Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

Proteases Immobilization and Pharmacological Aspects of Matrix Metalloproteinases

Jyoti Sarwan[1],Priyanka Pandey[2], Dr. J.C.Bose[3]

[1]Department of Biotechnology, Chandigarh University, Mohali

[2.] Amity University

Email- jagadeesh.e8748@cumail.in

Abstract

The immobilization of proteases can lead to several approaches like easy handling and recovery from a particular reaction medium and can be reused the enzyme in the continuous processing. Proteases also are being used in several application that is treatment of cancer, on antitumoral cells , for swelling and immune modulation like disorders and for digestive issues. Proteases have ability to survive in extremely high critical conditions that is why proteases are being used in industrial level as known as such crucial enzymes. Covalent binding can be used to immobilized the Proteases enzymes through an irreversible enzyme immobilizations.[Ali et al., 2016] To maintaining the stability of an enzymes is the most important part of the immobilization of an enzyme, it may create several problematic situations and sometimes halt to process too. Matrix metalloproteinases (MMPs) are also protease types, which are growing with their multiple uses. Several articles have been evidence for the MMPs roles. This review discuss with the structure and functions of the different MMPs. Mainly, the crisp points of the MMPs is signal transduction pathways, along with MMPs non- matrix degradation functions, in case of apoptosis. Inspite with MMPs complicated role with its physiology, we are focusing with therapeutics target. Its specificity to the inhibition is little bit difficult, but homeostasis play a great role in its functionality.

INTRODUCTION

Proteases can be defined as the enzymes that are required to degrade amino acids and peptides which are catalyzed hydrolytic biochemical or chemical reactions. The proteases enzymes have larger applications in Pharmaceutical as well as Industrial sector worldwide. Proteases can be extracted from several sources like soil, fruits, sunflower leaves and seeds etc. It was recorded that about 60 % of Proteases enzymes are involved in global market. Proteolytic enzymes are widely used in industries because they can be active upon variant range of temperature, pH, high stability and broad site specificity. The immobilization of proteases can lead to several approaches like easy handling and recovery from a particular reaction medium and can be reused the enzyme in the continuous processing. Proteases also are being used in several application that is treatment of cancer, on antitumoral cells , for swelling and immune modulation like disorders and for digestive issues. Proteases have ability to survive in extremely high critical conditions that is why proteases are being used in industrial level as known as such crucial enzymes. Covalent binding can be used to immobilized the Proteases enzymes through an irreversible enzyme immobilizations.[Ali et al., 2016] To maintaining the stability of an enzymes is the most important part of the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

immobilization of an enzyme, it may create several problematic situations and sometimes halt to process too. Some supports may be given to the enzyme to attaining covalent coupling of bonds, thermal stability of immobilized enzymes and leads to increase in half life of the enzymes. It is most vital part to be required in the reaction to maintaining both structural and functional properties of the elements that are being used on the commercial level. The support material plays the main role in the process of immobilization of the enzymes because a suitable support leads to the continues processing, it should be non reactive to the particles present the reaction, it should has high remaining activity , should be a ideal support to the enzyme and must be a cheapest as well easily available. The supports can be required in the reaction process usually, gamma alumina, cellulosic maxtrices , chitosan and alginates beads as capture enzyme.

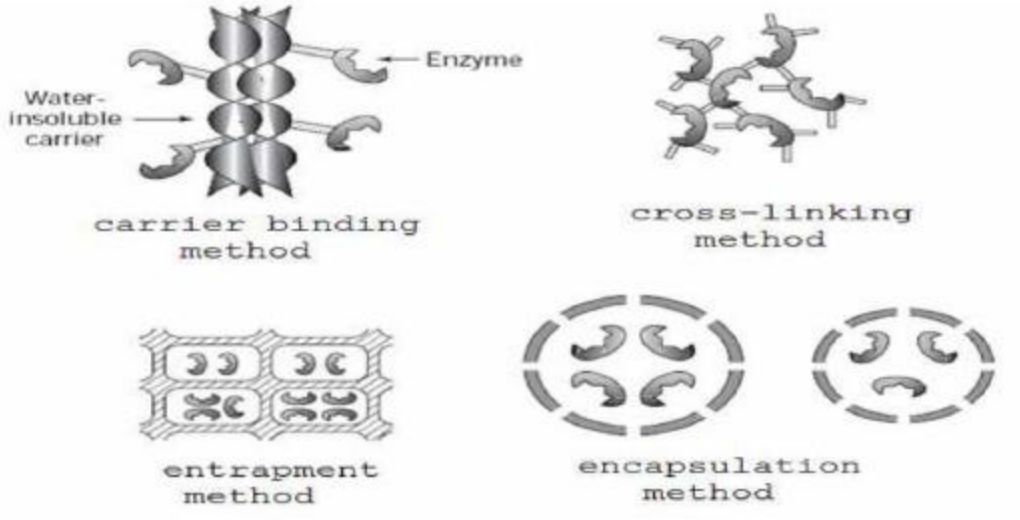

Fig.1. [Maqtari et al., 2019] It represented the encapsulation procedure of enzyme in immobilization.

Matrix Metalloproteinases are also called as matrixins or matrix metallopeptidases. They belong to the metzicin (larger families of proteases) super families (1), which consists of endopeptidases (2), serralysins, adamalysins and astacins. As name indicates, they are multi protein domain molecule, monitored by the Tissue inhibitors of metalloproteinases. Their dependent cofactors are metal ions which play lead role in regulating the degradation process of developmental DNA sequence and extracellular matrix. (3). Their central physiological functions are- wound healing, morphogenesis, tissue remodeling (3). In case of tissue remodeling process, they respond to the tissue injuries, in arthritis, cancer, myocardial infarction and chronic tissues ulcers. Their role can also be observed in – activating multiple biological active molecules, which can further degrade the extracellular matrix proteins; inactivating the cytokine/ chemokines (4); regulating the cleavage process of cell surface receptors; cellular behaviours (migration process like-adhesion and dispersion proliferation, angiogenesis, differentiation, host defense mechanism and apoptosis. The mechanism of MMPs activity at the extracellular matrix can be explained through cell surface docking as they provide the tether activity of MMPs in between the extracellular matrix (remodeling of extracellular matrix site) and cellular region. The process involves in the MMPs proteolytic activity through the adhesion receptor regulation. Collectively, it helps in extracellular

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

degradation as well as scaffolded ECM proteins (partially degraded) preservation, which may further assist in generating surviving signals via ease the emigration of ECM degradation(8).

IMMOBILIZATION

Immobilization with Sodium alginate gluteraldehyde: It is the most earliest method to encapsulate the enzyme and have been used for several years in industrial sectors. Calcium alginates or sodium alginates beads are generally prepared in which enzyme is being encapsulated. The solution of sodium alginate with 2% (w/v) is required to mix up with 200nM of solution of calcium chloride , sodium acetate of 25mM and 6.5% of gluteraldehyde to make final pH about 6.5 in continuous stirring. The beads will formed after the final solution has to be left for 12 hours at 9^{0} C for make appropriate hardening of gel beads. The following prepared beads has to be washed with distilled water so removal of excess gluteraldehyde can takes place and the activated alginated beads can be reacted with enzyme to encapsulate enzyme in that activated beads. This continues stirring of the reaction jar or flask lead to proper encapsulation of the enzyme in the reaction process. [Ali et al.,2016]

Immobilization with Gelatin alginate gluteraldehyde: The gel beads can be prepared using withsome previoushave been using for several years. The process of preparing of beads involving ,1g gelatin powder, 1g sodium alginate, 25mM of sodium acetate buffer and 5ml of glyerol to make pH of 4.5 of the solution at 50^{0} C. Whenever the mixture will prepare the next step would be dropping of mixture into iced solution having polygluteraldehyde and calcium acetate. The beads will be solidify after 12 hours at 9^{0} C. [Ali et al., 2016]

Agar immobilization of proteases : Proteases enzymes can also be immobilized by the agar method. In this method molten agar are used on the plates at 40 0 C and when agar get solidified it cuts into small pieces in the discs. 0.1M Sterile phosphate buffer at pH 7.0 will be added and distilled water will be used here for washing of excess buffer. After removal of excess buffer from the solution the agar immobilized proteases can be stored in the refrigerator at 4^{0} C for 1-2 hours. The proteases immobilized in the agar performed better on different pH values like 9, 9.5, 10, 10.5 and 11 than free enzymes.[Ellatif et al., 2020]

Nano-particle to encapsulate TEV-STRPT-Biotin –SPIONs : In this method Biotin can be labelled in the supermagnetic nano particles and this technique leads to several applications for various approaches of enzymes. The study reveals that solution of supermagnetic nanoparticles were prepared with the RayBiotech solution and these can be pelleted on magnetic separator and washing takes place with the help of PBS solution. Remove the extra PBS and transfer the pelleted beads into the conical flask. The next step involved the encapsulation of the microbial enzyme with pelleted beads and washing with PBS and incubation should be given at room temperature. The beads were then washed from PBS with 0.1% Tween-20. TEV-HALO-SPIONs: Chloroalkane functionalized nanoparticles (200 µL of a 20% slurry of macroporous cellulose encapsulated SPIONs (Promega)) were pelleted on a magnetic separator, storage solution removed by pipette, and washed with PBS (3 x 1 mL). After removal of the last PBS wash, pelleted beads were resuspended in 1 mL of PBS and transferred to a 15 mL conical tube. Clarified bacterial lysates (5 mL of a 3 mg/mL solution

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

in M-PER) were added to the beads, which were incubated at room temperature on an end-over-end rotor for 2.5 h. Beads were pelleted on a magnetic separator, lysate removed, and beads washed with PBS (3 x 5 mL; 10 min each). [Norris et al.,2019]

MATRIX METALLOPROTEASES

Structure and regulations of MMP:

❖ Matrix Metalloproteinases are also called as matrixins or matrix metallopeptidases. They belong to the metzicin (larger families of proteases) super families (1), which consists of endopeptidases (2), serralysins, adamalysins and astacins. As name indicates, they are multi protein domain molecule, monitored by the Tissue inhibitors of metalloproteinases. Their dependent cofactors are metal ions which play lead role in regulating the degradation process of developmental DNA sequence and extracellular matrix.(3)

❖ Their central physiological functions are- wound healing, morphogenesis, tissue remodeling (3). In case of tissue remodeling process, they respond to the tissue injuries, in arthritis, cancer, myocardial infarction and chronic tissues ulcers. Their role can also be observed in – activating multiple biological active molecules, which can further degrade the extracellular matrix proteins; inactivating the cytokine/ chemokines (4); regulating the cleavage process of cell surface receptors; cellular behaviours (migration process like-adhesion and dispersion proliferation, angiogenesis, differentiation, host defense mechanism and apoptosis.

❖ Controlled remodeling of the extracellular matrix (ECM) affects their functionality of the different cells or tissues with their alteration in the structure, composition and stiffness as many of the deteriorating enzymes are involved in the process. The results of which can lead to invasive cancer and fibrosis. These activities are controlled by the Matrix Metalloproteinases (MMPs) (5). They are the zinc vulnerable proteinases and constitute 23 representatives in human cells; all are capable in ECM degradation process (6).MMPs are activated through the proteolytic cleavage where they secrete zymogens inside the extracellular spaces (activated). Simultaneously, the protein cleavage process (especially by ser protease) and thiol group activation via oxidation results into the extracellular protein degradation (7).

❖ The mechanism of MMPs activity at the extracellular matrix can be explained through cell surface docking as they provide the tether activity of MMPs in between the extracellular matrix (remodeling of extracellular matrix site) and cellular region. The process involves in the MMPs proteolytic activity through the adhesion receptor regulation. Collectively, it helps in extracellular degradation as well as scaffolded ECM proteins (partially degraded) preservation, which may further assist in generating surviving signals via ease the emigration of ECM degradation(8).

❖ On the other side, some structural features of MMPs are same as ECM like- a single peptide for secretion, single catalytic domain in all members (consist of Znpp ion binding, substrate specificity conferring with carboxy terminal domain (9).

❖ There are total 5 subgroups of MMPs has been defined, they are

a) Collagenase (MMP-1, MMP-8, MMP-13 & MMP-14)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

b) Gelatatinases (MMP-2 &MMP-9)

c) Stromelysins (MMP-3, MMP-10 &MMP-11)

d) Elatase (MMP-12)

e) Matrilysin (MMP-17) (10)

❖ The activated form of the MMPs can attune the pro-form of MMP activation and degraded and inactivated protease inhibition through activation of Zymogen results into the global proteolytic potential modulation.

❖ MMPs play important role in regulating the multidirectional communication of cells and tissue homeostasis along with their immunity. In order to achieve the proper activity of the MMPs, its factors should be strictly regulated to avoid the homeostasis breakdown and further related destructive functionality.

❖ There are four levels processes through which MMPs controlled their catalytic activities. They are-

a) Transcriptional and post transcriptional gene regulation,

b) Compartmentalization,

c) Pro-domain removal with activation of pro-enzymes,

d) Specific (tissue inhibitors of matrix metalloproteinases) and no specific (□ □ □ macroglobulin) inhibition.

Gene Expressions modulation:

❖ Significant contribution mechanisms are involved in transcriptional and post-transcriptional MMPs gene expressions. Although, nitric oxide, cytokines or micro-RNA have been found to be beneficial in providing support to the post-transcriptional mRNA.

❖ Even the low levels of MMPs are individually regulated without any influencial over expressions of the chemokines, cytokines, growth factors and oncogene factors. Although in some cases implication of TNF-□ and interleukin-1can be seen.

❖ In general signal transduction of MMP gene expression regulates the MMPs promoter activities through cis element sharing however, co-regulation take place with multiple inductive stimuli, e.g. cytokines and growth factors; and co-repression through retinoid and glucocorticoid hormones (6). The promoters MMPs like- gelatinase (MMP-2/ MMP-9) collagenase (MMP-1/MMP-8) are remarkably clear in their method of activation.

❖ On the basis of cis-element configuration, there are three groups of MMPs has been defined(7) :

a) Ist group-

✓ They speak for almost promoters, constituting TATA box and AP-1 binding sites adjacent to the transcription site and PEA-3 binding site.

✓ PEA-3 binding sites regulate the MMP transcription through multiple growth factors (e.g. keratinocyte growth factors, platelet-derived growth factors, epidermal growth factors, vascular endothelial growth factors, transforming growth factors and cytokines) (8).

b) IInd group-

✓ Second groups (MMP-8,-11,-21) also contain TATA box and AP-1 binding sites. They are comparatively simpler from Ist group promoters.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

c) IIIrd group-

✓ Third group (MMP-1,-14, -28) does not constitute TATA box and AP-1 binding site as previous groups, rather they constitute Sp-1 family transcriptions factors with adjoining GC box.

❖ The regulation of the MMPs transcriptions are persuaded by epigenetic mechanisms. Epigenetic mechanisms are- acetylated histone induced chromatin remodeling/ or DNA methylation, cytosine's DNA methylation into cPg islands at promoter site, accompanied with repressive chromatin state and gene expression inhibition. Thus, MMP promoters hypomethylation results in enzyme expression enhancement observed in MMP-3 in colon cancer and MMP-9 in lymphoma cells (7) or other inflammatory disorders in osteoarthritis with MMP-3, -9,-13 expression in chondrocytes (10).

❖ Reversible histone acetylation (chief molecules- H3& H4) are directly related to the epigenetic chromatin remodeling and AP-1 proteins binding with expression of MMP-1 & MMP-13 induced by the IL-1□ □

❖ Post translational control of MMP expressions are evidenced through short miRNA (21-25 nucleotides) as well as non coding RNA. They act via mRNA degradation and translational inhibition.

Compartmentalization

❖ The pro-activation and specificity & efficiency of the proteolytic MMPs have strong impact of extracellular environment and locality within the pericellular space (11). Examples- in cell surface substrate enrolment process MMP-1 binding to a2b1 integrin, with regulation of a2 integrin interaction to both haemopexin plus linker domain of MMP-1(16), binding of MMP-9 to CD44 (17), and cholesterol binding to MMP-7 (matrilysin-1) results into the inclined substrate alteration and enhancement into the the fibronectin & pericellular laminin-332 degradation (14). Additionally, integrin a2b1 interaction with type I collagen regulate the enzyme secretion to the cell-matrix contact points (15).

Several ways to pro-MMP activation

❖ Synthesis of MMPs take place through removal of pro domain attached with zymogens (inactive pro-forms). The pro-domains anchors the "cysteine switch" lies close to the catalytic domain's borders, the residue of support the enzyme latency through interaction with zinc catalyze ions in order to prevent the cleavage and binding of the substrate (16).

❖ The MMP Zymogen activation leaves the cysteine residue through conformational change in the pro-domain and further zinc-water interaction take place to the active sites.

❖ The event initiation take place through three steps-

a. Another endoproteinase direct cleavage through pro-domain removal process,

b. Pro-domain allosteric reconfirmation, free cysteine modification (chemical modification) through reactive oxygen species or no physiological agents.

c. Auto proteolysis lead to pro-domain removal by allosteric control and free cistern reduction (11).

❖ In human, most of the MMPs (11 MMPs out of 24) are activated through intracellular processes with the help of furins or pro-protein convertases.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

❖ Furin is localized into the Golgi network and can be characterized as transmembrane substilisin proteinases (similar to the serine proteinases), that classifies the secretory pathway proteins along with secretory granules and cell surface.

❖ With appearing MMPs on the cell surface, their members start the catalytic action into the pericellular environment; remaining wait for their activation.

❖ The activating mechanism of pro-MMP is still yet to be cleared; however, in vitro mechanisms were discussed through chymase, plasmin, serine proteinases as well as other MMPs, e.g. MMP-3 & MMP-14 incubation (17). Therefore, alternate mechanisms were triggered to activate the zymogen. Activation of Pro-MMP is a step wise process, which include following steps-

a) Interference cysteine switch-zinc interaction through conformational change in pro-peptide,

b) Pro-domain removal through MMP intermediate processing (18) (19),

❖ A different mechanism for activation of zymogen is inserted through MMP molecule allostery. Therefore, molecular domain flexibility can subscribe through conformational transition of organized MMP inserted by exosites protein binding (20). It is evidenced by pro-MMP-9 incubation accompanied by □-hematin, which can further lead to the haemopexin-allosteric interaction results into the pro-domain cleavage at autocatalytic region (21).

❖ The MMP activation by reactive oxygen is driven through preferential oxidation of the thiol–zinc interaction and autocatalytic cleavage, followed by enzyme inactivation with extended exposure by modification of amino acids critical for catalytic activity, as shown in vitro for MMP-7 (22).

❖ In inflammation, phagocytes produced ROS productions which further monitor the activity of MMPs (23). However, In vivo activity of ROS in MMPs is yet to be cleared.

MMPs inhibition:

❖ It provide balance between active enzymes production and related inhibition, which further prevent the turnover of uncontrolled ECM, cell growth disregulation & migration, inflammation.

❖ Two MMPs inhibitors are TIMP & □ 2 macroglobulin. There are four MMPs family found in human, each constitutes with 190 amino acids, two domains: N-terminal & C-terminal which stabilization take place by three preserved disulfide bonds. N- Terminal is self-capable in inhibiting MMPs via catalytic zinc atom chelation, whereas, C-terminal function is no fully understood, except binding to a haemopexin domain. On the other side, □ 2 macroglobulin constitute of 725 homotetrameric macromolecule which is capable enough in almost endopeptidase categories, through enzyme entrap process, whose complexes are dissolved through endocytosis mediated by LDL receptor related protein-1. In blood and tissues, its role can easily see broad spectrum proteinase inhibitors. (24).

❖ Usually, MMPs can be inhibited by almost TIMPs, but, specificity differences can be seen.

a) The interaction of pro MMP-2 can easily seen with TIMP-2, -3 & -4; whereas, pro MMP-9 interaction with TIMP-1 & -3 (25); exception can be seen with pro MMP-2 which also interacts with TIMP-2 in activation mechanism. Biological relevance is still unclear with these complexes.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

b) The inhibition potency of MMP-19 and other membrane bounds (-14, -16, -24) are low with TIMP-1 whereas, more can be seen with MMP-3 & -7; which inhibition is low with TIMP-2, -3 (25).

c) Its expression can be seen in both manners constitutive as well as inducible as TIMP-2 can inhibits all MMPs due to its constitutive properties an so can be seen with TIMP-3 with its inhibitory profile of disintegrin & metalloproteinases (ADAMs).

❖ In inflammation, phagocytic immune cells derived ROS in higher concentration and are proven to be very potential in blocking all MMPs activities (11).

MMPs: molecular domain organization

❖ The MMPs constitutes of different families of enzymes, domain structures, which together participates with typical properties. On the basis of their substrate preference and domain structures, they can classified into 6 categories:

a) Collagenase
b) Stromelysins
c) Gelatinases
d) Matrilysins
e) Membrane type MMPs
f) others

❖ Synthesis of almost MMPs take place with inactive pro-enzymes, exception can be seen in case with membrane bound MMPs. The structures of al MMPs have similar configuration with N- and –C terminals, three domain conservations. In secretory pathways, N terminal with its catalytic domain and 80 amino acids, cleaved during transportation. The pro-domain constitutes PRCGxPD consensus sequence of “cysteine switch”. This domain further interacts in between catalytic domain (160-170 amino acids) and zinc ion.

❖ The gelatinases metalloproteinases (MMP-2 & -9) consist of three unique fibronectin type II molecules in respective catalytic domain, in order to obey the collagen binding domain. Collagen I constitute higher affinity of □-1 chain which further engender the collagen triple helix unwinding. On the other side, haemopexin domain of the C-terminal provides MMP-1, -8, -13, and MT-1 does not persuade any conformational change with the collagen fibrillar molecules, they easily leads to the cleavage site specific recognition with □-chain, which further give rise to three quarter N-terminal and one quarter C-terminal with conversion in the .body temperature. Haemopexin domain is also important in substrate recognition in case of TIMP interaction.

❖ In between the catalytic and haemopexin domain, flexible linker region take part cleavage process of enzymes through catalytic domain positioning towards the substrate.

❖ Furins or Pro- protein convertase activates almost one-third of the human MMPs through pro-peptide cleavage. These pro-catalytic domains of the furin consensus splitted during their passage of trans Golgi bodies.

❖ On the other hand, membrane type MMPs (MMP-14, -15, -16 & -24) are persuaded into transmembrane domain of plasma membrane and constitutes with ctosolic domain; whereas, MMPs -17 & -25 anchored by Glycosylphosphatidylinositol (GPI) of plasma membrane.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 3604 - 3615

Received 15 December 2020; Accepted 05 January 2021.

Application of Proteases on Commercial level [Ellatif and Abdelgalil 2020]

Industrial application of proteases	
Oil and fat industry	Large scale production of margarine.
Detergent	Protein stain removal
Starch and fuel	Yeast nutrition fuel
Food	Milk clotting, infant formula, flavor
Baking	Biscuits and cookies
Pulp and paper	Biofilm removal
Leather	Unhearing and beating
Textile	Degumming, texture development
Bio remediation	Waste treatment
Pharmaceuticals	Anti cancer and anti inflammation
Peptide synthesis	Enantioselective peptide synthesis
Therapeutics	Proteases mediated treatment, digestive aid , treat burns and wounds, dissolve thrombus, antimicrobial properties, to removal of scars , regenerate epithelia, preparation of vaccine for dermatophytosis therapy, silver recovery from x ray and photographic films.

CONCLUSION:

Multidirectional communications have been integrated in case of MMP with its role of immunology, cellular homeostasis, proliferations and differentiation, and so on. Homeostasis breakdown due to unregulated MMPs can interfere with the its physiology as well as pathology; hence, in order to get the gene expression regulation, transcriptional and other epigenetic; pro-enzymes activation and inhibition ratios are very important. In addition, if we discuss about the pathological conditions like, cancer and inflammatory diseases; even small groups have their own importance as they can regulate the whole gene expressions and also can also be enhanced the condition by targeting the antibodies blockers as well as selective inhibitors. However, the correlation of MMPs in different pathological conditions is still need to be explored.

Received 15 December 2020; Accepted 05 January 2021.

REFERENCES

[1]. Ali S, Sayed-EL, Azzouny A, immobilization and optimization of lettuce of alkaline proteases; 2016,7[5], page no 425-434

[2]. Elltif. A. S, Abdelgalil. A, Soliman. A, Amhed S, Encapsulation of Protease enzyme for domestic application; 2020, Vol 61, page no 117 -128.

[3]. Maktari .A.M, Naji. M. K, Ali .K.L, Extraction and immobilization of Proteolytic Enzymes from local Yemini Beans Seeds; 2019, Vol 5 [1], page number 1-7.

[4]. Noris .L.J, Patel .T, Cope. T, Covalent and non-covalent strategies for the immobilization of Tobacco Etch Virus (TEV) protease on superparamagnetic nanoparticles; 2019.

[5]. Emel, E., Sibel, S. and Ural, A., (2006): Polyacrylamide–gelatine carrier system used for invertase 1. immobilization. Food Chemistry, 97: 591-597.

[6]. Arıca, Y. and Bayramoglu, G., (2004): Reversible immobilization of tyrosinase onto polyethyleneimine-2. grafted and Cu (II) chelated poly (HEMAco-GMA) reactive membranes, Journal of Molecular Catalysis B: Enzymatic, 27: 255-265.

[7]. Saleem, M., Rashid, M., Jabbar, A., Perveen, R., Khalid, A. and Rajoka, M., (2005): Kinetic and thermodynamic 3. properties of an immobilized endoglucanase from Arachniotus citrinus. Process Biochemistry, 40: 849- 855.

[8]. Anisha, G.S. and Prema, P. (2008).Cell immobilization technique for the enhanced production of α-galactosidase by griseoloalbus. Bioresour Technol.99: 3325-3330.

[9]. Anjani, K.;Kailasapathy, K. and Phillips, M. (2007).Microencapsulation of enzymes for potential application in acceleration of cheese ripening. International Dairy Journal.17:79–86.

[10]. Anson, M.L. (1938).Estimation of pepsin, papain and cathepsin with hemoglobin. Journal of General Physiology. 22: 79-89.

[11]. Anwar, A.; Qader, S.;Raiz, A.;Iqbal, S. and Azhar, A. (2009).Calcium Alginate: A Support Material for Immobilization of Proteases from Newly Isolated Strain of Bacillus subtilis KIBGEHAS. World Applied Sciences Journal. 7(10): 1281-1286.

[12]. Banik, R.M. and Prakash, M., (2004).Laundry detergentcompatibility of the alkaline protease from Bacillus cereus. Microbiological Research.159: 135–140.

[13]. BBC Research. Report BIO030 F Enzymes in Industrial Applications: Global Markets. BBC Research; Wellesley, MA, USA: 2011

[14]. Bhaskar, N.;Sudeepa, E.S.;Rashmi, H.N. and Selvi, A.T. (2007).Partial purification and characterization of protease of Bacillus proteolyticus CFR3001 isolated from fish processing waste and its antibacterial activities . Bioresource Technology.98:2758–2764.

[15]. Bilal, M.;Iqbal, H.M.N.; Hu, H.; Wang, W. and Zhang, X. (2017).Enhanced bio-catalytic performance and dye degradation potential of chitosan-encapsulated horseradish peroxidase in a packed bed reactor system. Science of the Total Environment. 575: 1352.

[16]. Bommarius, A.S. and Paye,M. F. (2013).Stabilizing biocatalysts. Chemical Society Reviews. 42(15) 6534.

[17]. Boominadhan, U.;Rajakumar, R.;Sivakumar, P.K.V. and Melvin, M.J. (2009).Optimization of protease enzyme production using Bacillus sp. isolated from different wastes. Botanical Research International.2: 83-87.

[18]. Caruso, F.;Trau, D.;Mohwald, H. andRenneberg, R. (2000).Enzyme encapsulation in layer-by-layer engineered polymer multilayer capsules. Langmuir, 16:1485–8.

[19]. Verma RP, Hansch C (March 2007). "Matrix metalloproteinases (MMPs): chemical-biological functions and (Q)SARs" (PDF). Bioorg. Med. Chem. 15 (6): 2223–68. doi:10.1016/j.bmc.2007.01.011. PMID 17275314. Archived from the original (PDF)on 13 May 2015. Retrieved 21 October 2015.

[20]. Matrix Metalloproteinases: Its implications in cardiovascular disorders

[21]. *Van Lint P, Libert C (December 2007).* "Chemokine and cytokine processing by matrix metalloproteinases and its effect on leukocyte migration and inflammation". J. Leukoc. Biol. *82 (6): 1375–81.* doi*:*10.1189/jlb.0607338. PMID 17709402.

[22]. *Kirchhain, A.; Poma, N.; Salvo, P.; Tedeschi, L.; Melai, B.; Vivaldi, F.; Bonini, A.; Franzini, M.; Caponi, L.; Tavanti, A.; Di Francesco, F. (January 2019). "Biosensors for measuring matrix metalloproteinases: An emerging research field". Trends in Analytical Chemistry. 110: 35–50.* doi*:*10.1016/j.trac.2018.10.027.

[23]. Kirchhain, A.; Poma, N.; Salvo, P.; Tedeschi, L.; Melai, B.; Vivaldi, F.; Bonini, A.; Franzini, M.; Caponi, L.; Tavanti, A.; Di Francesco, F. (January 2019). "Biosensors for measuring matrix metalloproteinases: An emerging research field". Trends in Analytical Chemistry. 110: 35–50. doi:10.1016/j.trac.2018.10.027.

[24]. Bonnans, C., Chou, J., & Werb, Z. (2014). Remodelling the extracellular matrix in development and disease. Nature Reviews Molecular Cell Biology, 15(12), 786–801. doi:10.1038/nrm3904.

[25]. Woessner JF Jr (1991) Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 5:2145–2154.

[26]. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol. 2011;3:a005058.

[27]. Stamenkovic, I. (2003). Extracellular matrix remodelling: the role of matrix metalloproteinases. The Journal of Pathology, 200(4), 448–464. doi:10.1002/path.1400.

[28]. Vincenti, M. P., & Brinckerhoff, C. E. (2007). Signal transduction and cell-type specific regulation of matrix metalloproteinase gene expression: Can MMPs be good for you? Journal of Cellular Physiology, 213(2), 355–364. doi:10.1002/jcp.21208.

[29]. Ra HJ, Parks WC. Control of matrix metalloproteinase catalytic activity. Matrix Biol 2007; 26: 587–596.

[30]. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-b and promotes tumor invasion and angiogenesis. Genes Dev 2000; 14: 163–176. 18

[31]. Yamamoto K, Miyazaki K, Higashi S. Cholesterol sulfate alters substrate preference of matrix metalloproteinase-7 and promotes degradations of pericellular laminin-332 and fibronectin. J Biol Chem 2010; 285: 28862–28873.

[32]. Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. Arthritis Res 2002; 4: 157–164.

Received 15 December 2020; Accepted 05 January 2021.

[33]. 0 Pilcher BK, Dumin JA, Sudbeck BD, et al. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol 1997; 137: 1445–1457.

[34]. Ruangpanit N, Price JT, Holmbeck K, et al. MT1-MMP-dependent and -independent regulation of gelatinase A activation in longterm, ascorbate-treated fibroblast cultures: regulation by fibrillar collagen. Exp Cell Res 2002; 272: 109–118.

[35]. Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci USA 1990; 87: 5578–5582.

[36]. Itoh Y, Takamura A, Ito N, et al. Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. EMBO J 2001; 20: 4782– 4793.

[37]. Sela-Passwell N, Rosenblum G, Shoham T, et al. Structural and functional bases for allosteric control of MMP activities: can it pave the path for selective inhibition? Biochim Biophys Acta 2010; 1803: 29–38.

[38]. Geurts N, Martens E, Van Aelst I, et al. b-hematin interaction with the hemopexin domain of gelatinase B/MMP-9 provokes autocatalytic processing of the propeptide, thereby priming activation by MMP-3. Biochemistry 2008; 47: 2689–2699.

[39]. Fu X, Kao JL, Bergt C, et al. Oxidative cross-linking of tryptophan to glycine restrains matrix metalloproteinase activity: specific structural motifs control protein oxidation. J Biol Chem 2004; 279: 6209–6212.

[40]. Kassim SY, Fu X, Liles WC, et al. NADPH oxidase restrains the matrix metalloproteinase activity of macrophages. J Biol Chem 2005; 280: 30201–30205.

[41]. Strickland DK, Ashcom JD, Williams S, et al. Sequence identity between the alpha 2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. J Biol Chem 1990; 265: 17401–17404.