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Improved stability of urease by covalent linkage to poly (tetrafluoroethylene)

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ABSTRACT: Methods for the preparation of poly (tetrafluoroethylene) surfaces containing specific organic functionality are described. These surfaces serve as intermediates for subsequent reactions involving the enzyme urease.

Enhanced stability of urease covalently immobilized on poly (tetrafluoroethylene) (Teflon) support has been observed by using a novel immobilization protocol which links the enzyme via its surface-exposed carboxylic groups (rather than conventionally used amino groups) to phospholipid-coated surfaces. Silanization of the solid supports with [N-[11-(trifluoroacetamido) undecanoyl] amino] propyltriethoxysilane, followed by removal of the trifluoroacetyl protective group, furnished the required amino-functionalized surfaces. The supports were linked to amino-functionalized phospholipids which, in turn, were coupled to the carboxylic moieties of urease through diimide activation of the latter. For comparison, aminated supports without the phospholipid coatings were linked directly to carboxylic groups of urease. For comparison, urease was also immobilized to the derivatized surface via its amino moieties using as cross-linkers cyanuric chloride as well as a new reagent, phthaloyl chloride.

Spectrophotometric assays revealed that urease exhibited superior retention of activity after heating to temperatures up to 100^{o}C. This stability was largely independent of the nature of the support material. It was found that urease bound to lipid-coated silica or Teflon could be boiled in aqueous solution for 1 h with minimal loss of activity.

Key Words: Immobilized enzymes; Urease; Poly (tetrafluoroethylene); Teflon; Silica; Tungsten.

Introduction

Enzymes are specific catalysts that speed up the rates of chemical reactions (1). The general form of an enzymatic reaction is usually represented as:

$$E + S \leftrightarrow ES \rightarrow E + P \qquad [1]$$

where E is the enzyme, S is the substrate, ES is the enzyme-substrate complex and P is the product. When S is the only limiting substrate, the reaction rate is limited by the decomposition of the enzyme-substrate complex, leading to the Michaelis-Menten equation:

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$$V_o = \frac{V_{max}[S]}{K_m + [S]} \qquad [2]$$

where V_o is the reaction rate (mol s^{-1}), V_{max} is the maximum reaction rate, [S] is the substrate concentration and K_m is the Michaelis constant (i.e. concentration at which $V_o = 0.5\ V_{max}$). V_{max} depends on the amount of enzyme or the enzyme activity. This parameter can be determined by measuring the initial reaction rate at high substrate concentrations ([S] >> K_m). At low substrate concentration the reaction rate is linearly related to the substrate concentration.

$$\frac{Vo}{V_{max}} = \alpha[S] \qquad [3]$$

where $\alpha = K_m$

When immobilized enzymes are used, the overall reaction rate may also be limited by mass transport conditions (1).

Reaction rates are generally affected by temperature. The higher the temperature, the faster the reaction. This is also true for reactions catalysed by enzymes, up to a point. Enzymes are proteins which can be denatured at high temperatures and their enzymatic property is thereby destroyed. The temperature range for activity of enzymes generally is 10 – 50^{o}C. The optimum temperature for many enzymes in the human body is 37^{o}C.

Generally, stable attachment of proteins to a support is best attained by covalent binding. Prerequisites for this immobilization procedure are suitable functional groups on the protein and on the support (1-5). Functional groups in the protein are provided by amino acid residues, the most important of which are amino groups from L-lysine and carboxyl groups from L-aspartate or L-glutamate (5).

The amino acids essential for the catalytic activity of the enzyme should not be involved in the covalent linkage to the support. Enzymes immobilized in this fashion generally lose their activity (6). In addition, the affinity of the enzyme for the substrate may be affected by conformational changes (6).

Polymers such as polysaccharides, collagen-poly(glycidyl metacrylate) graft copolymers, poly(vinyl alcohol), nylon, polystyrene and polypropylene have been utilized as solid supports for urease immobilization (7 – 10). Silica, controlled-pore glass and carbon fibres are examples of non-metallic inorganic supports on which urease has been covalently deposited. In all these cases, urease was immobilized through its amino groups (10).

Enzymes exhibit unique catalytic properties which can be harnessed for a wide variety of applications for detection, analysis and production in the medical, pharmaceutical, chemical and other industries (11 – 13). An inherent disadvantage associated with enzyme biocatalysts is instability (13). The loss of activity as a result of environmental factors such as storage, elevated temperatures and organic solvents can, either singly or collectively, limit their practical applications (11 – 13).

The present work focuses on the immobilization of urease through its carboxylic groups onto amino-functionalized silica, tungsten/tungsten oxide and Teflon support materials either directly or through phospholipid cross-linkers. the ultimate aim of the work is to investigate the effects of phospholipid coating on thermal stability of covalently immobilized urease on polymeric supports.

Materials and Methods

Materials

Silica gel (Davisil, grade 645, 60 – 100 mesh, 150 A, 99+ % purity) and tungsten foil (0.5 mm thick, 99.9+ % purity) were supplied by Aldrich Chemical Company. Teflon tape (0.05 mm thickness, virgin material) was obtained from commercial plastic (DuPont) and was extracted with tetrahydrofuran for 24 hours and dried under vacuum at 60^{o}C to constant weight.

(Aminopropyl) triethoxylsilane, 11-aminoundecanoic acid, cyanuric chloride, (dimethylamino) pyridine, ethyltrifluoroacetate, phthaloyl chloride and triethylamine were purchased from Aldrich Chemical Company and used without further purification. Ethyl [(dimethylamino)-propyl] carbodiimide and urease (U2000) were supplied by Sigma Chemicals Company, lyso-1-palmitoyl-sn-glycerophosphatidyl ethanolamine was from Avanti Polar Lipids, Birmingham, U.K. and the urease substrate solution (0.2 mM EDTA, 17 mM urea, 0.008% bromocresol purple, pH 4.8) was obtained from Biochem, Brentwood, U.S.A. All other solvents were reagent grade.

Syntheses

These were carried out using the procedure described in the literature (1 – 4, 6,7,10) with slight modifications. The structures corresponding to the numbering used in the procedures as well as in the Results and Discussion sections are included in Figs. 1 – 3.

(1) Synthesis of [N-[N11-(Trifluoroacetamido)undecanoyl]-amino]propyltriethoxysilane (5, Fig. 1).

(a) N-(Trifluoroacetyl)-11-aminoundecanoic acid (2).

A mixture of 11-aminoundecanoic acid (1, 50 mmol), ethyl trifluoroacetate (78 mmol) and triethylamine (100 mmol) was stirred in methanol at room temperature under nitrogen for 24h. The excess volatile reagent was distilled off along with the solvent under water pump suction. The residue was extracted with dichloromethane and the organic layer washed with sodium bicarbonate (1% aqueous solution, 100 ml) and water (100 ml) in that order. After drying ($MgSO_4$), the solution was concentrated on a rotary evaporator to leave a white waxy solid residue, yield quantitative: FTIR $_{vc=0}$ 1721 ($NHCOCF_3$), 1704 (COOH), 3250 cm^{-1} (NH); FABMS (negative), m/z (rel intensity) 296 [M-H]- (100); FABMS (positive) m/z (rel intensity) 298 MH^+ (5), 280 $[MH\text{-}H_2O]^+$ (11), 116 ($C_7H_{18}N^+$) 100.

(b) Acid Chloride 3 of 2

This acid 2 (25 mmol) was stirred at room temperature with thionyl chloride (20 ml) for 6h, the excess reagent was removed by distillation under water pump suction to yield a light yellow oily residue in quantitative amount. This product was used as such for the next step: FTIR 1798 (vc=o of COCl), 1713 (vc=o of $COCF_3$), 3319 cm^{-1} (NH).

(c) Condensation of 3 with (Aminopropyl) triethoxysilane (APTEES, 4) to form 5.

A solution of APTES (10 mmol) and triethylamine (5 ml) in toluene (50 ml) was stirred with dropwise addition of the acid chloride 3 (10.5 mmol in toluene) over a period of 1h at room temperature under nitrogen. The mixture was stirred for a further overnight period and then filtered to remove triethylamine hydrochloride; the filtrate was subjected to rotary evaporation to give a light yellow oily residue (yield 85%) which was purified by vacuum distillation: bp 128 – 130^{o}C/0.1 mmHg; FABMS (negative) m/z (rel intensity) 499, [MH]-(100%); FABMS (positive): m/z (rel intensity) 501, MH^+ (5%); 455 (MH-EtOH]+(100%); FTIR, 1711 (vc=o of $COCF_3$), 1083, 1104 cm^{-1} (Si-O and 3315 cm^{-1} (NH).

2. Immobilization of urease on silica, oxidized tungsten and Teflon surfaces through the surface amino groups.

(a) Oxidation of tungsten foil.

This reaction was carried out essentially under the conditions described by Przybyt and Sugier (14). Pieces of tungsten foils (1 cm x 1 cm) were heated in an oven at 700^{o}C for 1h. The metal surface turned yellowish green due to the formation of a tungsten trioxide layer. The oxidized tungsten pieces were soaked

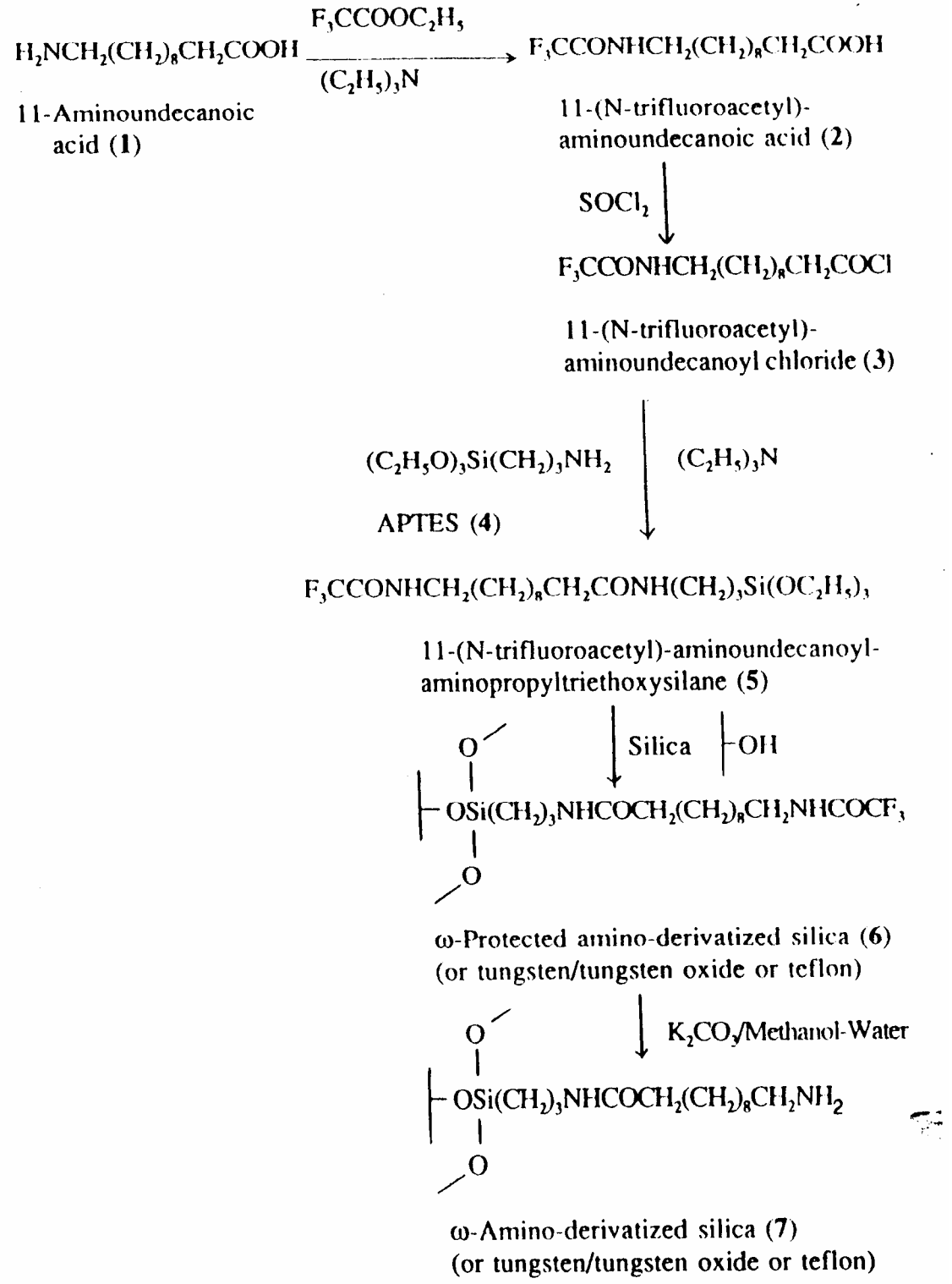

Fig. 1: Generation of ω-amino-derived silica, tungsten/tungsten oxide and Teflon surfaces.

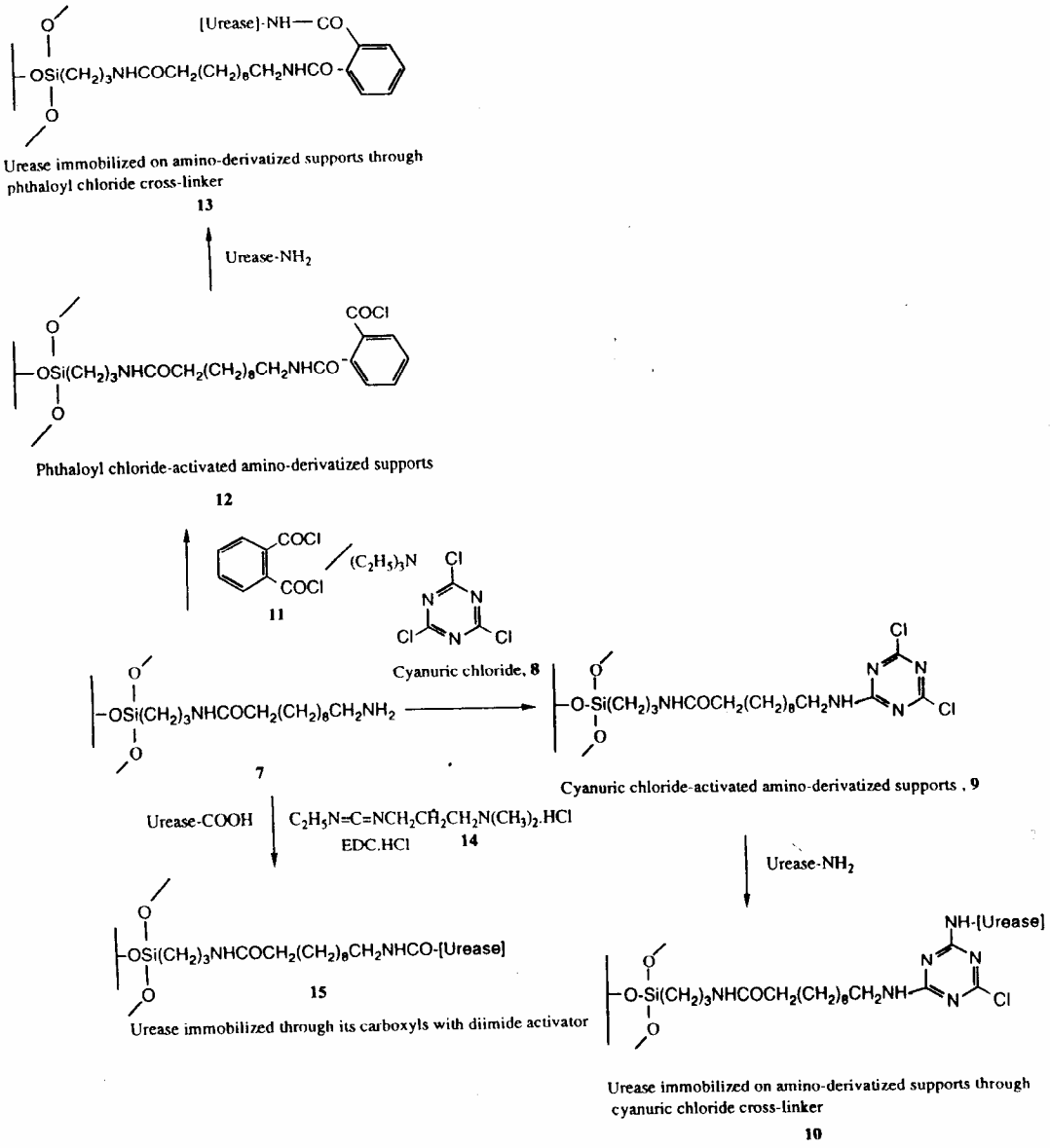

Fig. 2: Immobilization of urease through its ε-amino functionalities with cyanuric chloride and phthaloyl chloride cross-linkers and through its carboxylic functionalities by direct coupling to ω-amino-derived silica, tungsten/tungsten oxide and Teflon.

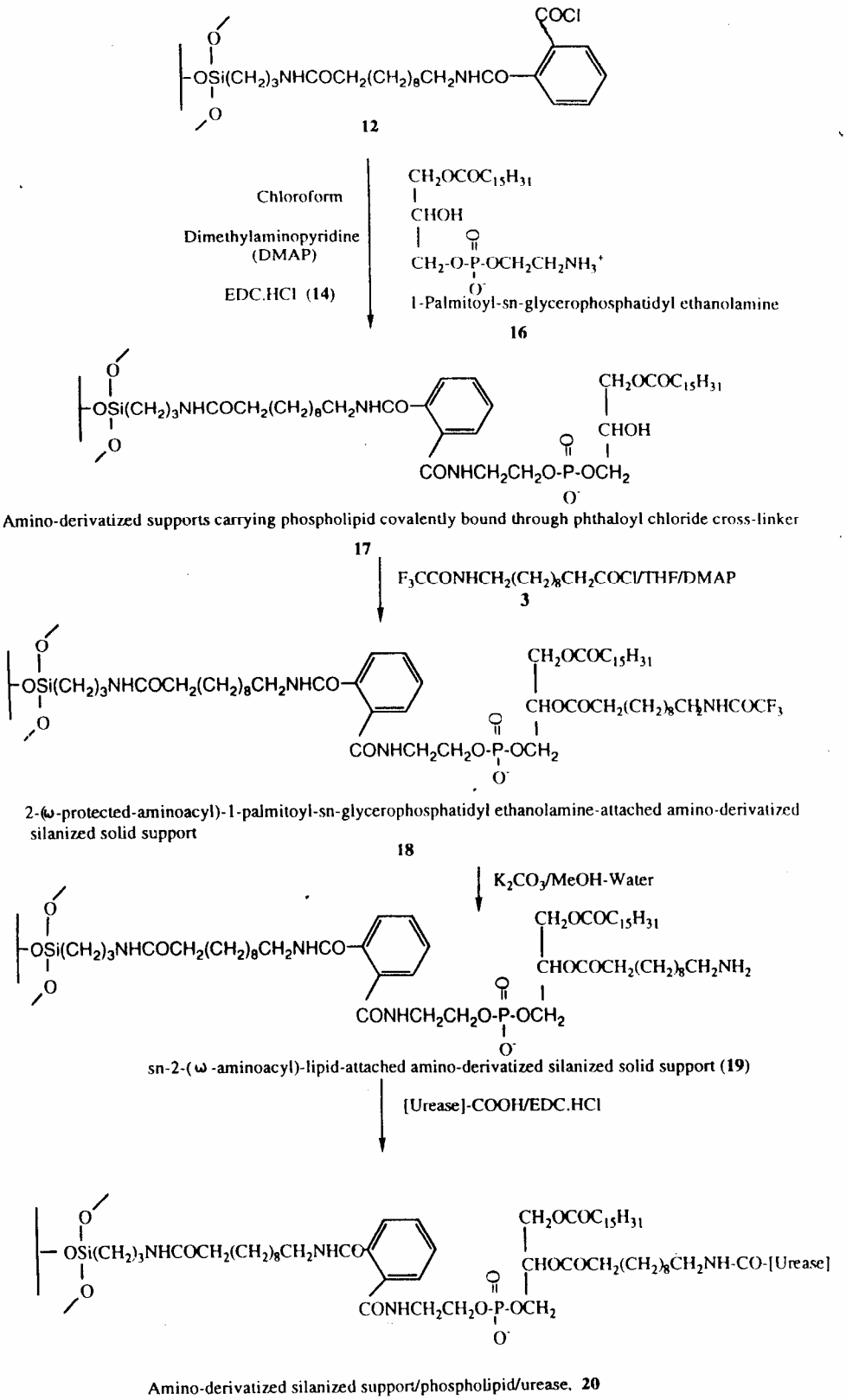

Fig. 3: Covalent binding of urease to ω-amino-derived silanized solid support through phospholipid cross-linker.

in sodium hydroxide (aqueous, 1M, 20 ml) for an overnight period and then washed thoroughly with deionised water.

(b) Surface modification of Teflon.

The introduction of hydroxyl groups onto the Teflon surface was accomplished by the protocol reported earlier (4). The Teflon was first cleaned by sonication in THF for 1h. A 3 – 4 nm sample of aluminium was vapour deposited onto the Teflon surface using a Key High Vacuum metal deposition unit. The deposited metal was removed by immersion in sodium hydroxide (0.1 M) and the Teflon washed thoroughly with water. The aluminium deposition and subsequent washing with NaOH were repeated twice. The Teflon was thoroughly washed with water and dried in an oven at 150^{o}C.

(c) Silanization of the hydroxylated supports with the silane 5.

Each of the support materials was suspended in toluene (50 ml) to which the trifluoroacetamido silane 5 (200 mg) was added, followed by triethylamine (1 ml). The mixture was stirred at room temperature under nitrogen overnight; the silanized support 6 was recovered and washed well with chloroform. After rinsing with acetone, the supports were dried in the oven at 150^{o}C for 3h.

(d) Removal of the trifluoroacetyl protective group from the surfaces 6 to form the surfaces 7.

The above silanized supports 6 were suspended in potassium carbonate (1:1 aqueous methanolic, 20 ml) and stirred for 24h. The deprotected amino-functionalized supports were recovered and washed thoroughly with deionised water and dried in a vacuum desicator.

(e) Direct immobilization of urease through its carboxyl groups on the amino-functionalized supports 7.

The supports 7 were suspended in water (2 ml) and ethyl [(dimethylamino) propyl] carbodiimide hydrochloride (50 mg) (14, Fig. 2) and urease (2 mg) added. The mixture was set aside for 48h in the refrigerator. The solution was carefully decanted to remove the supernatant liquid which was assayed for enzymic activity by spectrophotometry as described below. The solid supports 15 were washed repeatedly with deionised water and then dried and stored in a vacuum desiccator at room temperature.

(f) Immobilization of urease through its amino groups on the aminated supports using cyanuric chloride cross-linker.

The aminated support 7 was suspended in acetone (25 ml) to which cyanuric chloride (200 mg) (8, Fig. 2) was added. The mixture was set aside for 3h at room temperature under nitrogen. The activated supports were washed well with acetone and re-suspended in water (2 ml). Urease (2 mg) was added and the system allowed to stand for 8h in the refrigerator. The supernatant liquid was decanted carefully and assayed for enzymic activity by spectroscopy. The solid support 10 were thoroughly washed with water and stored in a desiccator at room temperature.

(g) Immobilization of urease through its amino groups on the aminated supports 7 using phthamloyl chloride cross-linker.

The aminated supports 7 were suspended in THF (25 ml) and phthaloyl chloride (200 mg) (11, Fig. 2) followed by the addition of triethylamine (1 ml). After stirring under nitrogen at room temperature overnight, the support were recovered and washed thoroughly with sodium bicarbonate (1%) and then with deionised water. The supports were re-suspended in water (2 ml) to which EDC.HCl (100 mg) (14, Fig. 2) and urease (2 mg) were added. After standing at 4^{o}C for 48h, the supports 13 were removed, washed with deionised water and stored in a desiccator at room temperature. The supernatant liquid was, as usual, assayed spectrophotometrically for enzymatic activity.

(3) Immobilization of urease through its carboxylic groups on amino-functionalized silica, tungsten and Teflon supports with phospholipid cross-linker.

(a) Covalent binding of the phospholipid 16 to phthaloyl chloride-activated amino supports.

The reaction of the aminated supports with phthaloyl chloride (11) was carried out as described under section 2g. The resulting supports were suspended in water (3 ml) and the lipid 16 (200 mg) was added in one portion along with EDC.HCl (100 mg) (14) and the mixture was stirred at room temperature under nitrogen for 48h. The substrates were recovered and washed thoroughly with methanol and dried in an oven at 50^{o}C.

(b) Removal of the protective group from the surfaces 18 to form the surface 19.

The supports 18 were suspended in aqueous methanol (1:1, 10 ml) and potassium carbonate (1g) was added. After stirring for an overnight period, the supports 19 were recovered and washed thoroughly with deionised water.

(c) Covalent attachment of urease through its carboxyl to the ω-amino moieties on the sn-2 acyl chain of the supports 19.

The supports 19 were suspended in water (3 ml) and treated with EDC.HCl (14) (100 mg) and urease (2 mg). The mixture was set aside for 48h at 4^{o}C and then the supernatant liquid was removed and assayed for residual enzyme activity. The solid supports 20 were thoroughly washed with deionised water and kept in a desiccator at room temperature.

Spectrophotometric Measurements

Calibration curves correlating the concentration of the enzyme urease with the absorbance of the pH-sensitive indicator dye bromocresol purple present in the substrate solution were initially obtained. A stock solution containing 2 mg urease in 100 ml of distilled water was prepared. Aliquots (ranging from 2 to 9 ml) of the stock solution were added to 1 ml of the substrate solution and the final volume adjusted to 10 ml. After standing for 15 min, a portion (3 ml) of the diluted enzyme/substrate mixture was delivered to a UV cell. Absorbance at 588 nm was then measured. A blank determination containing no enzyme was also carried out to ascertain the absorbance of the substrate solution at the same concentration and wavelength (588 nm). The results were plotted and the curve was used to estimate the concentration of the test solutions.

Each of the supports on which the urease was immobilised (10 mg) was suspended. After standing for 15 min, the solution was diluted to 10 ml with water and its absorbance measured.

The absorbance of the filtrates from the enzyme immobilization reactions were also determined in a similar fashion after diluting them to 10 ml (including the volume of the substrate). The concentration curve was recorded. Since the enzyme used for the immobilization reaction in each case was 2 mg, subtraction of the concentration of the non-immobilized enzyme from this amount gives the quantity of immobilization urease on the supports.

Instrumentation

The FAB mass spectra were recorded on a VG 70-250S double-focusing mass spectrometer operating at 8 kV and equipped with a VG 11-250 data system. Nitrobenzyl alcohol was used as the matrix for the spectra.

Infrared spectra were recorded on a modified Bomen Michelson 110-EFTIR at 4-cm^{-1} resolution using a diffuse reflectance cell (Spectra Tech) accessory. Typically, 100 signal-averaged scans requiring approximately 2 min to record were added for each spectrum.

Solid-state ^{13}C NMR spectra were obtained in natural abundance at a frequency of 75.3 MHz on a Chemagnetics CMX-300 spectrometer.

Results and Discussion

Preparation of the amino-functionalization solid supports 7 for the covalent coupling of urease.

The covalent attachment of urease to silica, tungsten and Teflon supports requires the improvisation of these material surfaces. The most suitable functional group for this purpose is the amino moiety which can be cross-linked to either the ε-amino groups on the lysine residues or the carboxylic groups on the aspartic/glutamic acid residues. The most convenient method for the introduction of this surface amino functionality onto the above solid supports is through silanization with an aminoalkyl-substituted silane. The principal requirement for effecting this silanization reaction with a chloro or alkoxysilane is that the solid supports carry surface hydroxyls. Silica, tungsten and Teflon do not, hence they must be chemically modified to introduce these moieties. In the present work, therefore, tungsten was thermally oxidised to form a thin surface film of tungsten trioxide which was then hydrated with dilute sodium hydroxide adopting the procedure reported by Przbyt and Sugier (14).

Since it has been demonstrated by earlier workers (4, 6) that urease bound to silica via (aminoalkyl) silanes exhibits greater activity with longer alkyl chain length, an amino C_{11} silane was used in the present work to coat the supports. It is well known that densely packed and well defined monolayer structures in the chain length range C10 – C18 could be formed on a variety of surfaces through either silanization or treatment with alkyl thiols (8). The synthesis of the key intermediate, viz. [N-[11-(trifluoroacetamido) undecanoyl]-amino] propyltriethoxysilane (5, Fig. 1), was accomplished by a three-step synthetic sequence. The amino group of 11-aminoundecanoic acid (1) has to be protected to prevent undesirable reaction during the synthetic steps and the trifluoroacetyl protective group selected in the current work could be readily removed under mild basic conditions not detrimental to the surface siloxane bond formed during the silanization reaction.

Silanization of the silica, tungsten/tungsten oxide and hydroxylated Teflon surfaces with the silane 5 was carried out under base-catalysed conditions employing triethylamine as catalyst. The resulting silanized surface (6, Fig. 1) was characterised by Fourier transformed infrared spectrometry and in the cases of the silica surfaces, additionally by solid-state ^{13}C and ^{31}P NMR spectroscopy. The trifluoroacetyl protective group was removed from 6 by treatment with aqueous methanolic potassium carbonate and the deprotected surface 7 was also characterised by the same techniques as above. Thus, while the trifluoro-acetylated surfaces 6 shows a strong band around 1720 cm^{-1} characteristic of the carbonyl of this protecting group, this band is absent in the deprotected surfaces 7 (see Fig. 4). Similarly, in the solid state ^{13}C NMR, a peak around 160 ppm is present, corresponding to the carbonyl carbon of this protective group (with the silica surface) which disappears upon deprotection (Fig. 5).

Covalent binding of urease onto the aminated surfaces 7.

The covalent attachment of urease to the amino-derived silica, tungsten and Teflon surfaces 7 was effected by the direct condensation of the enzyme through its carboxylic groups in aqueous solution in the presence of the water-soluble carbodiimide activator (14, Fig. 2). the reaction was carried out over a period of 48h at 4^{o}C. For comparative evaluation, urease was also bound covalently through its amino acid groups to the surfaces 7, making use of the commonly used cross-linker cyanuric chloride (8, Fig. 2) as well as a new reagent developed in the present work, viz. phthaloyl chloride (11, Fig. 2). The appropriate reactions are summarised in Fig. 2.

Ultraviolet spectrophotometric assays based on the residual enzyme activity in the supernatant layers as well as activity determinations of the immobilised enzyme indicated that the reaction with cyanuric chloride was sluggish compared to the one employing phthaloyl chloride (Table 1). The reaction yields were calculated as the percentage of initial urease (2 mg) immobilised on a fixed amount of solid support (10 mg). However, the best results were exhibited by the surfaces obtained by the carbodiimide coupling (66% total immobilization yield) method which makes use of the carboxyl groups of the enzyme.

The yields of the immobilized enzyme with the phthaloyl chloride cross-linker (11, Fig. 2) (~ 45%) were comparable to the total immobilization yield obtained with the ω-carboxyalkyl silylated surfaces (~ 45%), both procedures utilizing the urease amino moieties for covalent binding. It is interesting to note that Goldstein (10) reported total immobilization with amino acid and carboxylic groups of urease, respectively. In terms of weight, our current yields translate to 90 and 132 mg/g of silica for the phthaloyl chloride (amino-based) and diimide (carboxyl-based) reactions, respectively.

Table 1: Total immobilization yield of urease on various supports and amounts of active enzyme on these supports estimated by UV spectrophotometry.

Surface	Total Immobilization Yield	Init. Active Enzyme Yield (%)[a]	Residual enzymic activity[b] after standing at 25°C for			Residual active (%) upon heating to 100°C for 1h
			10 Days	21 Days	42 Days	
Silica/silane/urease (cyanuric chloride linker)	21	14	11	7	3	3
Silica/silane/urease (phthaloyl chloride linker)	44	42	61	32	22	18
Silica/silane/urease (carbodiimide coupling)	66	60	85	60	40	33
Silica/silane/lipid/urease	75	74	100	100	100	100
Tungsten/tungsten oxide/silane/urease	48	46	55	25	14	10
Tungsten/tungsten oxide/silane/lipid/urease	52	51	95	90	78	76
Teflon/silane/urease	45	43	88	51	45	28
Teflon/silane/lipid/urease	56	51	100	100	91	90

[a]Obtained by subtracting residual active enzyme in filtrate (UV) from the amount of enzyme used for immobilization.

[b]Expressed as percent of initial activity (taken as 100%).

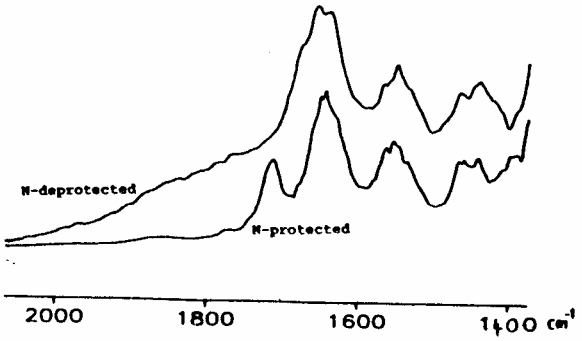

Fig. 4: FTIR spectra of N-fluoroacetyl-protected and N-deprotected [(11-aminoundecanoyl) amino]propyl-silylated silica surfaces.

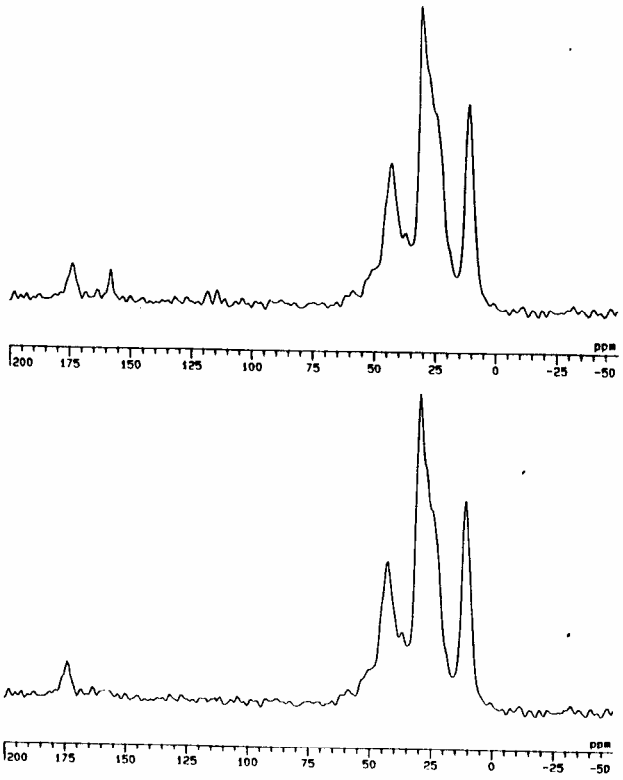

Fig. 5: Solid-state ^{13}C NMR spectra of N-trifluoroacetyl-protected (top) and N-deprotected (bottom) [(11-aminoundecanoyl) amino]propyl-silylated silica surfaces.

Thus, the total immobilization yield of urease is consistently ~50% higher when the enzyme is coupled through its carboxylic groups than when its amino moieties are utilized, and the current protocols for binding urease to aminated surfaces appear to be more efficient than the method used by Goldstein. The total immobilization yields of urease (via its carboxyl group) on the amino-functionalised tungsten and Teflon surfaces 7 are 48 and 45 percent respectively.

Immobilization of urease to silanized solid supports through phospholipid cross linkers.

The phthaloyl chloride cross-linker (11, Fig. 2) was utilized to effect the coupling between the surface amino groups on the surfaces 7 and the amino moiety on the head group of lyso-1-palmitoyl-sn-phosphatidylethanolamine (16, Fig. 3) to form the lipid-immobilized surfaces 17, in view of its successful use for the protocol shown in Fig. 2. The silica surface 17 was characterized by solid-state ^{13}C and ^{31}P NMR (Fig. 6). A second amino group was then introduced onto the surfaces 17 through the acylation of the sn-2-hydroxyl of the surface-bound lipid (Fig. 3) with the acid chloride 3 and subsequent deprotection of the trifluoroacetyl moiety to furnish the surfaces 19. These surfaces 19 were finally condensed with urease through its carboxyl groups in the presence of the diimide activator (14) to yield the surfaces 20. The intermediate surfaces 19 were characterized by FTIR (Fig. 7) which shows strong ester and amide carbonyl peaks at 1720 – 1730 and 1650 cm^{-1} respectively.

The total immobilization yields of urease on silica, tungsten and Teflon surfaces were about 10 – 15% higher than the corresponding yields registered in the absence of the lipid intermediates (20 vs 15). The yields were also higher than those in previous work (6) for the silica-silane-COOH + NH_2-urease scheme (53%).

Spectrophotometric studies of enzyme stability

the close correlation between the total immobilization yields and that determined by spectrophotometry indicates that most of the immobilized enzyme is in the active form irrespective of the nature of the support material (Table 1).

It is clear that upon dry storage at room temperature for 42 days, the enzymic activity of urease on the surfaces without lipid drops off considerably as previously reported (6). Thus, with the silica support and urease COOH immobilization (carbodiimide coupling), the residual activity after this period is 40% while for tungsten and Teflon it is 14 and 45% respectively. It would appear that the metallic support is the least efficient in preserving the activity of immobilised enzymes over extended periods of time. For silica with urease-NH_2 immobilization (cyanuric and phthaloyl chloride coupling) both the initial enzyme plus residual activity after storage were lower than for COOH immobilization.

The stabilising effect of phospholipids on the dry storage of immobilized urease is significant. Even the tungsten surface exhibits 78% residual activity after 42 days at room temperature if the lipid is present on the surface. For silica and Teflon residual activities are 100 and 90% respectively.

Urease, immobilized to silane-coated supports, was largely inactivated upon heat treatment (100^{o}C for 1h). On the other hand, urease immobilised on phospholipid coated supports displays considerable heat resistance. For silica and tungsten supports, after dry heat treatment (100^{o}C for 1h) the residual activities of the lipid-bound enzyme were 100 and 76%. For lipid-bound enzyme on Teflon, subjected to boiling in water for 1h, the residual activity was 90%.

The increase in enzyme stability that frequently occurs upon immobilization has been attributed to enhanced thermal stability at equilibrium or to decreased rates of irreversible denaturation as a result of a decrease in the conformational entropy of unfolding (6). In the present work, the superior stability of the lipid-containing matrices may be a consequence of these factors. Multiple attachment sites of the enzyme to the support plus non-bonded interactions of the enzyme with the lipid would favour enhanced stability both thermodynamically and kinetically (10).

Conclusion

Polymer surface functionalization is a strategy for enzyme immobilization. Various reports in the literature revealed that a variety of other immobilization protocols have demonstrated enhanced thermal stability of enzymes although none has been effective above 60^{o}C. With the use of lipid coated Teflon we have enhanced heat resistance of urease to about 100^{o}C. Further experiments to determine resistance to

hostile reaction conditions and to measure the enzyme activity at elevated temperatures should be carried out.

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