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IMMOBILIZATION AND APPLICATIONS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE: A REVIEW

Pramod Kumar Srivastava[1] and Siddhartha Singh[1,2]

[1]*Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India*
[2]*College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India*

□ *Immobilized enzymes have been used extensively in the fields of food industry, materials processing, textiles, detergents, biochemical and chemical industries, biotechnology, and pharmaceuticals. Studies on immobilization of glucose-6-phosphate dehydrogenase have been less extensive than those for other industrially applicable enzymes. Immobilization of glucose-6-phosphate dehydrogenase has been carried out for the formation of biosensors for the estimation of glucose, ATP, phosphate, and so on. The present review deals with the attempts made for immobilization of glucose-6-phosphate dehydrogenase and its applications for various purposes.*

Keywords biosensor, enzyme, glucose-6-phosphate dehydrogenase, immobilization

INTRODUCTION

Immobilized enzymes have been utilized for a number of practical applications, particularly in biomedical and biotechnological fields, along with industries, laboratories, large-scale synthesis, and so on. Immobilization of enzyme was introduced by Nelson and Griffin in 1916, and the first enzyme to be immobilized was invertase on aluminium hydroxide by using the process of adsorption.[1] However, attachment of enzyme on insoluble matrix was first performed by Grubhofer and Schleith in 1953.[2,3] The term "immobilized enzyme" was adopted in 1971 at the first Enzyme Engineering Conference.[4] Immobilization is simply the fixing of enzyme on or within solid supports, which give rise to heterogeneous immobilized enzyme systems. As compared to free enzyme, immobilized enzymes are more resistant and allow multiple reuse of enzyme, continuous operation of enzymatic processes, and so on.

Address correspondence to Siddhartha Singh, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India. E-mail: siddharthasingh786@gmail.com

The use of immobilized enzymes in medical application is less extensive as compared to industrial applications. At present, extracellular application of enzymes in medicine has been successful for the removal of toxic substances and treatment of life-threatening disorders within the blood circulation.[5] Glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate: $NADP^+$ 1-oxidoreductase; EC 1.1.1.49) catalyzes the first step of the pentose pathway, which involves the transformation of glucose 6-phosphate to 6-phosphogluconolactone concomitant with conversion of NADP to NADPH. The NADPH produced protects erythrocytes from oxidative damage.[6–8] Glucose-6–phosphate dehydrogenase deficiency is a common enzymopathy affecting more than 400 million people worldwide.[9,10] Since glucose-6-phosphate dehydrogenase deficiency is an X-linked disorder, males, being hemizygous, may be affected or unaffected.[11] By contrast, as females have two X chromosomes, they are either homozygous or heterozygous; hence, glucose-6–phosphate dehydrogenase deficiency is more frequently found in males, as homozygous females are extremely rare.[11] The heterozygotic female has a mosaic cellular pattern, with glucose-6-phosphate dehydrogenase normal and glucose-6-phosphayte dehydrogenase deficient population.[11] Clinical manifestations are generally milder in females and depend on the degree of mosaicism.[12] The enzyme deficiency is associated with a large number of clinical manifestations like malaria,[13–15] neonatal jaundice,[16] acute hemolytic anemia,[17,18] and so on.

Keeping in view the therapeutic importance for medical applications, various attempts have been made for the immobilization of glucose-6-phosphate dehydrogenase for several purposes. The immobilized form of enzyme has been used for the treatment of jaundice[19–21] and also used for various biosensors.[22,23] The enzyme has been used for the analysis of ATP activity,[24] which has important biomedical applications.[25] The present article reviews the attempts made for immobilization of glucose-6-phosphate dehydrogenase and its applications in various fields.

Immobilization and Applications of Glucose-6-phosphate Dehydrogenase

Glucose-6-phosphate dehydrogenase has been immobilized/coimmobilized on various supports (Table 1). Nylon tube supported enzymes have shown to have potential applications in automated analysis.[26] The secondary amide group of nylon can be converted to secondary imidate groups by *O*-alkylation,[27] which includes the introduction of a functional group into nylon without any depolymerization; the functional group reacts readily with amino compounds to produce a stable amidine link.[28] Hence, based on the initial reaction of the nylon imidate with bifunctional compounds

TABLE 1 Immobilization Techniques and Applications of Glucose-6-Phosphate Dehydrogenase

Source	Support for Immobilization	Application(s)	Reference
Baker's yeast	Nylon meshes	Voltammetric biosensors for determination of formate and glucose 6-phosphate	22
—	Poly (carbamoyl) sulphonate hydrogel on a Teflon membrane	Determination of glucose 6-phosphate	23
—	Clark type oxygen electrode and screen printed electrode	Amperometric ATP biosensor	24
—	Microchannels of porous alumina membrane	Study of xenobiotic metabolism	25
Yeast	Amino substituted nylon tube through glutaraldehyde and bisimidates	Automated analysis of glucose	28
Saccharomyces cerevisiae	CNBr-activated Sepharose 4B	—	30
Commercially available	Surface modified poly[bis(aryloxy) phosphazene] supported on porous alumina particle	—	33
Aquifex aeolicus	"Wired" onto the surface of graphite electrodes by using an epoxy-based poly(ethylene glycol) diglycidyl ether cross-linker.	Amperometric enzyme sensor	35
Baker's yeast (co-immobilized with glucose oxidase)	In silica hybrid film fabricated by hydrolysis of the mixture of tetraethyl orthosilicate and 3-(trimethoxysilyl) propyl methacrylate	Amperometric biosensor for detection of glucose and ATP	36
Leuconostoc mesenteroides	On glassy carbon disk electrode modified with 4-μL droplet of multiwalled carbon nanotubes and mixing enzyme and glutaraldehyde in phosphate-buffered saline	Digital sensing	38
Leuconostoc mesenteroides (co-immobilized with *p*-hydroxybenzoate hydroxylase, and hexokinase)	Clarke type oxygen electrode and on screen-printed electrode	ATP measurement	39

containing amino groups, enzyme immobilization can be carried out.[28] Hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) are used as a linked enzyme system in a highly specific assay for serum or plasma glucose[29] (Figure 1). The two enzymes were co-immobilized on an amino-substituted nylon tube through glutaraldehyde and bisimidates. Immobilization of enzyme was carried out by incubating

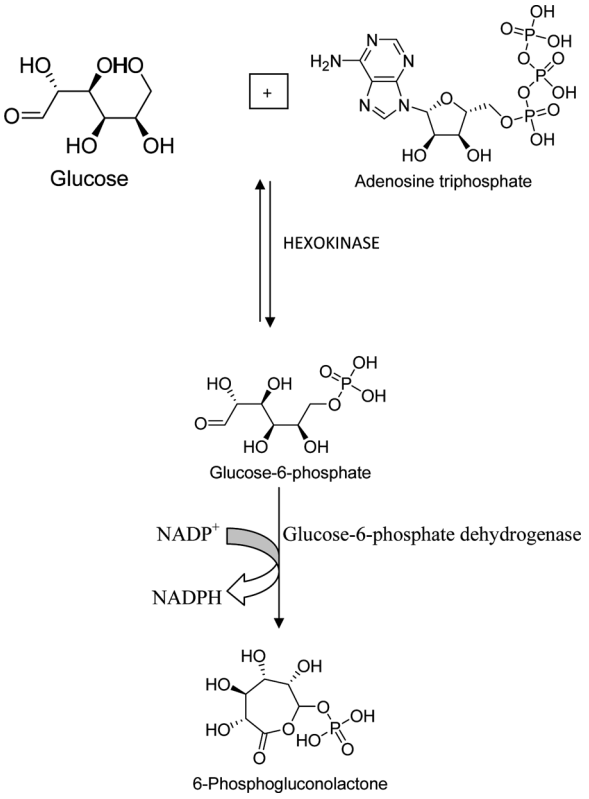

FIGURE 1 Reaction catalyzed by hexokinase and glucose-6-phosphate dehydrogenase.

the enzyme with activated amine-substituted nylon (either glutaraldehyde or bisimidate) at 4°C for 2 hr,[28] and the amount of immobilized enzyme was estimated by equating it to the amount of enzyme activity that is lost from the coupling solution.[28] The stability of the nylon tube supported glucose-6-phosphate dehydrogenase was much greater than that of the free enzyme. Despite low activity retention by glucose-6-phosphate dehydrogenase on immobilization, co-immobilized hexokinase and glucose-6-phosphate dehydrogenase on nylon tube showed excellent sensitivity and accuracy in determination of glucose.[28]

Goheer et al. (1976) immobilized the baker's yeast (*Saccharomyces cerevisiae)* glucose-6-phosphate dehydrogenase covalently on CNBr-activated Sepahrose 4B and full activity was retained over a period of 4 months.[30] Stable enzyme preparations with overall negative or positive charge were prepared by addition of valine or ethylenediamine to the CNBr-activated Sepharose 4B, and these enzyme preparations retain 40–60% activity even at higher temperatures (50°C for 15 min) and are completely stable over the entire range (25–50°C) of temperature, whereas the soluble enzyme

is inactivated under these conditions.[30] The pH optima of the positively and negatively charged enzyme were found to be 8 and 9, whereas the optimum pH of soluble and uncharged enzyme was 8.5.[30] Thus, immobilization of glucose-6-phosphate dehydrogenase on CNBr-activated Sepharose 4B had greatly increased the stability of the enzyme. The enzyme was also immobilized on CNBr-activated Sephadex G-25 but the preparation was unstable when compared to CNBr-activated Sepharose 4B.[30] Glucose-6-phosphate dehydrogenase has been insolubilized to glass beads by covalent bonding, and a batch recirculation reactor relatively free from concentration gradients and diffusion limitations has been used to determine the reaction rate.[31] A co-immobilized hexokinase and glucose-6-phosphate dehydrogenase method was assessed for D-glucose analysis on the Technicon SMAC[32] and the enzyme-containing coils were usable for 1 month for 12,000 tests. The method used on SMAC is judged to perform acceptably relative to the National Glucose Reference Method in the context of method usefulness.[32]

Glucose-6-phosphate dehydrogenase has also been covalently linked to a surface-modified poly [bis(aryloxy) phosphazene] supported on porous alumina particles.[33] Poly(diphenoxyphosphazene) was surface nitrated and then reduced to the aminophenoxy derivative. The aminophenoxy sites were then activated by reaction with cyanogens bromide, nitrous acid, or glutaric dialdehyde.[33] For immobilization of enzyme the amino groups were allowed to react with glutaric dialdehyde in a buffer solution of pH 1.4.[33] The activated support was then treated with glucose-6-phospahte dehydrogenase in HEPES buffer solution at pH 7.5.[33] The immobilized enzyme was found to retain a high degree of activity for 2150 hr, which was much greater than for free enzyme, which was only active for 260 hr.[33]

Biosensors have a significant impact on field monitoring in environmental analysis as they are reliable and can be made available at low cost. Inorganic phosphate is used for the measurement of eutrophism and is an analyte of interest for environmental monitoring with the help of biosensors. Keeping this in view, a reagentless enzymatic phosphate sensor was prepared by Parellada et al.[34] with a configuration based on the sequential action of three enzymes, phosphorylase A (converts glycogen to glucose-1-phosphate), phosphoglucomutase (converts glucose-1-phosphate to glucose-6-phosphate), and glucose-6-phosphate dehydrogenase (oxidizes glucose-6-phosphate in the presence of NAD^+ producing NADH), on the polymeric nature of co-substrate, and on introduction of an osmium-based mediator catalyzing the reversible oxidation of NADH.[34] NADH produced is again oxidized electrocatalytically by an osmium phendione mediator. Level of phosphate can be monitored, as the oxidation current of the mediator is proportional to phosphate concentration at a working potential of + 200 mV versus SCE.[34]

Voltammetric biosensors for the determination of formate and glucose 6-phosphate based on the measurement of dehydrogenase-generated NADH and NADPH were prepared by using immobilized formate dehydrogenase, glucose-6-phosphate dehydrogenase, ascorbate oxidase, and uricase.[22] The immobilization was carried out by mixing the enzymes with glutaraldehyde and bovine serum albumin in a disk of nylon mesh.[22]

Iyer et al. (2003) have developed an amperometric enzyme sensor capable of operating at high temperatures by utilizing a thermostable glucose-6-phosphate dehydrogenase from the hyperthermophilic bacterium *Aquifex aeolicus* "wired" on a surface of graphite electrodes by using an epoxy-based poly(ethylene glycol) diglycidyl ether cross-linker.[35] Response of the system was monitored through detection of the cofactor NADH, which was electrocatalytically reoxidized by a redox mediator osmium (1,10-phenanthroline 5,6-dione)$_2$-poly(4-vinylpyridine) at potential of +150 mV versus Ag/AgCl/KCl$_{sat}$.[35] The stability of the sensor is found to be much better (up to a temperature of 83°C) than the conventional system utilizing a mesophilic glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*.[35]

The use of a sol–gel technique for immobilization of enzyme and development of optical and electrochemical biosensor has received widespread attention. Co-immobilization of glucose oxidase and hexokinase/glucose-6--phosphate dehydrogenase in silica hybrid film fabricated by hydrolysis of a mixture of tetraethyl orthosilicate and 3-(trimethoxysiyl)propyl methacrylate was investigated for the development of amperometric biosensors.[36] Silica hybrid sol gel provided an excellent matrix for enzyme immobilization and the immobilized enzyme retained its bioactivity effectively.[36]

Glucose 6-phosphate biosensor based on co-immobilized *p*-hydroxybenzoate hydroxylase and glucose-6-phosphate dehydrogenase was also reported by Cui et al. (2006).[37]

A glucose-6-phosphate dehydrogenase- and salicylate hydroxylase-based Clark type electrode was also reported for interference-free determination of glucose 6-phosphate.[23] The enzymes were immobilized by entrapping them in a poly(carbamoyl) sulfonate hydrogel on a Teflon membrane. The enzyme glucose-6-phosphate dehydrogenase catalyzes dehydrogenation of glucose 6-phosphate, producing NADH. The NADH produced initiates irreversible decarboxylation and hydroxylation of salicylate in the presence of oxygen by salicylate hydroxylase, which results in a detectable signal due to the consumption of dissolved oxygen by salicylate hydroxylase for the measurement of glucose 6-phosphate.[23] The Teflon membrane used to fabricate the sensor avoids the interference from different amino acids and electroactive substances.[23]

Two types of amperometric ATP biosensor based on the co-immobilization of salicylate hydroxylase, glucose-6-phosphate dehydrogenase (G6PD),

and hexokinase are also reported.[24] The immobilization was carried on a Clark-type oxygen electrode and on a screen-printed electrode.[24] Covalent immobilization of human CYP2E1 and glucose-6-phosphate dehydrogenase was carried out in the microchannels of porous alumina for the xenobiotic studies.[25] A system was developed by stacking two independently modified membranes of silane–glutaraldehyde and silane–maleimide containing immobilized G6PD and recombinant human cytochrome CYP2E1, respectively, and a two-step enzyme reaction was designed by placing the membranes in a fluidic device at fast flow rates. Furthermore, the proposed system exhibited fast response allowing sufficient substrate transformation even in such short resident times of the order few seconds.[25]

Glucose-6-phosphate dehydrogenase was reported to immobilize on a glassy carbon disk electrode modified with a 4-μL droplet of multiwalled carbon nanotubes and mixing enzyme and glutaraldehyde in phosphate-buffered saline.[38] An enzymatic AND-gate based on electrode-immobilized glucose-6-phosphate dehydrogenase is used to catalyze an enzymatic reaction and the logic function is considered in the context of biocatalytic processes utilized for the biocomputing applications for "digital" sensing.[38]

Two types of ATP biosensors based on new combinations of enzymes and electrodes by using the co-immobilization of *p*-hydroxybenzoate hydroxylase, glucose-6-phosphate dehydrogenase, and hexokinase on a Clark-type oxygen electrode and on a screen-printed electrode were reported by Yue Cui (2010).[39] The principle for determination of ATP is: A phosphate group is transferred from ATP to glucose by hexokinase, forming glucose 6-phosphate, which is specifically dehydrogenated by glucose-6-phosphate dehydrogenase with consumption of $NADP^+$. The NADPH formed initiates the irreversible hydroxylation of *p*-hydroxybenzoate by *p*-hydroxybenzoate hydroxylase to consume dissolved oxygen, which results in a detectable signal on a Clark-type electrode and generate 3,4-dihydroxybenzoate. During the measurement of ATP, a detectable signal caused by the consumption of oxygen by *p*-hydroxybenzoate hydroxylase can be monitored by the Clark-type electrode and another detectable signal caused by the generation of 3, 4-dihydroxybenzoate by p-hydroxybenzoate hydroxylase can be monitored by a screen-printed electrode.[39] Both sensors show high performance characteristics with broad detection ranges, short measuring times, and good specificities.[39]

CONCLUSION

Immobilization of the enzyme was initially used only for biocatalysis, but now this is used for a variety of applications. Immobilization of glucose-6-phosphate dehydrogenase has not been a regular practice.

Co-immobilization of glucose-6-phosphate dehydrogenase has been carried out for preparation of amperometric and voltammetric biosensors for detection of ATP, glucose 6-phosphate, and so on. A vast number of methods of immobilization are currently available; however, an economical and small process of immobilization is still in demand. Independent immobilization studies of glucose-6-phosphate dehydrogenase on supports like sodium alginate, agar, gelatine, and so on may provide a pavement for biomedical applications of glucose-6-phosphate dehydrogenase for treatment of enzyme deficiency associated with a number of clinical manifestations and also for its use as biosensors. Although the progress in therapeutic use of immobilized enzyme is slow and somewhat staggered, the future prospect for application of immobilized enzyme in biosensing is promising. The current advancement in microprocessing and microelectronic devices has created a promising future for the application of glucose-6-phosphate dehydrogenase as biosensors.

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