overlooked. Urease is made by *Helicobacter pylori* (*H. pylori*) and comprises about 10% of the total bacterial protein [3], neutralizes the acidic environment of the stomach to allow *H. pylori* colonization in the gastric mucosa which is crucial in pathogenesis associated with the infection by *H. pylori* [4]. Although urease is known for its enzymatic activity, it acts importantly in the chemotactic motility of bacterium in a viscous environment [5], and takes action as bacterial surface proteins for adherence to CD74 on gastric epithelial cells which induces NF-κB activation and interleukin-8 (IL-8) production in the host [6].

Urease is a multimeric enzyme. Jack bean (*Canavalia ensiformis*) urease has a homohexameric structure assembled as two trimers of α subunits (α_3) [7] each of them has 840 amino acids [8]. The molecular mass of hexamer is suggested to be 545.34 kDa, 12 nickel ion included. The mass of the hexamer has also determined 590 kDa, using sedimentation method [9]. Homo-combinations of α subunits as α_2 and α_4 in mulberry (*Morus alba*) [10] and fungal *Coccidioides immitis* ureases [11], respectively, have also been reported. On the contrary, bacterial ureases are composed of heteromeric structures some examples are as follows: two distinct subunits (α; 61–66 kDa, β; 26–31 kDa), forming a dodecameric combination of $((\alpha\beta)_3)_4$ in *H. pylori* urease [12,13], three distinct subunits (α; 60–76 kDa, β; 8–21 kDa and γ; 6–14 kDa), forming $(\alpha\beta\gamma)_3$ in *Klebsiella aerogenes* [14] $(\alpha\beta\gamma)_4$ in *Staphylococcus saprophyticus* [15], and $(\alpha\beta\gamma)_5$ in *Staphylococcus leei* [16] ureases. Since, the alignment of different ureases shows high homology in their amino acid sequences, it can be concluded that they have diverged from a common ancestral enzyme [2]. In all different kinds of ureases, the active sites are

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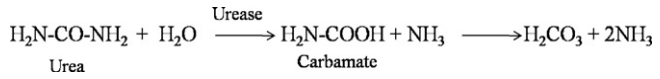


Scheme 1. Two steps urea hydrolysis by Jack bean urease. Second step proceeds spontaneously.

located in α subunits [2] and reaction mechanisms (where binuclear nickel centers are involved) are supposed to be similar [17]. Urease hydrolysis the urea to produce two ammonia and a carbonic acid in two steps; in the first step carbamate and the first ammonia are produced by the enzyme action, in the second step carbamate is hydrolysed spontaneously to produce second ammonia and carbonate [2] (Scheme 1).

Unfolding/refolding of globular proteins including Jack bean urease has been described as a complex process. The unfolding of urease is reversible, while the refolding is a three state process with stable intermediates [18,19]. It has been reported that treatment of urease by low concentrations of chemical denaturants (e.g., SDS or GdnHCl) [20], low pH [18,21], organic solvents [19,21–23], or urease thermal denaturation, results in protein aggregation [19,24] most probably due to partial or transient exposure [25] of hydrophobic surfaces in unfolded and abundantly in intermediate trimeric (T_I) or monomeric (M_I) species. Partially folded intermediates are thought to be appeared during refolding process of the unfolded structures and are characterized with aggregation potential [26,27]. A variety of enzyme immobilization techniques have been developed [28–31]. Recently, Krajewska reviewed a combination of techniques and support materials applied for urease immobilization [32]. Among numerous methods, conventional adsorption of urease is associated with the disadvantages of low loading and poor stability due to enzyme leakage which results in low sufficiency in various applications [33]. However, reversible denaturation has been reported for adsorptive immobilization of carbonic anhydrase [34] and of primary importance for this study, urease [35] using heat denaturation and acidic pH environment, respectively. Ureases in immobilized form are of most promising applications: firstly urea removal in medical, industrial, agricultural, environmental and food processing fields, secondly analytical approaches include not only urea quantification, but also the analysis of substances that act as inhibitors of the enzyme. Nowadays, immobilized ureases are broadly employed in urease-based biosensors, as analytical devices [28,36–39].

Here, dioxane enhanced adsorptive immobilization of urease on alkyl modified nano-porous silica are presented. The adsorptive interactions between accessible hydrophobic surfaces of the protein intermediate species of the molecular population of the enzyme and extended hydrophobic surfaces of the substituted alkyl chains on matrices are formed in competition with protein aggregation process using dioxane as an organic solvent. Dioxane is capable of accepting hydrogen from water (without evidence of hydrogen donating ability), hence, it is a water miscible organic solvent (without phase forming behavior) [40–42]. Meanwhile, dioxane decreases solvent polarity efficiently and can impact protein structure, despite on the difficulties in having a controlled unfolding – an additional difficulty using this enzyme as a model. Moreover, dioxane may be facilitating the entry of the enzyme into the pores on the support [43]. Therefore, present report addresses the efficiencies of the novel dioxane-induced loading urease on octadecyl substituted porous-silica for adsorptive immobilization of urease through both dioxane enhanced surface hydrophobicity of the enzyme, and improved interactivity of the substituted octadecyl moieties on the porous silica.

2. Experimental

2.1. Materials

Porous silica with an average pore size of 66 nm, octadecyl trichlorosilane (OTS) and dioxane were purchased from Merck (Darmstadt, FRG). 8-anilino-1-naphthalen sulfonic acid (ANS), sodium nitroprusside (sodium pentacyanonitrosyloferrate III) and urease (EC 3.5.1.5) from Jack beans were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical reagent grade. Distilled water was used in all experiment. Potassium phosphate buffer (100 mM), pH 7.6 was prepared in dioxane–water ratios in the range of 0–50% (v/v).

2.2. Protein assay

The protein content was estimated using Bradford method [44], in which bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) was used as standard. Bound protein was estimated after acid or detergent induced protein desorption from product of immobilization. 200 mg enzyme-loaded support material was treated with 200 μl HCl 0.08 M or 8.5 mM SDS for 30 min at 90 °C. Then supernatants were assessed for protein content against control (same substituted matrix, without immobilized protein was treated with acid or detergent, to consider the interference with protein assay method) [45,46].

2.3. Urease assay

Enzyme assay was performed by Berthelot alkaline phenol–hypochlorite method to examine the efficiency of adsorptive immobilization. This method is based on the released ammonia (NH_3) which reacts with hypochlorite (OCl^-) to form a monochloramine [47]. This product then reacts with phenol to form blue-colored indophenols whose absorbance is measured at 625 nm. In brief, 10 μl enzyme was incubated with 140 μl urea at final concentration of 25 mM in phosphate buffer solution pH 7.6, 100 mM for 15 min at 37 °C. The liberated ammonia was estimated using 500 μl of solution A (contained 5.0 g phenol and 25 mg of sodium nitroprusside) and 500 μl of solution B (contained of 2.5 g sodium hydroxide and 4.2 ml of sodium hypochlorite in 500 ml of distilled water) at 37 °C for 30 min and the absorbance was measured at 625 nm against the control. The immobilized urease also was taken to be assayed using direct assay method. 50 mg of immobilization product was washed twice then incubated in the presence of 200 μl urea 25 mM in phosphate buffer 100 mM, pH 7.6 under gentle mixing for 15 min at 37 °C. The absorbance of supernatant (due to released ammonia) was read at 625 nm against the control. In both cases specific activities were determined based on milligram protein present in assay cocktail or adsorbed on matrix for free or immobilized enzyme, respectively.

2.4. Unfolding analysis

For dioxane-induced unfolding studies, the solutions of urease were prepared in potassium phosphate buffer at different ratios of dioxane:water at the range of 0–50% (v/v). Structural investigation on the enzyme unfolding was carried out at UV-region of the wavelength using Camspec M550 spectrophotometer with the final concentration of enzyme at 0.2 mg ml^{-1}. Also, both intrinsic and extrinsic fluorescence analyses as a function of different ratios of dioxane were carried out in Hitachi (Model MPF-4) spectrofluorimeter in a quartz cuvette with 1 cm path length at 25 °C using 0.1 mg ml^{-1} enzyme. For each experiment, appropriate blank solution was used for baseline correction of fluorescence intensity. The intrinsic fluorescence was recorded in the emission wavelength

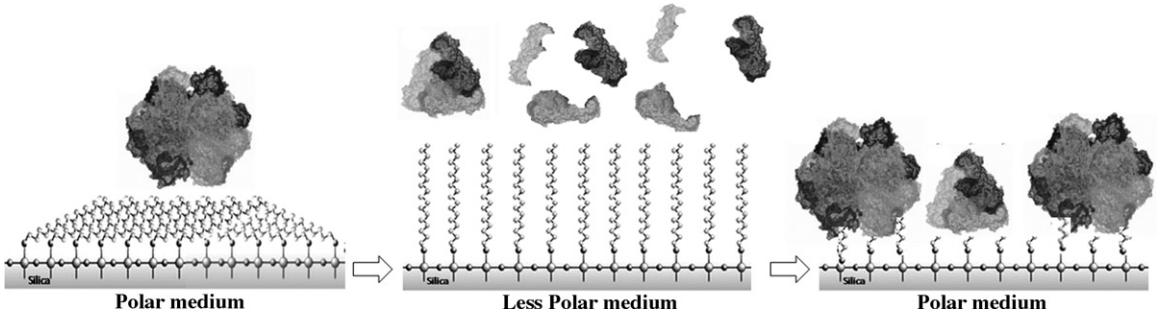


Fig. 1. Dioxane-induced unfolding/refolding approach enhances the surface hydrophobicity of the urease and improves the physical exposition of substituted hydrophobic alkyl moieties on silica, in order to improve urease adsorptive immobilization. Substituted hydrophobic alkyl moieties on silica were not available efficiently in polar conditions, decreasing polarity condition improves physical exposition of them to interact efficiently with urease refolding intermediates in polar medium.

ranging from 300 to 500 nm after excitation of the enzyme at 280 nm. The slit width for both excitation and emission was set at 5 nm. For extrinsic fluorescence analysis, 8-anilino-1-naphthalene sulfonic acid (ANS) was used as the reporter of hydrophobic pockets. Urease samples in the presence of different ratios of dioxane and final concentration of ANS at 20 μM were prepared. The ANS excitation wavelength was taken at 350 nm and emission spectra were collected between 450 and 600 nm. Also, ANS fluorescence emission spectra were collected in the absence of the urease to provide differential analysis of the spectra. The maxima of the differential spectra (ΔFI) as a function of dioxane concentration were constructed. In all the structural investigations, inflection points were determined using linearization of the function by computing multiple derivatives of the functions.

2.5. Circular dichroism measurements

Circular dichroism measurements were carried out on AVIV (Model 215) spectropolarimeter. All measurements were performed at 25 °C. Far-UV measurements were recorded in the range of 200–300 nm using enzyme at concentration of 0.2 mg ml^{-1} with 1 mm path length cell. The data were analyzed by CDNN software.

2.6. Porous silica alkylation

Porous silica was characterized using Brunauer, Emmett and Teller (BET) approach as the most widely used technique for estimating surface area and pore size. The parameters including; average grain size, pore size, pore volume and surface area for this matrix were estimated at 22.01 μm, 66 nm, 0.488 cm^3 g^{-1} and 33.62 m^2 g^{-1}, respectively. The alkylation of porous silica was carried out as described in our previous work through refluxing of octadecyltrichlorosilane (OTS) in dried toluene in the presence of anhydrous matrix overnight [48]. The alkylation was approved using FTIR spectroscopy (Equinox 55, Bruker Company) and the mole number of alkyls per gram of porous silica was calculated by thermogravimetric analysis (TGA) using a TAinst Q50, thermal analysis instrument at 221.2 μmol g^{-1}.

2.7. Urease immobilization

For enzyme immobilization, enzyme at 0.3 mg ml^{-1} was prepared in 100 mM phosphate buffer pH 7.6, consisted with different concentration of dioxane in the range of 0–50% (v/v). 100 mg of octadecyl modified porous silica was hydrated, then 200 μl enzyme solution in desired polarity condition due to the appropriate concentration of dioxane was added on the matrix and incubated for 2 h at 4 °C under rotary mixing. The samples were then centrifuged for 5 min at 13,000 rpm in 4 °C (Heraeus Labofuge 400R) and washed with distilled water (dioxane free) to restore the folded protein in immobilized state.

2.8. Stability analysis

Stability of immobilized preparations was investigated at 4 °C in both of single-usage (in-storage) and multi/repetitive-usage (in-use) modes against free enzyme. In in-use mode, an immobilized preparation (at triplicate) withdrawn at intervals, equilibrated to room temperature, checked for activity, washed twice using 1 ml phosphate buffer 100 mM, pH 7.6 followed by low speed centrifugation and pouring out the supernatant. The washed adsorbent stored at 4 °C for the next assay.

3. Results and discussion

Here, we are reporting the immobilization of Jack bean urease through adsorptive interactions between protein refolding intermediate species and extended hydrophobic surfaces of the substituted alkyl chains on porous silica governed by hydrophobicity in competition with protein aggregation process using dioxane as an organic solvent (Fig. 1). Dioxane induced structural changes of urease using ultra-violet spectroscopy and intrinsic spectrofluorimetry were studied and results are shown in Figs. 2 and 3, respectively. It is known that the structure of protein may be influenced by the polarity of the environment. Therefore, tryptophan absorbance or excitation at 280 nm may provide valuable information on the structural states of the urease. In both strategies,

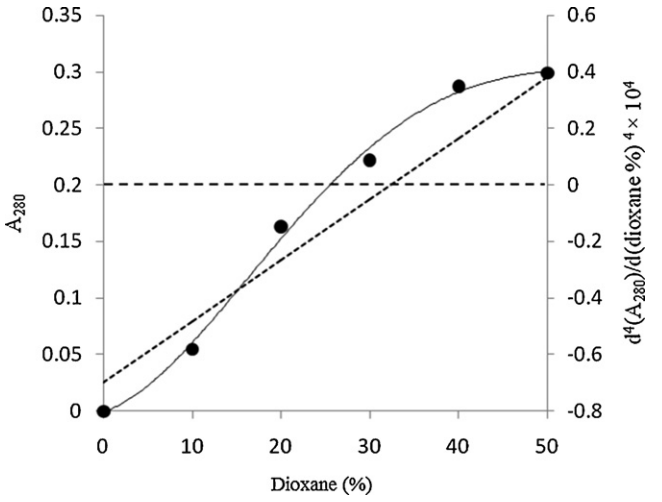


Fig. 2. UV analysis of the urease at 280 nm as a function of dioxane concentration (solid line/left axis). Multi derivative plot has included with the *x*-axis intercept (dash line/right axis).

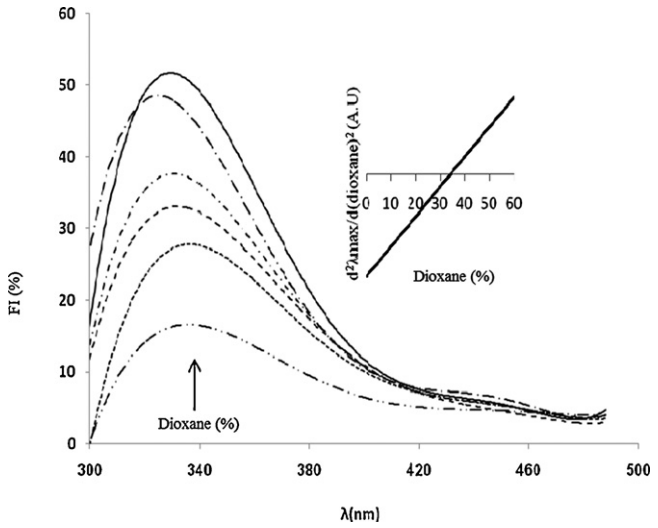


Fig. 3. Intrinsic fluorescence emission spectra of urease at 0–50% dioxane in phosphate buffer 100 mM, pH 7.6. Inset to figure shows linearized function as a derivative of the emission maxima with *x*-axis intercept. All the measurements were made at 25 °C using the 0.1 mg ml^{-1} enzyme.

multiple derivatives determine the inflection points of primary plots at ~30% dioxane concentration. The fluorescence probe ANS, as a reporter of the hydrophobicity is widely used to assess the surface hydrophobicity of proteins [49,50]. Because the emission of ANS is influenced by solvent polarity, a differential analysis of ANS fluorescence due to binding to the hydrophobic pockets of urease was carried out at different percent ratios of dioxane (Fig. 4). Again, the pattern of the fluorescence emission as the function of dioxane concentration has shown inflection point close to 30% of organic constituent of the solvent system obtained through a derivative plot and is presented as an inset to Fig. 4. Moreover, the observed blue shift in ANS fluorescence emission maximum due to ANS localization in hydrophobic pockets of the enzyme molecule at different percent of dioxane indicates an enhanced surface hydrophobicity of the urease under dioxane induced unfolding/refolding process. The observed minimum intensity in ANS fluorescence emission up to 20% of dioxane may probably be due to the strengthening of the hydrogen bonds, which decline the protein surface hydrophobicity. Consequently rising dioxane concentrations to more than 20% favored translocation of hydrophobic portions to the less polar medium thermodynamically, which increased the protein surface hydrophobicity. The aforementioned explanations are supported by the influence of protein α-helicity from dioxane content of the solvent system using CD analysis (Table 1) and even the extent of immobilization on modified matrix which will be discussed later. The α-helix content of the urease was found to be close to the native value at 30% dioxane. The collection of evidences indicates the presence of molten globule intermediate at 30% dioxane. This state is characterized by the presence of substantial secondary structure arranged in a native-like overall fold and an efficient exposition of a hydrophobic core to the solvent [51]. Adsorptive immobilization of urease on hydrophobic support as a function of dioxane concentration was studied and the results are presented in Fig. 5. Considering the trend of immobilized activities as a function of dioxane concentration, the immobilization is decreased by increasing dioxane concentration (most probably due to the inhibitory effect of dioxane on hydrophobic interactions) with the exception of concentration of dioxane which results in enzyme structure change enough to offer an exceptionally higher hydrophobic surface at ~30%. Urease has low tendency of binding to hydrophobic support in its native form [52]. However, hydrophobic sites of inter-

Table 1
α-Helicity of urease as a function of dioxane content in phosphate buffer 100 mM, pH 7.6 using far-UV circular dichroism analysis. The spectra of urease at 0.2 mg ml^{-1} were recorded in the wavelength region 200–300 nm and CDNN software was used for data analysis.

Dioxane (%)	0	10	20	30	40
α-Helicity (%)	5.7	6.6	6.9	5.2	3.4

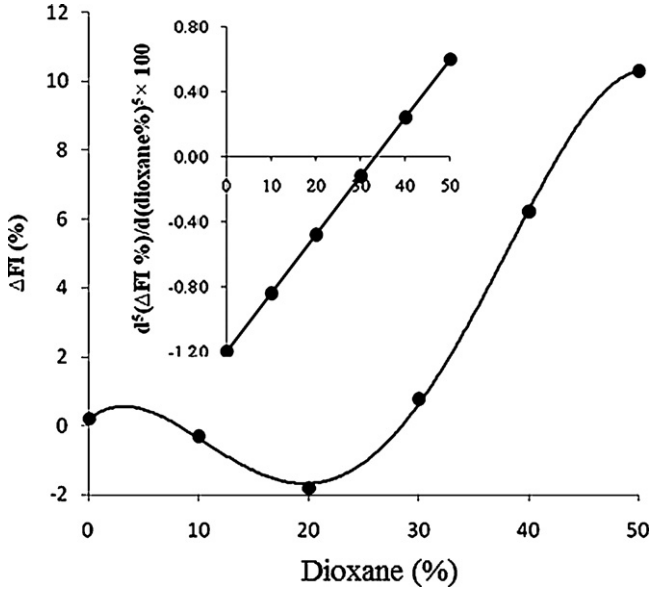


Fig. 4. Differential ANS fluorescence emission intensities (ΔFI) as a function of dioxane concentration are presented after determining maxima from emission spectra in the presence and absence of urease. Inset to figure shows multiple derivatives of the function to determine the inflection point of the function at *x* axis intercept. Fluorescence spectra of 20 μM ANS in potassium phosphate buffer (100 mM), pH 7.6 was prepared in dioxane–water ratios in the range of 0–50% (v/v). The ANS excitation wavelength was taken at 350 nm and emission spectra were collected between 450 and 600 nm.

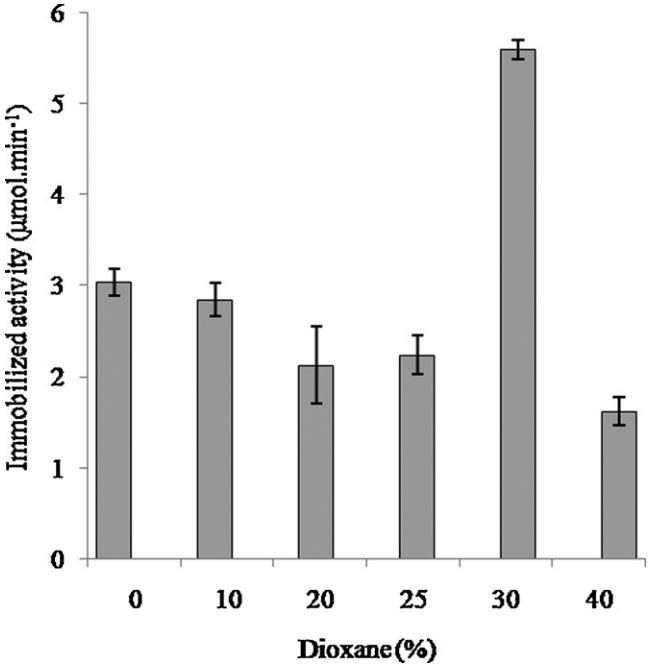
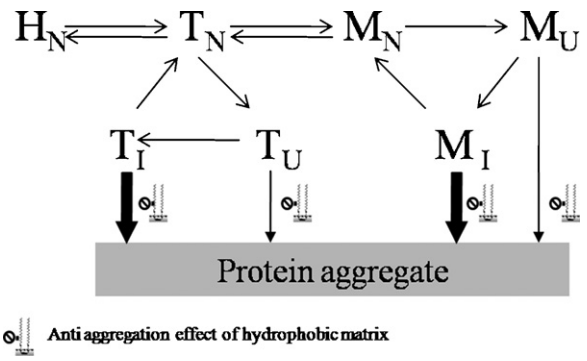


Fig. 5. Adsorptive immobilization of urease on octadecyl silica as a function of dioxane concentration ($n = 3$). The immobilized urease was assayed using direct assay method in phosphate buffer 100 mM, pH 7.6. For more details see Section 2.



Scheme 2. Postulated interrelationships between dissociation, unfolding and refolding species of Jack bean urease. Hexameric (H), trimeric (T) and monomeric (M) states in native (H_N and T_N), unfolded (T_U and M_U), and intermediate (T_I and M_I) states have been presented. Refolding intermediates are thought to be mainly involved in protein aggregation process. In the presence of the exposed hydrophobic alkyl chains (induced by dioxane), aggregation is suppressed and refolding is progressed by interfering of the alkyls to result active immobilized urease species.

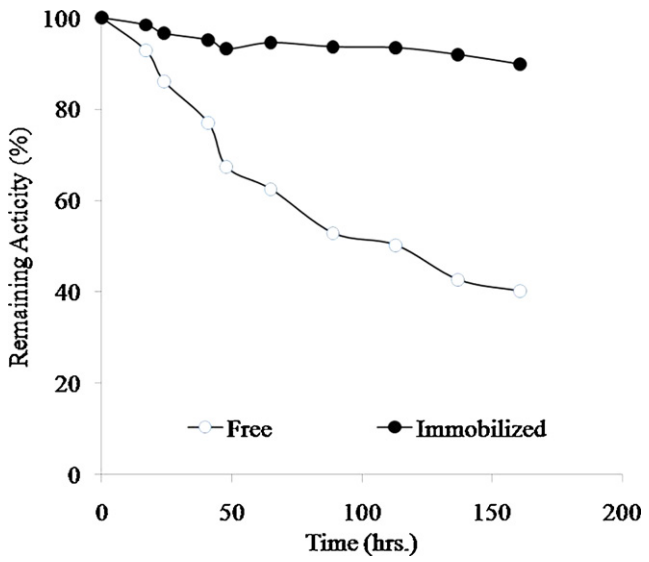


Fig. 6. Stability analysis of multi-used single aliquot of immobilized urease and free ureases as a function of storage time, in 100 mM phosphate buffer, pH 7.6, at 4 °C. Samples were withdrawn at various times, equilibrated to room temperature, assayed, washed twice then stored at 4 °C for the next assay.

mediates are exposed to hydrophobic interfaces during refolding process in water. Based on our structural observations, maximum urease surface hydrophobicity can be achieved at ~30% dioxane. Moreover, substituted alkyl chains on nano-porous silica which are in collapsed state in aqueous condition are thought to be raised to expose at dioxane treated condition. Therefore, both the enzyme and adsorbent are improved to favor more efficient adsorption process in the dioxane-enhanced immobilization strategy and immobilization yield is about doubles. Observed dramatic immobilization decrease in 40% dioxane concentration may be just because improvement on protein surface hydrophobicity is not able to compensate further decrease in medium polarity, so solvent preventive effect exceeds the enzyme adsorptive interactions. Such action of "solvent effect" can be understood from sigmoidal pattern of ANS fluorescence (reporter of surface hydrophobicity) as a function of dioxane concentration (decreasing solvent polarity) (Fig. 4). Moreover, at 40% dioxane concentration, α-helix content was estimated to be far lower than the helix content in native state (Table 1) therefore, protein might be far enough from the optimum state for immobilization or molten globule state.

Scheme 2 is postulated to describe the relationships between urease hexameric (H) and dissociation products as trimeric (T) and monomeric (M) states in native (H_N and T_N), unfolded (T_U and M_U), and intermediate (T_I and M_I) states, with special emphasis on aggregation potential for later states of the enzyme. Partially folded intermediates (T_I and M_I) are thought to be accumulated in equilibrium and are generally pertained to the molten globule states during refolding process of the unfolded structures [26,27,35]. The trimer structure of urease has been reported with the same activity as the hexamer; as a result, a little activity loss occurs during hexamer–trimer transition ($H_N \leftrightarrow T_N$) [53]. Dissociation of Jack bean urease to functionally active trimmers using organic solvent [54] and even monomeric state has been reported [55]. However, the next transitions to unfolded states (T_U and M_U) make them prone to be aggregated either directly or through refolding intermediates (T_I and M_I) and bring about a dramatic decrease in activity. In the presence of the exposed hydrophobic alkyl chains (induced by dioxane), aggregation is suppressed and refolding is progressed by interfering of the alkyls to result active immobilized urease species in the forms of hexameric or even trimeric states (Scheme 2).

The adsorption product was without leakage or leaching, as confirmed by successive washing steps. Fig. 6 compares the remaining activities of multi-used single aliquot of the immobilized urease (in-use) and free (soluble) urease as a function of storage time, at 4 °C up to 160 h. Activity loss of the immobilized enzyme was observed to be far less than the free enzyme (the half live is improved about 6.5 times). However, almost no activity loss was observed in the case of single-used (in-storage) immobilized preparations at 4 °C up to 160 h which were assayed in appropriate intervals. The multimeric structure of the urease is in the risk of dissociation which makes it prone to be denatured. Therefore, observed remarkable stabilization upon adsorptive immobilization by reversible denaturation strategy is deduced, if in part, by the involvement of multipoint interactions between enzyme species and support [32,35,56].

Effect of dioxane concentrations on yields of immobilization is presented in Table 2. In spite of mass transfer limitations,

Table 2
Specific activity analysis of free and immobilized ureases. Immobilized preparations have featured by folds of activation. The activity of immobilized urease has been determined by direct assay method (for detail see Section 2). To determine the protein loading on the silica, protein desorption were performed using acid and detergent.

Dioxane (%)	Protein[a] (μg)	Yield protein (%)	Activity[b] (μmol min^{-1})	Yield activity (%)	Specific activity[c] (μmol min^{-1} mg^{-1})
0	5.20	8.86	3.03 ± 0.15	121.15	569.92
10	5.00	8.14	2.84 ± 0.19	113.62	578.48
20	3.88	6.39	2.12 ± 0.42	84.80	546.96
25	3.84	6.40	2.24 ± 0.21	89.72	584.20
30	10.91	18.19	6.15 ± 0.11	223.45	563.20
40	2.76	4.60	1.62 ± 0.15	84.97	589.12

[a] Determined through detergent desorption strategy (for details, please see Section 2). Protein loaded was 60 μg per 100 mg matrix.
[b] Activities were determined as micromoles of liberated ammonia per minute (μmol min^{-1}). Initial (free) activity was resulted at 2.5 μmol min^{-1}. The immobilized enzyme was determined through direct assay method (n = 3, for details, please see Section 2).
[c] The activity of free enzyme was calculated at 41.6 μmol min^{-1} mg^{-1}.

Table 3
Urease activation upon immobilization on alkyl-substituted porous silica.

Immobilized *vs.* free urease	Specific activity (μmol min^{-1} mg^{-1})	Folds of activation
Free urease	41.60	1.00
Immobilized urease (acid desorption)[a]	290.62	6.99
Immobilized urease (detergent desorption)[b]	563.20	13.54

[a] Adsorbed protein (mg) was determined after desorption from immobilized preparations using acid (HCl, 0.08 M). For details see Section 2.
[b] Adsorbed protein (mg) was determined after desorption from immobilized preparations using detergent (SDS, 8.5 mM). For details see Section 2.

urease was revealed to be activated at ~7 or ~13.5 folds by immobilization, based on applied acid or detergent desorption strategies, respectively (Table 3). This observation means that immobilized enzyme probably has gained improved functional conformation upon refolding process on adsorbent; therefore, presumably positive structural alterations overcome the mass transfer limitations due to immobilization. Activity improvement by adsorptive immobilization of urease from Jack bean has also reported by other groups e.g., Azari et al. [35] and has recently reviewed [32]. As a result, substituted octadecyl moieties on porous silica are assumed to be perfectly exposed at optimum concentration of dioxane which improves the surface hydrophobicity of the matrix to interact well with solvent-exposed hydrophobic sites on the discussed intermediate structures during the course of refolding process. Moreover, it can be supposed that dioxane may importantly involve facilitating the entry of enzyme on the hydrophobic pores. Therefore, compared to the previous reports [27,34,35], polarity based dioxane treatment has dual effects which ensures appropriate enzyme–matrix interactions.

4. Conclusions

Urease immobilization is an important task in applied points of views, especially for construction of urease based sensory devices. Since, silica is a widely used matrix in electronics such as field effect transistors, porous silica have been used in this article for urease adsorptive immobilization after alkylation. We validated the efficiency of the reversible denaturation for adsorptive immobilization by hydrophobic interactions and the extension of this approach to improve the utilization of dioxane as water miscible organic solvent. Moreover, improved immobilization provides further evidences for enhanced hydrophobicity of the multimeric urease at defined concentration of dioxane. Considerable stabilization and activation of urease observed on immobilization, ensures the efficiency of this immobilization strategy, for applied approaches and even further cases.

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