

## Chapter 2

# Literature Review of Cellulase and Approaches to Increase Its Stability

**Abstract** Cellulose is now as an important composite of lignocellulosic material. Cellulose is made up of D-glucose molecules linked together by  $\beta$ -1, 4-glucosidic bonds have complex and diverse structures. Although cellulase has several applications in brewery, animal food, and laundry, pulp and paper industries, its application in bioethanol production attracted many researchers recently. Bioconversion of cellulose to glucose is done by different cellulase enzymes extracted from various organisms. *Trichoderma reesei* is a well-known fungi species which produce very efficient cocktail of cellulases. One of the key enzymes in this procedure is cellobiohydrolase which attacks to the end sides of cellulose. However, due to high cost of enzymes bio ethanol is not commercialized yet. One approach to overcome this obstacle is to lower enzyme usage by increasing its stability and efficiency, the most common way to enhance enzyme stability is introducing disulfide bonds. Rational protein engineering tools helped to design more stable enzyme to decrease cellulase production. Recently by advances in computer science the protein can be computationally engineered and the effect can be simulated prior to any lab experiment.

**Keywords** *Trichoderma reesei* • Cellobiohydrolase • Cellulose structure • Bioconversion • Protein engineering

## 2.1 Cellulose and Cellulases Structure

Cellulose despite a typical chemical arrangement made up of D-glucose molecules linked together by  $\beta$ -1, 4-glucosidic bonds have complex and diverse structures. The polymeric chain which is linear may have more than 10 thousand insoluble glucose molecules. The chains are not isolated, but, instead, stranded by to each other in a parallel style to form crystalline micro fibrils. Micro fibrils differ on the origin, the size and the crystallinity. Moreover, physical treatments can disturb crystallinity and the grade of polymer production as well [1].

In the natural world and in the area of industry cellulases are highly essential enzymes, since they have a significant activity in the carbon cycle globally which are able to break down cellulose that is not soluble to sugars molecules that are soluble. The most varied class of enzymes that catalysing the hydrolysis of a substrate are cellulases. Cellulases can be classified into three main classes including: endoglucanases, exocellulases, and processive endoglucanases which are dissimilar structurally and functionally. The cellulases have a catalytic domain (CD) and a substrate-binding unit which this property is similar to other enzymes hydrolysing substrates that are not soluble. Three diverse methods are exist used by microorganisms that degrade cellulose: secretion of a set of free cellulases that work together (synergistic enzyme), production of cellulosomes which are several enzyme complexes, and an unidentified mechanism that does not need processive cellulases that were critical in the two mentioned way [2].

## **2.2 Applications of Cellulase**

Broad elementary and practical investigation for the period of the 1970s and 1980s proved that the enzyme-induced bio-conversion of lignocellulose to sugars that can be solved was relatively challenging. Nevertheless, studies on hemicellulases, cellulases and pectinases shown their biotechnological applications in different industries, such as brewery, food, animal feed, textile and laundry, pulp and paper, agriculture and wine [3].

### ***2.2.1 Application of Cellulase in Biofuel Production***

With the unavoidable reduction of the world's petroleum source, a growing universal attention to substitute, resources of energy that is not based on petroleum has been increasing. Over the past two decades years as petroleum provides 97 % of the energy spent for transportation governments and industries global has been dynamically recognizing, rising and commercializing technology for substitute fuels for transportation. There is an increasing use of ethanol as fuel for transportation in the United States. The ethanol produced during fermentation can be expensive and government subsidy is required to keep the price down. Almost all ethanol fuel is created throughout fermentation of sucrose in the Brazil or corn glucose in United States, but any other country with a noteworthy budget based on agriculture are able to use present technology for making petroleum ethanol. This is likely as, throughout the past 20 years, technology for ethanol making from non-food-plant resources has been established to the purpose that massive amount of fuel will be a fact in the few years later. Therefore, agricultural products including wheat or rice straw, corn Stover (corn cobs and stalks), waste sugar cane, the paper

potion of municipal waste, forestry and paper mill castoffs, and dedicated energy crops together are called 'biomass' can be transformed to ethanol that can be used as a fuel [4].

The main problem for commercialization of ethanol made by fermentation is much costly compared to the native gasoline. Latest rises in the comprehensive value of oil seem to be aiding to bridge the fee breach among gasoline and ethanol. The price of ethanol is related, in part, to the unpreventable loss of partial of the carbon throughout microorganism's fermentation of sugars. Though ethanol production from cane sugar is a relatively easy procedure, trouble raises when these procedures need enzymes to hydrolyse starch to glucose former to fermentation when ethanol is made from corn or wheat starch. Ethanol production from biomass involves even broader treating to release the polymeric sugars in hemicellulose and cellulose that account for 20–35 % and 23–53 % of biomass, individually. Cellulose is a polymer made by beta-linked glucoses, while hemicellulose is a meaningfully diverged chain of arabinose and xylose that has glucose, mannose and galactose as well. Usually acids hydrolysis of these carbohydrate polymers such as cellulose, amylose, hemicellulose and starch (contributed either by the biomass or added externally) and enzymes. Concentrated sulphuric acid used in the hydrolysis of the biomass carbohydrates. The sugars are parted from the acid once hydrolysed, and then are fermented to ethanol. The pre-treatment techniques aim to diverse the lignin matrix and carbohydrates while chemical demolition of sugars throughout fermentation is vital for producing ethanol. Improvement of a faultless pre-treatment technique is problematic, given that 'biomass' contains sources such as hardwood and softwood trees, agricultural remains products like corn Stover, and paper that cannot be recycled. Scientists have tested numerous pre-treatment procedures such as steam explosion and hot water, and also alkaline and solvent pre-treatments and many valued shapes of acid pre-treatment regarding to the existence of various feedstocks. Newly the yields and kinetics of the countless acid-based batch and flow-through techniques have been analysed, and obviously the flow-through processes make greater sugar yields available and grounds less sugar degradation, but consequences an additional sugar solution. The pre-treatment procedure is considered simply to start the decay of the biomass and partly hydrolyse the polymers of carbohydrates, in order to making them ready to enzymatic attack. For the first time cellulases were used in a sequential procedure (pre-treatment → cellulase hydrolysis → ethanol fermentation). On the other hand, the saccharification (procedure of depredating a complex carbohydrate such as starch or cellulose into its subunits) and fermentation (SSF) procedure affords important reduction in expenses because cellulase hydrolysis happens throughout glucose fermentation. The procedures presently used include fermentation of all biomass sugars in a simultaneous saccharification cofermentation (SSCF) procedure [4]. In order to prepare substrate for fermentation pre-treatment technique is required which might use several ways for treating biomass. Recently acid based pre-treatment are use although it was very challenging when biomass used as substrate of fermentation.

## 2.3 *Trichoderma reesei* Known as the Crowned King of Cellulolytic Fungi

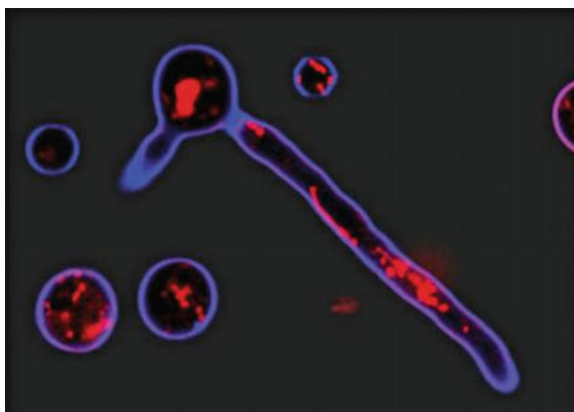
The finding of the fungus *Trichoderma reesei* during the period of World War II, when the allied militaries in the South Pacific hurt the quick damage of cotton exhaustions and camps. The unseen ‘enemy’ was recognized as a tiny fungus *T. viride* strain QM 6a. Broad investigations of this fungus were conducted, causing the choice of hyper cellulolytic fungal mutants, with the high-yielding strains *T. viride* QM9414 and MCG77. In 1977, the fungus was given new name *T. reesei* in honour to E.T. Reese, who discovered it in the 1940s. The report of Mandels and Sternberg (in 1976) express information on more than 14,000 discovered and screened fungi by seeing their cellulase activity; no serious competitors to *T. reesei* were found. The ‘king’ of cellulolytic fungi was at the end of the day crowned. Diverse cellulase hyperproducing strains of *T. reesei* have been settled; the *RUT C30* strain is one of the most powerful and best characterized strains, and it has become a reference strain among *T. reesei* high cellulase producers [5]. A surprisingly pitiable repertoire of genes for cellulases and hemicellulases has discovered by the genome sequencing of *T. reesei*. Though ten cellulases including eight endoglucanases and two cellobiohydrolases belonging to different glycoside hydrolase families have been identified in the *T. reesei* genome, only four main cellulases are generally secreted in remarkable amounts by this fungus: CBH I (Cel7A), CBH II (Cel6A), EG I (Cel7B) and EG II (Cel5A) [6]. These normally characterize up to 90–95 % of the whole secreted protein, CBH I making up 50–60 % and CBH II 20 % of the whole cellulases.

A number of hyper thermophilic and thermophilic bacteria live at great temperatures that can go above 100 °C. These microbes are a latent basis of extremely thermo stable cellulases and other enzymes, which are improved, appropriate to strict progression environments and might take greater exact activities. Although bacterial cellulases have interesting and beneficial features, bacteria cannot be compared to mutant strains of fungi by the level of protein production. This feature might come to be the major difficulty during using cellulases from bacteria in the assembly of second-generation biofuels. As a matter of fact, cellulases from bacteria with useful properties can be heterologously expressed in fungi as members of fungal simple enzyme systems. For consolidated bioprocessing, specific bacterial species are suitable precisely the straight microbial transformation of lignocellulosic biomass to ethanol, butanol, organic acids and further valuable yields (Fig. 2.1).

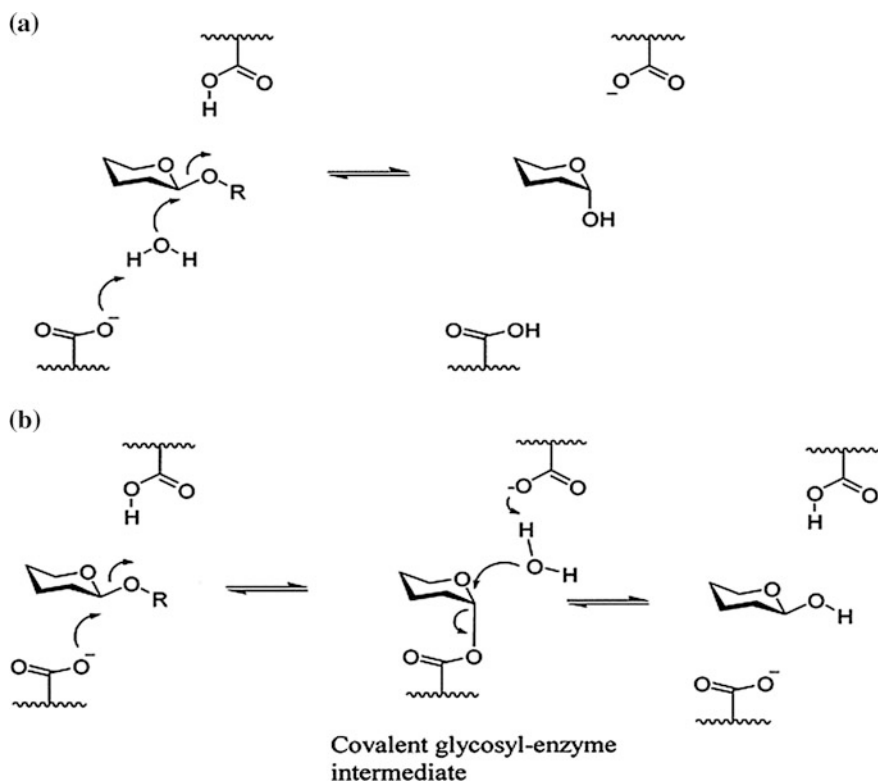
## 2.4 Catalytic Mechanisms of Glycoside Hydrolases

By acid-base catalysis glycoside hydrolases cut glycosidic bonds. Catalysis can be accomplished with either inversion or net retention of the anomeric configuration (when two molecule have similar formula but only the conformation at hemiketal

**Fig. 2.1** In this microscope image of the fungus *Trichoderma reesei*, proteins are stained red, and white chitin, a component of the cell walls, is stained blue (<http://machinedesign.com/news/using-fungus-make-fuel>)



carbon is different) of the substrate (Fig. 2.2). Inversion is a typical shift reaction. A catalytic acid provides protonic support to a group that leaves (glycoside hydroxyls have a high  $pK_a$  and thus make poor leaving groups) at the same time, in order to



**Fig. 2.2** Inversion and retention process. **a** Inversion, **b** retention

discharge proton from a water molecule for nucleophilic replacement at the anomeric core catalytic base. The base and acid both are normally placed some 7–13 Å separately in order to provide somewhere to stay the nucleophilic water ‘below’ the pyranoside ring. In many cellulase systems, the identification of the catalytic base remains controversial.

The mechanism of retention is a binary movement basically as defined by Koshland in 1953. By oxacarbenium ion-like conversion conditions a covalent glycosyl-enzyme intermediate is made, and then hydrolysed. This involves two vital residues, an enzymatic nucleophile and a catalytic acid/base which first serves as a typical Brønsted acid (the atom that donates hydrogen according to Brønsted-Lowry definition), giving proton to the leaving group to support departure then functions as a base, lose proton from the incoming water nucleophile for the second step. The nucleophile and acid/base are always found some 5–6 Å separately on all systems studied to date. It is worth commenting that, the stereochemistry of catalysis is preserved within each family as catalytic mechanism is dictated by the place of functional groups on the protein. Both inverting and retaining cellulases are identified [7].

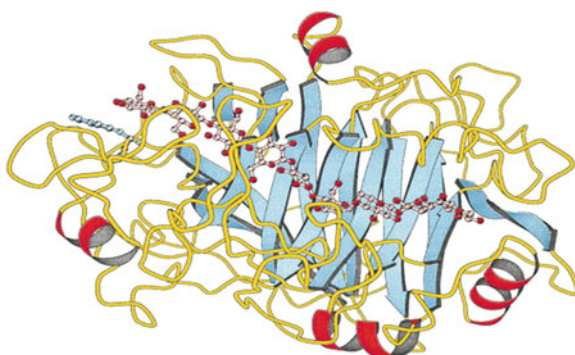
## 2.5 An Introduction to Cellulase Family 7

Cellulases from family 7 (family 7 is a family of glycoside hydrolases which is classified among glycoside hydrolases, based on sequence similarity and carry out catalysis with net retention of configuration), as explained before. From this family three-dimensional (3D) structure of several cellobiohydrolases and endoglucanases are identified. The 3-D structure contains a double  $\beta$ -sandwich. Alike to family 6, the structural difference among the cellobiohydrolases and the endoglucanases is exposed in the size and nature of the loops neighbouring the substrate binding sites: these loops are stretched and form a sealed off tunnel in the cellobiohydrolases.

The 3-D structures of both cellobiohydrolases (CBH I and CBH II) and endoglucanases from family 7 exposed a ‘trio’ of carboxylates in the active centre. By mutation of the residues Glu212-Asp214-Glu217, to their isosteric amide counterparts, the functions of them (formerly CBH I), were investigated in *T. reesei* Cel7A. The three point mutations meaningfully reduced the catalytic activity of the enzyme, though all keep a number of residual activities. On the minor chromophoric substrate 2-chloro-4-nitrophenyl  $\beta$ -lactoside the  $k_{\text{cat}}$  (catalytic constant) standards were fall down to 1/2,000, 1/85 and 1/370 of the native activity, respectively, whereas the  $K_m$  (Michaelis constant which shows enzyme’s affinity for a substrate) (values stayed basically unaffected. On insoluble bacterial microcrystalline cellulose (BMCC) no noteworthy activity was distinguished for the Glu212Gln and Glu217Gln mutants, whereas the Asp214Asn mutant retained residual activity. From many studies it is now obvious that the two glutamates work correspondingly as the catalytic nucleophile and catalytic acid/base (Table 2.1).

**Table 2.1** Catalytic residue equivalences in family 7 [1]

Enzyme	Organism	Catalytic acid/base	Catalytic nucleophile
Cel7A (CBH I)	<i>T. reesei</i>	Glu217	Glu212
Cel7B (EG I)	<i>T. reesei</i>	Glu201	Glu196
Cel7B (EG I)	<i>H. insolens</i>	Glu202	Glu197
Cel7B (EG I)	<i>F. oxysporium</i>	Glu202	Glu197



**Fig. 2.3** The CBH I catalytic domain with a cello oligomer bound secondary-structure elements are colored, *blue arrows* for  $\beta$  strands; *red spirals* for  $\alpha$  helices; *yellow coils* loop areas. The cello oligomer is highlighted in *pink* as a ball-and-stick object [8]

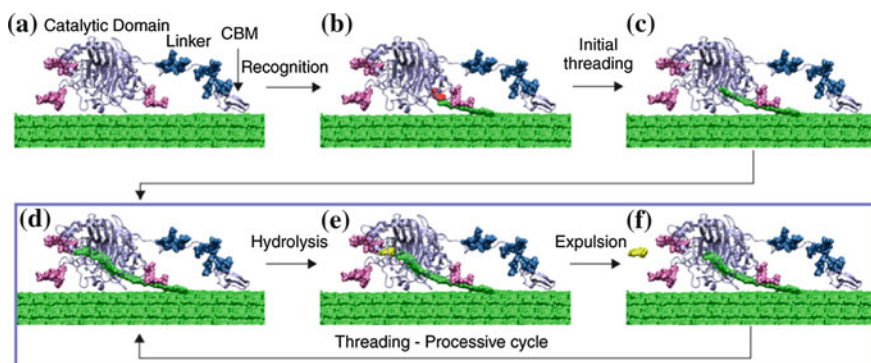
Cellulose chain was bound alongside the 50 Å substrate binding tunnel. Active site variations Glu212Gln and Glu217Gln were crystallized together with cello-oligosaccharides and the structures disclose glucose moieties covering sub sites as well. The binding manner witnessed matches to that predicted throughout productive binding of a cellulose chain and provides the theory that hydrolysis by *T. reesei* Cel7A continues from the reducing end of a cellulose chain (Fig. 2.3).

## 2.6 Cellulose Biodegradation Procedures

Cellulose biodegradation begins by binding of cellulase to cellulose by binding sites which recognize reducing end of cellulose then degradation of cellulose begins in catalytic tunnel and hydrolysis occurs. Summarized interaction of the cel7a can be observed in the Fig. 2.4.

## 2.7 Protein Engineering as a Solution

Enzymes are naturally designed protein that catalysts and are able to work in physiological states. On the other hand, biotransformation involves the enzymes in conditions that may proceed considerably from physiological condition. It is very



**Fig. 2.4** **a** Cel7A binding to cellulose, **b** recognition of a reducing end of a cellulose chain, **c** beginning threading of the cellulose chain into the catalytic tunnel, **d** threading and formation of a catalytically active complex, **e** hydrolysis in a processive cycle and **f** product expulsion and threading of another cellobiose (shown in yellow in **e** and **f**). Image reproduced with publisher's permission [9]

difficult to engineer enzyme that would optimally perform under extreme conditions. Alternatively, many enzymes are capable of catalysing a number of substrates and/or transforming some substrates, they are more promiscuous catalysts [10].

In the decades since the inception of protein engineering three approaches have been taken. Early work was focused on rational design and thereby restricted to enzymes where there was a considerable knowledge of structure. In the next generation, the emphasis was on the development of diversity via random mutagenesis to create large libraries. In the last decade this type of library has developed such that a greater diversity is used as the starting point, also using natural diversity [11].

### 2.7.1 Rational Design Approach

The latest approach for engineering enzymes is rational protein design (RPD). RPD contains a set of molecular biology methods, for example site-directed mutagenesis (SDM), according to structure of enzyme investigations. The correct reorganization of residues in charge for substrate-enzyme relations, particularly, docking, stability and activity is vital for the use of RPD in a directed way. Occasionally, it is essential to do sequence homology or alanine scanning of linked species mutual with biophysics data. Progresses in the likelihood of the consequence of SDM have been made so, at the same time the effect of mutation on ligand affinity, stability, and pK(a) values can be assessed and predicted for several mutants in one step. Due to the fact that RPD desires the information of the interest enzyme structure and/or its sequence in numerous and linked to species, the crystallography and spectroscopic analysis of numerous enzymes have been a great device to practice in modelling by computer. This method relays on the improvement completed in



determination of structure, better modelling rules and new visions into structure–function relationships. Then again, progresses in modelling, mainly calculations of free energy perturbation and molecular dynamics (MD) can calculate the suitable mutations for the development of enzyme selectivity.

On the other hand computer simulation investigations were established for the enhancement of enzyme catalysis. Recently, in MD simulations, estimated free energy and hydrogen bond energy calculations were integrated to reveal the structure–activity interactions. Similarly, the integration of diverse prediction for structure methods can be used in RPD of enzymes as is the case of molecular docking, fragment molecular orbital method (FMO) calculation, Quantitative structure–activity relationship (3D-QSAR), Comparative Molecular Field Analysis (CoMFA) modelling [12].

Since, structural data are not available always, a dissimilar idea as consensus sequence design (CSD) was established. CSD is an interesting idea for making protein more stable, that exploits amino acid conservation in sets of homologous proteins to recognize likely good mutations and it does not rely on the accessibility of data of structure. Data-driven CSD is according to the typical hypothesis that the rate of a certain residue in a multiple sequence alignments (MSA) of homologous proteins contacts with that amino acid's participation to protein stability. On the other hand, its achievement relays on the phylogenetic variety of the sequence set obtainable. The practice of this technique shows that a phylogenetically balanced CSD can lead in mesostable proteins noteworthy stabilization of secondary structural motifs. Enhancing the amino acid alphabet used for making random by taking into account structural details and differences in functions, and considering covariation in the plan procedure are further plans that could be exploited to make the most of stabilization while conserving activity [13].

Newly, RPD by MD simulations was used in thermo stability enhancement without decreasing enzyme performance taking considering properties of protein's surface as a substitute on core of protein features including core packing and cavity filling. Thus, flexible residues in the surface tolerant to mutations are practical objectives for thermo stabilization and that local-interaction stabilization of cavity lining residues by means of the MD technique can be an operative substitute to the typical cavity filling technique. Methods which employ computers can perhaps guide the RPM of proteins making DE more useful. On the other hand, the exploitation of molecular possible tasks can be cooperative to calculate the influences of mutations on protein stability and structure for collections of enzyme variants created in silico. Numerous illustrations of the combination of DE and RD might be found in the literatures to increase stability, adaptation to cold, new activities and creates, substrate specificity using sequence base enzyme remodel between the rests [14].

## 2.8 Advantages of Enzyme Thermo Stabilization

Enzyme thermo stabilization is very much of interest by industry and research. Keeping the protein stable at temperatures that would normally denature and destabilize it can be a main objective. Or a second demand can be to retain the protein stable at sensible temperatures, but for longer time (increasing the half-life). These two sides of stability required to somehow a different practice to the problem, but typically both effects are found together in thermo stable proteins. Thermo stable enzymes are needed to breaking down numerous organic compounds to working sources of energy. For instance the breakdown of cellulose for the aim of generating economically possible ethanol, a procedure that at present is too costly [15].

## 2.9 Some Thermo Stabilize Mutations

There are many mechanisms for enzyme thermo stabilization including; engineering: amino acid composition and intrinsic propensity, disulfide bridges, hydrophobic interactions, aromatic interactions, hydrogen bonds, ion pairs, prolines and decreasing the entropy of unfolding, intersubunit interactions and oligomerization, conformational strain release, helix dipole stabilization packing and reduction in solvent-accessible hydrophobic surface, docking of the n and c termini, and anchoring of loose ends, metal binding, nonlocal versus local interactions, post translational modifications and Extrinsic Parameters.

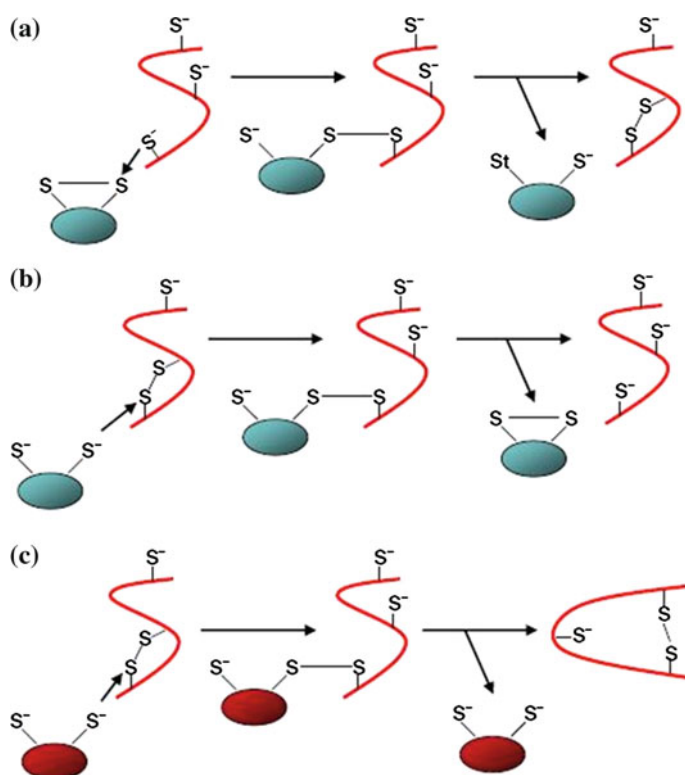
### 2.9.1 *Introducing Disulfide Bonds as a Strategy to Increase Stability*

In most extracellular proteins disulfide bonds are present, where they seemingly make stable the native conformation by decreasing the entropy of the unfolded state or by lowering the unfolding level of permanently denatured proteins This procedure property makes disulfide bond cross-linking an smart approach for engineering extra stability for conformation into proteins by site directed mutagenesis [16].

A disulfide bridge that the covalent bond is made have to be broken for a fully unfolded state and the breaking of covalent bonds is an energetically unfavourable procedure. The best of a recent characterized protein containing disulfide bridge has an ideal stability and activity beyond 100 °C, which is a probable gage that the adding of this bond can have potential effect in stabilization in more than 100 °C. Though, due to oxidation, at these temperatures cysteine is quite instable, thus this strategy probably is not used much in nature [17].

### 2.9.1.1 Mechanism of Disulfide Bond Formation

The disulfide bond in vivo formation mechanisms has been broadly investigated in eukaryotic and prokaryotic organisms in the past 20 years [18]. A sequence of thiol/disulfide replacement responses among cysteine thiolate ( $S^-$ ) and oxidizing disulfide bond ( $S-S$ ) by the nucleophilic attack that are done by enzymes inside the cells commonly results disulfide bond formation. Three classes of chemical interactions are available including: isomerization, reduction, and oxidation, which are shown in Fig. 2.5. A disulfide bond is presented to the protein substrate by responding with the oxidase (Fig. 2.5a). In the same way, a formed disulfide bond can be reduced by the reductase in the protein (Fig. 2.5b). The reorganization of disulfide bond inside a protein molecule can happen through two unlike mechanisms: a two-step procedure with a reduction reaction first (Fig. 2.5b) after an oxidation reaction (Fig. 2.5a) or a single-step isomerization procedure (Fig. 2.5c). Two oxidoreductase catalyzed reactions involved in the two-step mechanism: a reduction of a disulfide bond to two thiols and a catalyzed oxidation of thiols to make fresh disulfide bonds. The single-step mechanism contains only one isomerize which make the mixed



**Fig. 2.5** **a** Oxidation, **b** Reduction, and **c** Isomerization. The oxidoreductase and isomerase enzymes are represented by blue and red ovals, respectively

disulfide bond by isomerizing a disulfide bond. Reliant on the conformation of the protein molecule, the diverse disulfide bond might reply with an close cysteine residue, causing the creation of a novel disulfide bond [19].

## 2.10 Molecular Dynamic Simulation as a Strong Approach to Evaluating Structure Stability

Since the first molecular dynamics (MD) simulations of a protein described, it has been 25 years. Throughout this period, in the investigation of biomolecules, matching to experimental methods dynamics simulations have become a well-known tool.

Three major areas of application can be found in bio molecular dynamics simulations nowadays. Firstly, MD simulation is used to bring biomolecular structures alive, giving insights into the natural dynamics on different timescales of biomolecules in solution. Secondly, MD simulation affords thermal averages of molecular properties. Based on the ergodic hypothesis, individuals are able to simulate a molecule or group of molecules under a specific environment for a period of time and the macroscopic properties can be calculated by time-averaging all the possible state of the molecules in a system and the set of all the possible states of the molecular system is known as ensemble. This is applied to calculate, for illustration, the specific properties of solutions and the free energy changes for biochemical procedures including ligand binding. Thirdly, MD can discover which conformations of a molecule or a complex are accessible thermally. This method is used for discovering conformational space, for example, in ligand-docking uses. Furthermore, available conformations of a molecule or a complicated are thermally can be find by MD simulation [20]. This way is used for discovering conformational space, for example, in ligand-docking applications. In addition data from experiments used for dynamics calculations, MD can suitably combine these data from experiment with the data about the overall properties of molecular structure that is embodied in the hundreds of factors of a molecular mechanics force field [21].

The MD field catches benefit from the apparently constant developments in computer hardware; simulations that were difficult for past processors can be done these days by normal office workstations.

Over the last decades molecular dynamics simulations have developed from a technique to investigate the dynamics of fluids of solid spheres and Lennard–Jones units to a multipurpose technique to investigate a comprehensive collection of systems at atomic level. The simple technique contains explaining the contacts of all atoms in a certain arrangement by a fairly empirical probable purpose, in this system the energy on whole atoms is able to be calculated and combined in time, by time steps of the order of 1,015 s. A trajectory is resulted as a main outcome and holds the movements of whole atoms in time, more than billions of time steps. Investigation of these movements provides vision into the system that is being

considered. Such simulations give entire information of the motions molecules in the system, and hence those and numerical mechanics, contact to thermodynamic properties. There are, however, major restrictions. A significant attention for the simulation application and to evaluation of the accuracy of simulations is the length and time scale on which the procedures of interest happen. Methodical limits related to the precision of the empirical possible work are existing as well, the comparatively shortens of the systems that can be simulated, and difficulties with exactly incorporating noteworthy variables including pH, transmembrane likely alterations, and little ion concentrations. The initial configuration of a simulation might bias the outcomes in unwanted approaches as well [22].

Simulation has many application for example in physics many system can be build up and simulated, the vast amount of date are produced in this field of science. The example of the simulation can be seen in the following papers which study behavior of ring resonators. The ring resonators are suitable for many applications in micro and nano optical communication. Optical soliton is a self-reinforcing solitary wave that maintains its shape while it travels at constant speed. Optical solitons are seen by a cancellation of nonlinear and dispersive effects in the medium which can be a fiber optic. In a Kerr effect medium such as fiber optics, high intensity of light causes a phase delay having similar temporal shape as the intensity. This nonlinear phenomenon occurs for a beam called self-phase modulation (SPM), which is generated by its intensity. Optical Chaos occurs in many nonlinear optical systems. One of the most common examples is a ring resonator. Chaotic behavior has been considered as a nonlinear property in physics, electronics, and communication. When a high-intensity short pulse is coupled to optical fiber, the instantaneous phase of optical pulse rapidly changes through the optical Kerr effect. The SPM and cross-phase modulation (CPM) effects change the phase of the pulse as a function of its intensity. Here, we derive the soliton equations based on solving the nonlinear Schrodinger and Maxwell equations. The main performance characteristics of ring resonators are transmittance, free spectral range, finesse, Q-factor, and group delay, which have been demonstrated [23–33]. It is interesting that these studies have application in biology by rapping bio cells [34].

## References

1. M. Schülein, Protein engineering of cellulases. *Biochim. Biophys. Acta* **1543**(2), 239–252 (2000)
2. A.L. Rodrigues, A. Cavalett, A.O.S. Lima, Enhancement of *Escherichia coli* cellulolytic activity by coproduction of beta-glucosidase and endoglucanase enzymes. *Electron. J. Biotechnol.* **13**, 1–9 (2010)
3. M.K. Bhat, Cellulases and related enzymes in biotechnology. *Biotechnol. Adv.* **18**(5), 355–383 (2000)
4. J.R. Mielenz, Ethanol production from biomass: technology and commercialization status. *Curr. Opin. Microbiol.* **4**(3), 324–329 (2001)
5. A.V. Gusakov, Alternatives to *Trichoderma reesei* in biofuel production. *Trends Biotechnol.* **29**(9), 419–425 (2011)

6. V.V. Morozova, A.V. Gusakov, R.M. Andrianov, A.G. Pravilnikov, D.O. Osipov, A. P. Sinitsyn, Cellulases of *Penicillium verruculosum*. *Biotechnol. J.* **5**(8), 871–880 (2010)
7. G.J. Kleywegt, J.Y. Zou, C. Divne, G.J. Davies, I. Sinning, J. Ståhlberg, T. Reinikainen, M. Srisodsuk, T.T. Teeri, T.A. Jones, The crystal structure of the catalytic core domain of endoglucanase I from *Trichoderma reesei* at 3.6 Å resolution, and a comparison with related enzymes. *J. Mol. Biol.* **272**(3), 383–397 (1997)
8. C. Divne, J. Ståhlberg, T.T. Teeri, T.A. Jones, High-resolution crystal structures reveal how a cellulose chain is bound in the 50 Å long tunnel of cellobiohydrolase I from *Trichoderma reesei*. *J. Mol. Biol.* **275**(2), 309–325 (1998)
9. G.T. Beckham, Y.J. Bomble, E.A. Bayer, M.E. Himmel, M.F. Crowley, Applications of computational science for understanding enzymatic deconstruction of cellulose. *Curr. Opin. Biotechnol.* **22**(2), 231–238 (2011)
10. O. Khersonsky, D.S. Tawfik, Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu. Rev. Biochem.* **79**, 471–505 (2010)
11. J.M. Woodley, Protein engineering of enzymes for process applications. *Curr. Opin. Chem. Biol.* **17**(2), 310–316 (2013)
12. Q. Zhang, J. Yang, K. Liang, L. Feng, S. Li, J. Wan, X. Xu, G. Yang, D. Liu, S. Yang, Binding interaction analysis of the active site and its inhibitors for neuraminidase (N1 subtype) of human influenza virus by the integration of molecular docking, FMO calculation and 3D-QSAR CoMFA modeling. *J. Chem. Inf. Model.* **48**(9), 1802–1812 (2008)
13. J.C. Joo, S.P. Pack, Y.H. Kim, Y.J. Yoo, Thermostabilization of *Bacillus circulans* xylanase: computational optimization of unstable residues based on thermal fluctuation analysis. *J. Biotechnol.* **151**(1), 56–65 (2011)
14. A. Illanes, A. Cauerhff, L. Wilson, G.R. Castro, Recent trends in biocatalysis engineering. *Bioresour. Technol.* **115**(2012), 48–57 (2012)
15. C. Vieille, G. Zeikus, Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. *Microbiol. Mol. Biol. Rev.* **65**(1, 517), 1–43 (2001)
16. O.R. Siadat, A. Lougarre, L. Lamouroux, C. Ladurantie, D. Fournier, The effect of engineered disulfide bonds on the stability of *Drosophila melanogaster* acetylcholinesterase. *BMC Biochem.* **7**, 12 (2006)
17. D.B. Volkin, A.M. Klibanov, Thermal destruction processes in proteins involving cystine residues. *J. Biol. Chem.* **262**(7), 2945–2950 (1987)
18. J. Messens, J.-F. Collet, Pathways of disulfide bond formation in *Escherichia coli*. *Int. J. Biochem. Cell Biol.* **38**(7), 1050–1062 (2006)
19. L. Zhang, C.P. Chou, M. Moo-Young, Disulfide bond formation and its impact on the biological activity and stability of recombinant therapeutic proteins produced by *Escherichia coli* expression system. *Biotechnol. Adv.* **29**(6), 923–929 (2011)
20. T. Hansson, C. Oostenbrink, W. van Gunsteren, Molecular dynamics simulations. *Curr. Opin. Struct. Biol.* **12**(2), 190–196 (2002)
21. E. Olkhova, Molecular dynamics simulations and hydrogen-bonded network dynamics of cytochrome c oxidase from *Paracoccus denitrificans* (2004)
22. W.L. Ash, M.R. Zlomislic, E.O. Oloo, D.P. Tieleman, Computer simulations of membrane proteins. *Biochim. Biophys. Acta* **1666**(1–2), 158–189 (2004)
23. S.E. Alavi, I.S. Amiri, H. Ahmad, A.S.M. Supa'at, N. Faisal, Generation and Transmission of 3×3 W-Band MIMO-OFDM-RoF signals using micro-ring resonators. *Applied Optics* **53**(34), 8049–8054(2014)
24. A. Nikoukar, I.S. Amiri, S.E. Alavi, A. Shahidinejad, T. Anwar, A.S.M. Supa'at, S.M. Idrus, L.Y. Teng, in *Theoretical and Simulation Analysis of The Add/Drop Filter Ring Resonator Based on the Z-transform Method Theory*. Presented at the The 2014 Third ICT International Student Project Conference (ICT-ISPC2014), Thailand (2014)
25. I.S. Amiri, J. Ali, Simulation of the single ring resonator based on the Z-transform method theory. *Quantum Matter* **3**(6), 519–522 (2014)

26. I.S. Amiri, S.E. Alavi, S.M. Idrus, A. Nikoukar, J. Ali, IEEE 802.15.3c WPAN standard using millimeter optical soliton pulse generated by a panda ring resonator. *IEEE Photonics J.* **5**(5), 7901912 (2013)
27. I.S. Amiri, S.E. Alavi, H. Ahmad, A.S.M. Supa'at, N. Fisal, Numerical computation of solitonic pulse generation for Terabit/Sec data transmission. *Opt. Quant. Electron.* (2014)
28. I.S. Amiri, S.E. Alavi, N. Fisal, A.S.M. Supa'at, H. Ahmad, All-optical generation of two IEEE802.11n signals for 2×2 MIMO-RoF via MRR system. *IEEE Photonics J.* **6**(6) (2014)
29. Optical wired/wireless communication using soliton optical tweezers. *Life Sci.* **10**(12) (2013)
30. A. Nikoukar, I.S. Amiri, J. Ali, Generation of nanometer optical tweezers used for optical communication networks. *Int. J. Innovative Res. Comput. Commun. Eng.* **1**(1), 77–85 (2013)
31. S. Alavi, I. Amiri, S. Idrus, A. Supa'at, J. Ali, Chaotic signal generation and trapping using an optical transmission link. *Life Sci. J.* **10**, 186–192 (2013)
32. I.S. Amiri, F.J. Rahim, A.S. Arif, S. Ghorbani, P. Naraei, Single soliton bandwidth generation and manipulation by microring resonator. *Life Sci. J.* **10**, 904–910 (2013)
33. I. S. Amiri, A. Nikoukar, A. Shahidinejad, T. Anwar, The proposal of high capacity GHz soliton carrier signals applied for wireless communication. *Rev. Theor. Sci.* **2**, 2327 (2014)
34. N. Suwanpayak, S. Songmuang, M.A. Jalil, I.S. Amiri, I. Naim, J. Ali, P.P. Yupapin, Tunable and storage potential wells using microring resonator system for bio-cell trapping and delivery. *Int. Conf. Enabling Sci. Nanotechnol.* (0), 1–2 (2010)

In Silico Engineering of Disulphide Bonds to Produce  
Stable Cellulase

Barati, B.; Sadegh Amiri, I.

2015, VIII, 48 p. 34 illus., 30 illus. in color., Softcover

ISBN: 978-981-287-431-3