HCTL Open International Journal of Technology Innovations and Research (IJTIR)
http://ijtir.hctl.org
Volume 14, April 2015
e-ISSN: 2321-1814, ISBN (Print): 978-1-62951-946-3

Applicability of Nanomatrices Immobilized α- amylase in Biotechnology

Om Prakash[1] , Saumya Khare[2]

sksaumya24@gmail.com[2]

Abstract

Recent advancement in nanotechnology has provided us diverse nanostructured materials for wider applications. Nanomaterials are emerging innovative field that have attracted considerable attention for the enzyme technology. The current demand is to develop and implement new technologies in order to enhance enzyme immobilization. The entrapment of enzymes in suitable matrices facilitates enhanced catalytic activity, stability, catalyst recovery, loading ability and reusability etc. These unique properties are inevitable and cost effective for large scale application in industrial biotechnology. The present review delineates some of these aspects in the field of nanotechnology for enzyme stabilization.

Keywords

Amylase, Immobilization, Nano-particles, Enzymes

Introduction

Enzymes are ubiquitous biological catalyst that has remarkable history. The rapid growth in protein engineering has provided ability to manipulate catalytic activity along with chemo selectivity, region selectivity and especially stereo selectivity which are of immense importance for the industrial applications. These generally operate under myriad of mild physico-chemical conditions. Unlike other conventional chemical catalyst, biological catalyst catalyzes the chemical reaction with lower activation energy thereby increasing the turnover number[1]. The wider applications of enzymes for various industrial purposes are generally hampered due to lower operational stability, recovery and reusability [2]. The enzymes having enhanced stability, prolonged shelf-life and reusability provide efficient application with reduced utilization of enzyme [3].These limitations of enzymes can be overcome by adopting suitable immobilization techniques.

[1,2] Department of Biochemistry, Faculty of Science, Banaras Hindu University, U.P., India. 221005, oprakash01@yahoo.co.in[1]

The immobilized enzymes are more preferred in comparison with their free-counterparts. The ideal kinetic and biochemical properties of enzymes are coupled with their carrier molecule having desired chemical and mechanical properties for the efficient immobilization. These provide higher efficiency, immobilization (%) and operational stability [4]. Thus, immobilization techniques have paved the way for the successful large scale commercialization of these environmental friendly biocatalyst. Nelson and Griffin for the first time discovered immobilization of enzymes when sucrose was hydrolyzed by invertase absorbed to charcoal surface [5].However, immobilized enzymes are being used since 1966 for the industrial application [6].

Recent breakthroughs in nanotechnology have emerged as a new area of fundamental sciences. Extensive research has made various nanostructures affordable for the efficient enzyme immobilization by offering versatile and excellent matrice for the purpose. The major goal of nano imobilization is to achieve better enzyme recovery, operational stability and reusability.In current scenario nanoparticles stabilized enzymes are receiving global attention. In the last couple of years, there are many reports relevant to fabrication of nanoparticles for the enzyme immobilization [7-10].

With the upsurge of enzyme applications, there is increasing demand for the suitable enzyme which utilizes inexpensive production techniques [11]. The α-amylase are endo-acting glycoside hydrolases which hydrolyzes α-1,4-O-glycosidic bond present in polysaccharides to produce glucose, maltose and maltotriose units [12]. These are second most important industrial enzyme which accounts for 30% of the world's production. Their major applications are in the starch based industries and in detergent formulations. Although amylase is immobilized on various matrices, the primarychallenge is to develop nanoformulation based enzymatic immobilization techniques. So the present review is intended to discuss some of these aspects of nanoparticles for the enzyme immobilization.

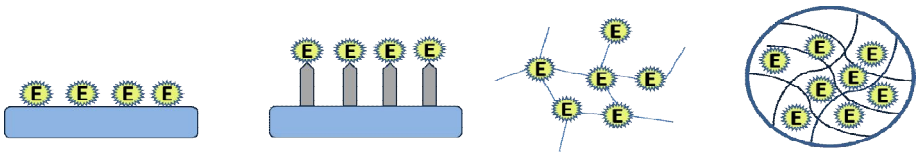
Methods of enzyme immobilization

In the last few decades, immobilization techniques are rapidly growing but it still needs some further advancements. The term 'immobilization' can be referred as confinement of enzyme molecules in a certain space with retention in enzyme activity and ease to reuse. Immobilization reduces the protein contamination of the product. These also provide facile route for the enzyme separation along with their desired storage and operational stability [13-16].The enzyme stability is time and temperature dependent. The retention in enzyme activity through a series of cycles can be attributed to the immobilization processes. The catalytic activity of enzyme changes according to the support matrices. The properties of immobilized enzyme may be altered when enzyme and substrate reacts in the microenvironment as compared to the bulk environment. These changes occurs due to alteration in the three dimensional structure of protein (enzyme) when linked with the support matrices [17,18].

In general, there are three principle methods for the enzyme immobilization. This includes binding of enzyme to suitable support or carrier, cross-linking and entrapment or encapsulation [19,20], [Fig.1].These approaches of immobilization, possess their own advantages and disadvantages [21].The binding of enzyme to a support is achieved through various physical and chemical methods [22].The materials such as activated

charcoal, alumina, ion exchange resins, hydroxyapatite, silica, chitin, chitosan,alginate etc., are used for adsorption. In physical method weak interaction (van der Waals forces, hydrogen bonding and ionic interactions) occur between enzyme and the adsorption surface [23, 24]. Since covalent binding of the enzyme to the support is much stronger than an ionic binding, therefore,it prevents leaching of enzyme from the surface [21].

Immobilizing an enzyme via crosslinking is a carrier free process. These involve aggregation of enzyme molecules to form a large three dimensional structures [25].The covalent binding generally occurs between enzyme and bifunctional cross-linker such asglutaraldehyde.The cross-linked enzyme provides poor mechanical stability, reproducibility and lowretention of activity. However, in recent years cross-linked enzyme aggregates (CLEA) have emerged as a novelmethod for the carrier free immobilization.



A: Physical adsorption B: Covalent binding C:Crosslinking D: Entrapment
Figure 1: Common methods of immobilization.

Entrapment is caging of enzyme molecules in a network of gel or fibre by covalent or non-covalent bonds [26].The problem of enzyme leakage can be prevented by controlling the pore size of the polymeric network which allows the free diffusion of substrates and products [27,28].These provide high enzyme loading, offering cost-effective and fast process of immobilization with less mass transfer. Entrapment can be achieved by entrapping an enzyme molecule e.g. via sol-gel matrices. Moreover, the additives such as polyethylene glycol, polyvinyl alcohol, and albumin play a key role in enzyme stabilization [29]. Meanwhile, entrapment via nanostructured supports such as electrospun, nanofibers and pristine materials have provided a wealth of diverse applications [30].

Role of nanoparticles in enzyme stabilization

New frontiers in nanotechnology have manifested their immense role in biotechnological processes. In comparison with traditional immobilized enzyme carrier, nanostructure offers stable immobilization. In present era, several nanostructures such as nanotubes, nanosphere, nanocomposites, nanoparticles [NPs] etc., have received considerable attention as enzyme carrier. Owing to intrinsic large surface area, extreme small size and electronicproperties of NPs, offer effective enzyme loading. The nanostructured materials have great potential to control the environment of enzymes to nanoscale level [21].These nanoparticles have their profound applications for immobilizing various biomolecules such as proteins, enzymes, antibodies, and anticancer agents, etc.[31].The present review reveals the immobilization of amylase onto polymeric and metallic NPs. These nanoimmobilized enzymes also have various biotechnological applications [Table 1].

Silver Nanoparticles

The noble silver nanoparticles offer diverse applications in optics, electronics, bio-labelling, antimicrobials, sensors and catalysts [32]. The Ag-NPs revealed its major application for the enzyme immobilization which relies on its ideal characteristics [33,34].There are many reports relevant to amylase immobilized silver nanoparticles. The amazing silver nanoparticle upon interaction with enzymes facilitated faster hydrolysis of

starch, for its efficient use in food industries [35].Further, similar report outlined that interaction of enzyme and Ag-NPs lead to structural changes of protein molecule and its surface modifications. This entails an increase in catalytic activity due to stabilization of enzyme on the silver nanoparticles [32]. In the presence of silver nanoparticle the rate of hydrolysis increases, ensuring that enzyme has successfully immobilized on the surface of nanoparticles [36]. The polyaniline-assisted Ag nanocomposites are reported for coupling α-amylase. The nanocomposite immobilized enzymes exhibited high thermostability with varying pH, large surface area and effective enzyme loading for starch hydrolysis and their role in food industry [37].

BSA Nanoparticles

Nowadays, bioactive polymeric nanoparticles are deliberately employed for improving enzyme immobilization. The nanoentrapment of amylase onto BSA matrix offers stable enzyme system. This favors protection against microbes for its efficient utilization in drug delivery, food and detergent industries. The encapsulation method was used for bindingα-amylase with BSA NPsusing hydrophobic ion pairing (HIP) complexation. The addition of BSA increases the rate of stabilization with improved stability and particle size to100 nm [38]. Recently BSA NPs were also used for amylase encapsulation from *Cicerarietinum* and *Pearl millet*. The chemical modification of nanoparticles is achieved by utilizing glutaraldehyde, almond oil, and n-butanol. The alkaline protease plays a vital role in controlled and sustained release of enzyme from oil driven emulsified BSA NPs. The emulsified nanoparticles show elevated encapsulation, storage stability, reproducibility and thermal stability. Moreover, these ideal properties of amylase encapsulated into emulsified BSA NPs have their excellent role in detergent formulations [39,40].

Magnetic nanoparticles

Beside various other supports, magnetic nanoparticles are extensively used for the enzyme stabilization. Application of magnetic nanoparticles has given prominence in biomedical field for drug delivery, hyperthermia treatment, cell separation, biosensors and enzymatic assays[41,42] including biocatalyst applications,[43] environmental remediation etc. The magnetic nanoparticles have certain advantages such as lack of toxicity, biocompatibility, and most interesting their magnetic properties offering small size [44].This provides large surface area allowing easy recovery and reusability for the immobilized enzymes [45].

Currently, many enzymes including amylase are immobilized onto magnetic nanoparticles [45,46]. Magnetic nanoparticles basedon magnetite (Fe_3O_4) and maghemite (Fe_2O_3) are employed for the enzyme stabilization. The covalently bonded amylase onto Fe_3O_4 magnetic nanoparticles were used for immobilization via carbodiimide activation. These covalently attached enzyme and support modification involved multiple reaction steps for effective enzyme activity [47].The porcine pancreatic α-amylase immobilized onto magnetic (Fe_2O_3) NPs enhances the starch hydrolysis. This occurred due to improved catalytic activity of enzyme bound to magnetic nanoparticle with higher thermal stability [48].The novel approach is adopted for preparation of magnetic CLEAs of alpha amylase via amino functionalization of magnetite nanoparticles. These aggregated magnetic CLEAs are easily separated with efficacy from reaction mixture using magnetic field.

Efficacious thermal, storage stability and reusability of magnetic CLEAs opens an alternative way for the stable CLEAs preparation [49].

Silica nanoparticles

The enzymes stabilization on solid supports is of great interest. Among various inorganic materials, silica are efficiently exploited for enzyme immobilization. The promising application of α-amylase immobilized on silica nanoparticles in detergent formulation were achieved with enhanced storage stability. The nm size of silica and similar sizes of enzyme molecules provided high surface areas, ordered structures, high stability to chemical and mechanical forces, and resistance to enzymatic attack [50]. The silica and laponite nanoparticles upon interaction with α-amylase were also used for removing starch based soil [51].The colloidal silica NPs and functionalization of silica NPs via3-aminopropyl is also reported for amylase immobilization [52, 53].

Conclusion

The recent development in nanotechnology has given new opportunities for enzyme immobilization. Although, the use of chemical catalyst has been replaced by enzymes for industrial purposes, commercialization of immobilized enzymes is still a limiting factor. For industrial point of view, reusability and operational stability are key factors for its efficient application. The extensive array of research on enzyme stabilization has enabled advanced techniques to face major challenges, such as improved operational stability and reusability in order to reap maximum advantages.The rigorous efforts are dedicated for stabilizing various enzymes on nanomatrices. Nanoparticles offers highly efficient andcost-effective enzyme carrier with promising applications in various industries.The alpha amylase immobilized onto surface of nanoparticles, offers enhanced storage stability, thermostability and reusability which promises its potential application in food and detergent industries.

Table 1:Application of nanoimmobilized α-amylase

S No.	Type of nanoparticle	Method of attachment	Application	Reference
1	AgNPs	Stabilizatio+ n of enzyme by thiol linkage	Rapid degradation of starch	[35]
2	Polyaniline assisted AgNPs	Covalent coupling of enzyme with PANI/Ag nanocomposite	Starch hydrolysis	[37]
3	BSA NPs	Encapsulation (hydrophobic ion pairing complexation)	Starch hydrolysis	[38]
4	Coconut oil driven emulsified BSA NPs	Encapsulation	Additive in washing detergents	[39]
5	Magnetic Fe_2O_3 NPs	Adsorption	Starch hydrolysis	[48]
6	Si NPs	Physical adsorption	Laundary detergents	[50]
7	3- aminopropyl functionalized Si NPs	Crosslinking	Starch hydrolysis	[53]
8	Silica and laponite NPs	-	Cleaning performance of enzyme toward starch soil	[51]

References

[1] Bairoch, TheEnzyme database in 2000. Nucleic Acids Res. 28, 304–305 (2000)

[2] M. Hartmann and D. Jung. J. Mater. Chem. 20, 844-857 (2010)

[3] J. Kim, J. Grate and P.Wang. Chem. Eng. Sci. 6, 1017-1026 (2006)

[4] W. Tischer and V. Kasche. Trends Biotechnol. Aug, 17, 8, 326-35 (1999)

[5] J. M. Nelson and E. G. Griffin. J. Am. Chem. Soc. 38, 5, 1109-1115 (1916)

[6] T. Tosa, T. Mori, N. Fuse and I. Chibata. Enzymologia. 31, 214-224 (1966)

[7] M. Soleimani, A. Khani and K.Najafzadeh. J. Mol. Catal. B: Enzym. 74, 1-5 (2012)

[8] S. Demir, S. B. Gok and M. V. Kahraman. Starch/Starke. 64, 3-9(2012)

[9] M. J. Khan, Q. Husain and S. A. Ansari. Appl. Microbiol. Biotechnol. 97, 1513-1522 (2013)

[10] G. A. Petkova, K. Zaruba, P. Zvatora and V.Kral. Nanoscale. Res.Lett. 7, 287 (2012)

[11] A. Pandey, P. Selvakumar, C. R. Soccol and P. Nigam. Curr. Sci. 77, 149-162 (1999)

[12] M. M. Dehkordi and F. A. Javan. Journal of Biology and today's world. 1, 1, 39-50 (2012)

[13] R. A. Sheldon. Biochem. Soc. Trans. 35, 6, 1583-1587 (2007)

[14] P. Asuri, S. S. Karajanagi, E. Sellitto, D. Y. Kim, R. S. Kane and J. S. Dordick. Biotechnol. Bioeng. 95, 5, 804-811 (2006)

[15] N. Saifuddin, A. Z. Raziah and A. R. Junizah. J. Chem. 2013,676815 (2012)

[16] N. R. Mohamad, N. H. C. Marzuki, N. A. Buang, F. Huyop and R. A. Wahab. Biotechnol. Biotechnol. Equip. 29, 2, 205220 (2015)

[17] M. Sharma, V. Kumar and C. S. Pundir. Indian J. Biotechnol. 7, 328-332 (2008)

[18] S. Nisha, S. ArunKarthick and N. Gobi. Che. Sci. Rev. Lett. 1, 3, 148-155 (2012)

[19] A. A. Homaei, R. Sariri, F. Vianello and R. Stevanato, J. Chem. Biol. 6, 185-205 (2013)

[20] R. A. Sheldon and S. V. Pelt. Chem. Soc. Rev. 42, 6223-6235 (2013)

[21] E. T. Hwang and M. B. Gu. Eng. Life Sci., 13, 1, 49-61 (2013)

[22] U. Hanefeld and L. Veum. Chem. Commun. 8, 825-831 (2006)

[23] D. Brady and A. Jordan. Biotechol. Lett. 31, 1639-1650 (2009)

[24] T. Jesionowski, J. Zdarta and B. Krajewska. Adsorption.20, 801-821 (2014)

[25] T. Xie, A. Wang, L. Huang, H. Li, Z. Chen, Q. Wang and X. Yin. Afr. J. of Biotechol. 8, 19, 4724-4733 (2009)

[26] B. D. Singh. Biotechnology expanding horizons. Kalyani, India (2009)

[27] D. Dinelli, F. Morisi, S. Giovenco, and P. Pansoli, UK patent No.1,361,963 (1974).

[28] V.Varalakshmiand D. S. Prasad. JECET. 2, 3, 840-853 (2013)

[29] R. R. Naik, M. M. Tomczak, H. R. Luckarift, J. C. Spain and M. O. Stone. Chem. Commun.15, 1684-1685 (2004)

[30] S. Datta, L. R. Christena and Y. R. S. Rajaram. 3 Biotech.3, 1-9 (2013)

[31] K. J. Dussan, O. H. Giraldo, and C. A. Cardona, Editors. Application of magnetic nanostructures in biotechnological processes: biodiesel production using lipase immobilized on magneticcarriers. Proceedings of the European Congress of Chemical Engineering, (2007) September; Copenhagen, Denmark

[32] V. Ernest, M. J. Nirmala, S. Gajalakshmi, A. Mukherjee and N. Chandrasekaran. Journal of Bionanoscience. 7, 1-5, (2013)

[33] L. S. B. Upadhyay and N. Verma. Bioprocess. Biosyst. Eng.37, 2139-2148 (2014)

[34] G. A. Petkova, K. Zaruba, P.Zvatora and V. Kral. Nanoscale Res. Lett. 7, 287 (2012)

[35] V. Ernest, P.J. Shiny, A. Mukherjee and N. Chandrasekaran. Carbohydr. Res. 352, 60-64 (2012)

[36] M. Falkowska and E. J. Molga. Pol. J. Chem. Tech. 16, 3 (2014)

[37] M. J. Khan, Q. Husain and S. A. Ansari. Appl.Microbiol.Biotechnol.97,1513-1522 (2013)

[38] S. K. Raja Namasivayam, S. kumar and S. Stanley. Global Journals Inc. (US) (2014)

[39] K. Rani and C. Chauhan. World J. of Pharm. and Pharm. Sci.3,12 (2014)

[40] K. Rani, C. Gupta and C. Chauhan. AJPCT.2321-2748 (2015)

[41] Q. A. Pankhurst, J. Connolly, S. . K. Jones, and J.Dobson. J. Appl. Phys. D 36,167-181(2003)

[42] P. Tartaj, M. D. P. Morales, S. Veintemillas-Verdaguer, T. Gonzalez-Carreno and C. J. Serna. J. Phys. D. Appl. Phys 36, 182-197 (2003)

[43] L. Zeng, K. Luoand Y. Gong.J. Mol. Catal. B. Enzym. 38, 24-30 (2006)

[44] P. A. Johnson, H. J. Park, and A. J. Driscoll. Enzyme Stabilization and Immobilization: Methods and Protocols, Methods in Molecular Biology, Edited S. D. Minteer (2011), vol. 679, pp 183-191.

[45] D. A. Uygun, N. Ozturk, S. Akgol and A. Denizli. J. Appl. Polym. Sci. 123, 2574-2581, 2012 (2011)

[46] A. K. Johnson, A. M. Zawadzka, L. A. Deobald , R. L. Crawford and A. J. Paszczynski. J Nanopart Res.10, 1009-1025 (2008)

[47] R. Jalal, Z. MortazaviMilani and E. K. Goharshadi. Editors. Immobilization of α-amylase onto magnetic nanoparticles by shaking method. 1st National Iranian new chemistry congress, (2011) May 5-6, Shriaz.

[48] M. J. Khan, Q. Husain and A. Azam. Biotechnol. Bioprocess.Eng. 17, 377-384 (2012)

[49] S. Talekar, V.Ghodake, T.Ghotage, P. Rathod, P. Deshmukh, S. Nadar, M. Mulla and M. Ladole. Bioresour. Technol. 123, 542-547 (2012)

[50] M. Soleimani, A. Khani and K. Najafzadeh. J. Mol. Catal. B: Enzym. 74, 1-5 (2012)

[51] W. P. Lee, A. Martinez, D.Xu, A. Brooker, D. W. York and Y. Ding.Particuology. 7, 459-465 (2009)

[52] G. R. Behbehani, M. Soleimani, A. Khani, L. Barzegarand S. Bagheri. Pak. J. Chem.(2012)

[53] S. Kumar and S. K. Khare. Enzyme Res. (2015).

Authors

Dr. Om Prakash is presently working as Professor of Biochemistry in the Department of Biochemistry, Banaras Hindu University, India. He did his M.Sc (1974) and Ph.D. (1978) from Banaras Hindu University. His current area of research is enzymology and enzyme technology. He has successfully worked on purification and immobilization of Urease and Amylase for analytical applications. He has organized four seminars/ symposia.

He is also a life member of "Society of Biological Chemists, India", Bangalore, "The Indian Society of Agricultural Biochemists", Kanpur and "Society for Plant Biochemistry and Biotechnology", New Delhi. He has been awarded Honorary Fellowship (FISAB) in 2006 and excellence award of the Indian Society of Agricultural Biochemists at ICBFH 2010. He has also served as Course Coordinator, 2nd Refresher course in Life Sciences (Interdisciplinary), organised by UGC Academic Staff College, B.H.U. during November 19 to December 9, 2013. He has published one book and more than 60 research papers in various journals of national and international repute.

Saumya Khare is pursuing Doctorate degree in Biochemistry at Banaras Hindu University. She has qualified GATE (2013). She has obtained her postgraduate degree in Botany (2012) from Banaras Hindu University. Her present work is on Physico-chemical characterization and immobilization studies of amylase.