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A Review on Formulation of Enzymatic Solution for Biopolymer Hydrolysis

Bala IA[1*], Abdullahi MR[1], Bashir SS[2] and Idris IM[3]

[1]Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria
[2]Department of Physical Sciences, Rabiu Musa Kwankwaso College of Advanced and Remedial Studies, Tudun Wadar Dankade, Kano, Nigeria
[3]Department of Chemistry, Nigerian Police Academy, Wudil, Kano, Nigeria

Review Article

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***For Correspondence**

Bala IA, Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria, Tel: +23464666023.

E-mail: iabala.chm@buk.edu.ng

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ABSTRACT

In the drug industry, biopolymers play a key role in administering drugs to patients. The efficiency and activity of drug depends on its solubility-hence the need for an excellent hydrolytic decomposition of biopolymeric drug carriers. Biopolymers are natural polymers from living organisms. Human body contains many enzymes which help in metabolic activities. The effect of enzymes on effectiveness and efficacy of drugs have been studied. In this review, those studies have been analysed and presented critically with the aim of bringing to surface both positive and negative impacts.

INTRODUCTION

Biopolymers are organic polymers produced by living organism [1]. They are generally formed naturally through complex metabolic pathways within the cells of organisms during growth cycles. Therefore, they are also called natural polymers. Biopolymers are vast class of polymers which include carbohydrates, polypeptides, DNA etc. [2]. Biopolymers are good candidates for research due to their low toxicity, stability, renewability and biodegradation of some like starch, cellulose etc., into simple molecules such as methane, water or carbon dioxide. However, some biopolymers are long lasting like collagen, chitosan etc. [1]. Biopolymers are widely used in various fields example cellulose which is the most abundant biopolymer on earth is produced mainly by plants is the chief ingredient in paper and pulp industries. Collagen which is a biopolymer formed by animals has extensive biomedical applications in areas such as drug delivery, wound healing, tissue engineering etc. Shrimps, lobster, craps and insect shells are made up of a polysaccharide called chitin. Chitin is also obtained from fungal cell walls. The moisturizing and water-retaining capacity of chitin favors its use in cosmetic industries [3,4].

Biopolymers are classified based on their method of production or source **(Scheme 1)** as:

Polymers derived from animal or vegetable biomass example are polysaccharide, protein etc.

Polymers obtained from chemical reactions of renewable bio based monomers, e.g., polylactic acid.

Polymers derived from microorganisms such as polyhydroxyalkanoates, xanthan, pullulan etc. [2,5].

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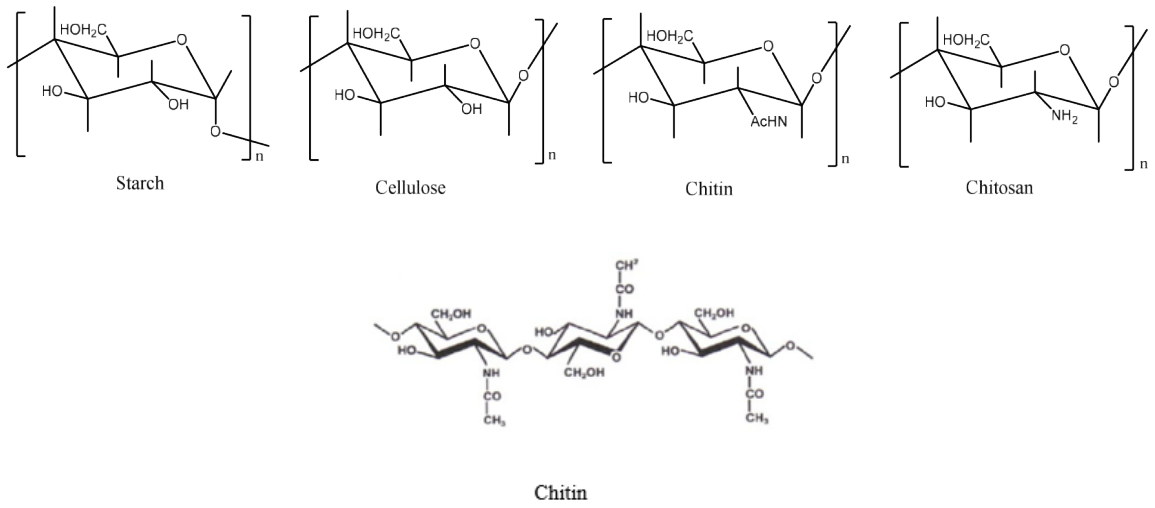

Scheme 1. Basic chemical structure of natural polymers.

HYDROLYSIS OF BIOPOLYMERS

Hydrolytic decomposition of biopolymer to produce non-toxic degradation products is of practical importance especially in drug delivery. Drug carries made from polymeric materials that are not easily wetted by aqueous solution could not circulate long enough in the blood to reach its target [6].

Kiss et al. have studied the hydrolytic decomposition of polymers surfaces in aqueous media [6]. Films of D,L-poly(lactic acid), and two of its copolymers D,L- poly(lactic/glycolic acid), and D,L-PLGA with 85/15 and 50/50 rations were used soaked in water in order to study the wettability of the polymers via dynamic contact angle measurements. All the polymeric films were found as the decomposition progressed to be more soluble (decrease in dynamic contact angle). In 50-80 days the solubility of copolymers were found to be very significant compared to the homopolymer. Enzymatic hydrolysis studies of starch from lyophilised Quercus-rubra L. root tissue with amyloglucosidase or Takadiastase carried out by Bruce et al. revealed that maximum hydrolysis needed high enzyme activity: 2.4 U amyloglucosidase or 48 U Takadiastase per mg of starch [7]. 5 U and 70 U of amyloglucosidase and Takadiastase respectively should be used in route analysis per mg of starch. The starch hydrolysis was found to be independent of prolong geletinisation, hydrolysis and tissue weight but, dependent on sufficient enzyme. MacGregor et al. have found that at 35°C α-Amylase I the minor α-Amylase component in malted barley is better in solubilizing small and large starch granules obtained from normal and waxy barleys than major component, α-Amylase II [8].

Table 1. Enzymatic hydrolysis of biopolymers.

Biopolymer	Enzymes	Activity
Starch [7]	Amyloglucosidase, Takadiastase	+
Chitin [9]	*Lecanicillium fungicola*	+
Beef protein [13]	Papain, bromelain, actinidin and zingibain	-
Myoglobin [14]	Calpain, proteasome and cathepsin B	+
Collagen [17]	Zingibain, papain, cucumin	+

However, the two α-Amylases hydrolysed faster the smaller starch granules and the starch granules obtained from waxy barley. The smaller and larger granules gave different degradation products **(Table 1)**.

Laura et al. conducted enzymatic hydrolysis of chitin [9]. The chitin hydrolysis using Lecanicillium fungicola was carried out with UFL at 40°C and pH 5 also with 619 and 355 U/mg of protein of Endo and NHase respectively. The yield of chitin oligosaccharides produced as well as the processing time was reported to have improved by the use of acidified media for the enzyme. 2.77 and 4.44 mmol/L were the maximum chitin oligosaccharide concentrations obtained for α and β deacetylated chitins with degrees of deacetylation (DDA) of 55 and 50% respectively. Varum et al. studied the hydrolysis of O-glycosidic linkages and N-acetyl linkages of partially N-acetylated chitosans in concentrated and dilutes HCl [10]. The rate of hydrolysis of glycosidic linkages and the rate of de-N-acetylation in dilute acid were found to be equal. However, in concentrated HCl the glycosidic linkage was found to hydrolyse more than ten times faster than the N-acetyl linkages. The hydrolysis of N-acetyl linkages was considered to be SN2 with the limiting step being the addition of water to the carbonium ion, while the hydrolysis of glycosidic linkages regarded as SN1 where the rate-limiting step is the formation of carbonium ion. 152.2 ± 8.1 and 1158.1 ± 9.8 kJ/mol were reported to be the activation energies for acid hydrolysis of two nearly full de-N-acetylated chitosans (FA=0.002 and FA<0.0003). Two partially N-acetylated chitosans (FA=0.47 and FA=0.62) activation energies for the acid hydrolysis was 130.4 ± 2.5 and 134.3 ± 3.1 kJ/mol respectively. Acid hydrolysis studies of chitin using gel filtration conducted by Einubu11 to determine the amount of mono-

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mer, dimer, trimer and tetramer as a function of time revealed that tetramers hydrolysed nonrandomly, therefore the glycosidic bonds next to one of the end residue hydrolysed 2.5 and 2 times faster than the other glycosidic linkages in fully N-acetylated and fully N-deacetylated tetramer, respectively. However, in the same study, Einubu reported the rate constants for the hydrolysis of glycosidic linkages of fully N-acetylated oligomer were reported to be 50 times faster than that of fully glycosidic linkages of fully de-N-acetylated oligomer. Due to the vast application of enzymatic solution in hydrolysis, surface activity studies of immobilised enzymes on self-assembled monolayer could help greatly in surface enhancement activity. Therefore, hydrolytic studies of polypeptides in self-assembled monolayer will have positive impact in sensor, medical, tissue engineering, food etc. fields.

ENZYMATIC ACTIVITIES

Collagen is one of the main components of extracellular matrix of connective tissue. It is related to some human disorders such as scleroderma, keloids, tumour growth etc. [12]. Minh et al. in their studies on activities of papain, bromelain, actinidin and zingibain protease preparation on proteins in beef connective tissue and my fibril showed that the enzymatic preparations hydrolytic activities on substrates depends on the assay used [13]. The activity was investigated using extracted collagen from the proteins via time course sampling then display on 1D-SDS-PAGE. Actinidin protease preparation was most effective against myofibril proteins while zingibain protease preparation better at hydrolysis proteins from the connective tissues. The result validate the potential of actinidin and zingibain in meat tenderisation applications. The collagenolytic activity of the protease preparations was studied with Azocoll: an azo dye-labelled collagen in phosphate buffer. The collagen hydrolysis by the four commercially protease preparations were found to be in the order: zingibain>bromelain>papain>actindin with the activity taking place mostly in the first hour of the incubation, however zingibain demonstrated gradual decrease in activity with the first 3 hours. Further hydrolysis did not take place after the addition of aliquot of commercial protease preparation after 24 hours of incubation. Bromelain protease preparation showed activity towards Azocoll substrate. Hasina et al. carried out enzymatic and fluorogenic assay on collagens from HeLa cells and check tissue. The fluorogenic assay using 3,4dihydroxyphenylacetic acid (3,4-DHPAA) was found to quantified as low as 0.18 μg/ml collagen coupled with enzymatic degradation of the collagen [12]. 3,4-DHPAA selectively detect N-terminal Gly-containing peptides (NGPs) in the presence of sodium borate and NaIO4. The sensivity of 3,4- DHPAA FL assay was 20, 10 and 5 times higher than conventional methods spectrofluorometric (OPA), immunological and colorimetric (Siriusred dye) methods respectively. 10.7 ± 1.3 and 0.93 ± 0.02 mg per μg of total proteins were the concentration of total collagens estimated by 3,4-DHPAA FL assay of cheek tissue and HeLa cells respectively. Volle et al. reported the inhibition of endopeptidase activities by myoglobin and hemin [14]. The study which was carried out via two methods. In method one the addition of myoglobin and hemin to the incubation mixture took place just before starting the reaction while in method two they were added to the incubation mixture just after the reaction was stopped. Both myoglobin and hemin were found to have inhibitory activity against papain, calpainscathepsin B, trypsin and three major activities of 20S proteasome: chrymotrypsin-like, trypsin-like and PGPH hydrolytic activites. This was in agreement with Rosell et al. and Spanier et al. findings [15,16]. However, the authors suggested that the inhibition activities by myoglobin and hemin are artefact due to the inhibitions found when they were not incubated with the enzyme but added after the reaction has finished. Naveena et al. reported the increased in solubility of collagen in enzymatic solution (cucumis, ginger extracts and papain) of Biceps femoris muscle of buffalo (4-5 years age) within 48 h at 4°C [17]. Improvement in tenderness, flavour and juiciness were observed in enzyme-treated samples. The findings were supported by Kumar et al. findings. The authors suggested that ginger and cucumis could be use as substitute to papain [18].

NANOPARTICLE-PROTEIN INTERACTIONS

Nanoparticles are the particles between 1 and 100 nanometers in size. Nanoparticle being small object behaves in term of transport and properties as a whole unit [19]. They have wide range of applications in electronics, medicine, biosensors etc. Monolayer- protected nanoparticles are good scaffold for studying protein interactions. Nanoparticle-protein interactions have great importance in formation of functional self-assemblies hence protein and nanoparticle are fantastic building blocks. Small molecules are normally use for the inhibition of enzymes active site. Protein surface recognition has evolved as a complimentary method for the inhibition of enzymatic activity [20]. Rotello et al. in their study of NPs inhibition of enzyme activity have developed a mixed monolayer protected NPs that have anionic termini for recognition and inhibition of an enzyme (α- chymotrypsin (ChT)) with an active site surrounded by cationic residue rings [21]. They reported the inhibition of ChT via two-step process: fast reversible inhibition then a slower irreversible step leading to denaturing of ChT on the surface of NP. An efficient complementary electrostatic interaction between ChT and NP was reported with a Kapp of 10 ± 1.3 nM and a binding five molecules to a NP stoichiometrically. The nanoparticle inhibition potency depended on the ionic strength of the aqueous medium due nature of the electrostatic interaction [20,22]. Larsericsdotter et al. in their thermodynamic studies of protein reported the decrease in stability of protein adsorbed on silica nanoparticles via electrostatic interaction [23]. This was in agreement with Rotello et al. findings [21]. The inhibition of enzymes on the surface of nanoparticle is not only as a result of blocking of ChT active site by the NP scaffold but also connected to the electrostatic interaction between NP and the substrate as reported by Rotello et al. in another study [24]. Positively charged NPs have excellent application

in drug, protein and gene delivery due to their internalisation into cells via charge-mediated endocytosis [25]. Studies have being done on positively charged NPs in the area of inhibition of negatively charged NPs and molecular recognition. Example

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Verma et al. reported complete inhibition of β-Gal activity by trimethylammoniunfunctionalised gold NPs [26]. Gold NPs with photocleavable monolayers have being reported to switch the inhibition of enzymes by UV light [27]. Gold NPs have been reported by Zheng and Huang to bind specifically to glutathione-Stransferase and streptavidin [28]. Lin et al. in their study of interactions of carbohydrate-encapsulated gold NPs with concanavalin A (Con A) found that Mannose-functionalised gold demonstrated high affinity to Con A with nanomolar dissociation constants. However, the authors confirmed that the interaction is being interfered by the size of the NP and the linker of the mannose ligands [29]. Interaction between proteins and monolayer-protected NPs generate highly organised 1D, 2D and 3D assemblies [20]. Mann et al. directed self-assembly of NPs into macroscopic materials by attaching IgE or IgG antibodies that have specificity for dinitrophenyl (DNP) and biotin respectively to NPs [30]. The NPs formed 3D network following addition of bivalent antigens with appropriate double-headed functionalities. Heterogeneous aggregates and filaments of compactly packed gold and silver particles were reported when anti DNP IgE-modified gold NP, anti-biotin IgG-conjugated silver colloid and DNPbiotin bivalent antigen were mixed in a right ratio. Otsuka et al. reported the construction of a lactose-conjugated gold nanoparticle in order to target agglutinin which is a bivalent lectin with β-D-galactose specificity [31]. Lectin-induced aggregation was improved by the lactose on the gold NPs. Therefore the aggregation was proportional to lectin concentration. Construction of biotinylated gold NPs was reported by Perez-Luna et al. [32]. Aggregation of the gold NPs was investigated in the presence of streptavidin. The biotinylated gold NPs aggregated as revealed by the shifting of surface plasmon resonance peak as well as broadening of the absorption spectrum of the NPs. Production of linear arrays of NP and self-assembly of protein via biotin-streptavidin interaction have being reported by Li et al. [33].

CONCLUSION

Biopolymers have a wide range of applications. As there medical applications rapidly evolved, there is a growing need for environmentally benign and non-harmful polymeric materials for drug delivery, tissue engineering and other health related operations. Pharmaceutical industry could provide and expand market for modified biopolymers. Biopolymer being renewable are cheaper than synthetic polymers which are nonbiodegradable due to among others resistance against microbial attack. Two major concerns for food industry are food quality and safety to human health; therefore, some biopolymers such as chitosans are good candidates for food industry-food packaging.

REFERENCES

1. Tanase EE, et al. Biopolymers based on renewable resources A review. Scient Bull Ser F. Biotechnol. 2014;18: 188-195.
2. Rao MG, et al. A Comprehensive review on biopolymers. Scient Rev Chem Comm 2014;4:61-68.
3. Singh AV. Biopolymers in Drug Delivery: A Review. Pharmacol Online 2014;1:666-674.
4. Daniel G, Ovidiu T. Biopolymers used in food packaging: A REVIEW. Acta Universitatis Cibiniensis Series E: Food Technol. 2012;16:3-19.
5. Ruban SW. Biobased Packaging-Application in Meat Industry. Vet World. 2009;2:79-82.
6. Kiss E, Vargha-Butle, EI. Novel method to characterise the hydrolytic decomposition of Biopolymer surfaces. Colloid Surf B: Biointerfaces. 1999;15:181-193.
7. Bruse EH, Richard ED. Starch measurement in plant tissue using enzymatic hydrolysis. Physiologia Plantarum. 1979;47:51-157.
8. MacGregor AW, Balance DL. Hydrolysis of large and small starch granules from Normal and waxy barley cultivars by alpha-Amylases from barley malt. Cereal Chem. 1980;57:397-402.
9. Laura R, et al. Enzymatic hydrolysis of Chitin in the production of oligosaccharides using *Lecanicillium fungicola* chitinases. Process Biochem. 2006;41:1106-1110.
10. Varum KM, et al. Acid hydrolysis of chitosan. Carbo Poly. 2001;46:86-98.
11. Aslak E. Characterisation of chitin and a study of its acid-catalysed hydrolysis. PhD Thesis. Norwegian University of science and Technology, Department of Biochemistry, 2007.
12. Yasmin H, et al. Amplified and selective assay of collagen by enzymatic and fluorescent reactions. Scient Rep. 2014;4:4-8.
13. Ha M, et al. Characterisation of commercial papain, bromelain, actinidin and zingibain protease preparations and their activities toward meat proteins. Food Chem. 2012;134:95-105.
14. Volle B, et al. Myoglobin inhibition of most protease activities measured with fluorescent substrates is an artifact. Meat Sci. 1999;52:81-87.
15. Rosell CM, et al. Myoglobin as an endogenous inhibitor of proteolytic muscle enzymes. J Agri Food Chem. 1996;44:3456-3456.
16. Spanier AM, et al. Endogenous cathepsin B inhibitior activity in normal and myopathic red and white sketetal muscle. Muscle and Nerve. 1982;5:313-320.

e-ISSN:2319-9849
p-ISSN:2322-00

17. Naveena BM. Meat Tenderization of buffalo meat using plant proteases from Cucumis trigonus Roxb (Kachri) and Zingiber officinale roscoe (Ginger rhizome). Meat Sci. 2004;68:363-369.

18. Kumar M, Berwal JS. Tenderisation of spent hen meat with Cucumis trigonus Roxb (Kachri). Indian J Poult Sci. 1998;33:67-70.

19. Robert T, et al. Small particle, big impacts: A review of the diverse applications of nanofluids. J Appl Phys. 2013;113:011301.

20. You C, et al. Monolayer-protected nanoparticle-protein interactions. Curr Opin Chem Biol. 2005;9:639-646.

21. Rotello VM, et al. Inhibition of chymotrypsin through surface binding using nanoparticle-based receptors. Proc Natl Acad Sci USA. 2002;99:5018-5023.

22. Verma A, et al. Effect of ionic strength on the binding of α-chymotrypsin to nanoparticle receptors. Langmuir. 2014;20:4178-4181.

23. Larsericsdotter H, et al. Thermodynamic analysis of proteins adsorbed on silica particles: electrostatic effects. J Coll Inter Sci. 2001;237: 98-103.

24. Rotello VM, et al. Monolayer-controlled substrate selectivity using noncovalent enzyme-nanoparticle. J Am Chem Soc. 2004;126:13572-13573.

25. Song HT, et al. surface modulation of magnetic nanocrystals in the development of highly efficient magnetic resonance probes for intracellular labelling. J Am Chem Soc. 2005;127:9992-9993.

26. Verma A, et al. Tunable reactivition of nanoparticle-inhibited β-galactosidase by glutathione at intracellular concentration. J Am Chem Soc. 2004;126:13987-13991.

27. Fischer NO, et al. Light-induced inhibition of chymotrypsin using photocleavable monolayers on gold nanoparticles. Chem Commun (Camb) 2004:2866-2867.

28. Zheng M, Huang X. Nanoparticles compromising a mixed monolayer for specific binding with biomolecules. J Am Chem Soc. 2004;126:12047-12054.

29. Lin CC, et al. Quantitative analysis of multivalent interactions of carbohydrate-encapsulated gold nanoparticles with concanavalin A. Chem Commun (Camb) 2003:2920-2921.

30. Shenton W, et al. Directed self-assembly of nanoparticles into macroscopic materials using antibody-antigen recognition. Adv Mater 1999;11:449-452.

31. Otsuka H, et al. Quantitative and reversible lectin-induced association of gold nanoparticles modified with α-lactosyl-ω-mercapto-poly(ethylene glycol). J Am Chem Soc. 2011;123:8226-8230.

32. Aslan K, et al. Controlled and reversible aggregation of biotinylated gold nanoparticles with streptavidin. J Phys Chem B. 2004;108:15631-15639.

33. Li H, et al. DNA-templated self-assembly of protein and nanoparticle linear arrays. J Am Chem Soc. 2004;126:418-419.