

Carbonyl-Induced Enzyme Inhibition: Mechanisms and New Perspectives

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Abstract: While agents that cause carbonyl-induced modification include sugars, lipids and industrial and pyrogenic compounds, much of the existing literature describes the process of glycation. Protein glycation is recognized as a major post-translational modification that attends the pathogenesis of diverse diseases. Glycation arises from the reactivity of common carbohydrates, their metabolic intermediates and their oxidized byproducts. The hyperglycemia associated with diabetes and the life-long exposure to pro-glycating agents bring about an environment that favors the modification of diverse proteins resulting in macro- and micro-angiopathy and the neuropathy of misfolding disorders such as Alzheimer's disease. Numerous structural and catalytic proteins have been shown to be targets of glycation. The literature documents the potent inhibitory effects of glycation with very insightful suggestions on mechanisms of action. The current review describes the way carbonyl-containing (and particularly glycyating) agents react with protein residues elucidating mechanisms that include two broad categories: direct reaction (1) with active site residues and (2) with residues distinct from the active site. The consequence of active site modification involves obvious steric and chemical changes that are likely to be prohibitive. The modification of residues distinct from the active site suggests inhibitory mechanisms more subtle and complex. The current review presents new perspectives in this emerging field that has implications beyond enzyme inhibition, such as the cellular impact of protein insolubility and aggregation.

Keywords: Glycation, advanced glycation endproducts (AGEs), enzyme inhibition, post-translational modification; active site.

INTRODUCTION

Carbonyl-induced modification includes sugars and oxidized lipids typically referred to as glycation (or glycooxidation to reflect autooxidation of carbohydrates) and lipoxidation, respectively. Industrial and pyrogenic compounds (i.e. from smoking and air pollution) represent additional carbonyl-containing agents capable of modifying macromolecules. Protein glycation (the unwanted modification of proteins that occurs in aging) provides a unique approach to studying enzyme inhibition and may give insights into the specificity of tissue decline in diabetes and in aging. A large number of proteins are affected by glycation. The susceptibility to glycation appears to be a function of: 1) the type of glycyating agent [1]; 2) the presence of substrate and cofactors [2]; 3) the levels of anti-glycation compounds like carnosine [3]; 4) and metabolizing enzymes like glyoxalases, aldose/aldehyde reductase, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase [4-6]. An enzyme's susceptibility to glycation is also attributed to the protein's structural characteristics, for example, the number and type of accessible and reactive amino acid residues.

The classical model for glycation involves an initial Schiff base reaction between the glucose carbonyl and the protein's lysyl amino group rearranging to an Amadori product called fructosyl-lysine. Strecker degradation of this adduct create protein carbonyls, and auto-oxidation of unreacted sugars yield dicarbonyls, which also react with proteins particularly arginines. Sodium borohydride is used

to stabilize the early glycation product in order to identify the specific hit site(s) on the enzyme. Following prolonged incubation in a redox active environment and/or with transition metal ions, diverse products form and these are called advanced glycation endproducts (AGEs).

A vast array of carbonyl-containing agents (Table 1) exists in vivo with varying degrees of reactivity and specificity. This list while incomplete is nonetheless representative of the literature. These agents may be useful as probes for examining specific changes in catalytic function of enzymes. Modification of functional groups may identify the crucial residues involved in catalysis. The use of synthetic reagents has been the method of choice in studying enzyme inhibition and examples include N-ethylmaleimide for sulfhydryls [7], carbodiimides for carboxyls [8], diethylpyrocarbonate for histidine [9] and fluorescein isothiocyanate for amines [10]. Endogenous glycyating agents also exhibit some degree of specificity. Many are quite small making them easily accessible to surface residues and to active site crevices. Generally, the targets for modification for the mono-carbonyls are the protein terminal -amino groups and the -amino groups of lysyl residues. However, nucleophilic centers of other amino acids provide potential hit sites (i.e. arginine, histidine, cysteine and tryptophan). The glycyating agents react by virtue of their carbonyls though considerable structural heterogeneity exists suggesting that enzyme selectivity can be engineered in designing specific inhibitors.

Interestingly, AGEs may play a beneficial role in the immune response and in tissue remodeling following an injury. From genetic deficiency studies and knock-out models one appreciates the delicate balance of production and destruction of AGEs. Patients with deficiencies in glyoxalase

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present with an unusually high level of AGEs [11], putting them at risk for nephropathy. On the other hand in the NADPH oxidase knock-out in mice phagocyte-derived AGEs are diminished [12] and thus may interfere with immune response. Generally, protein glycation is an unwanted event that typically has undesirable consequences.

Table 1. List of Diverse Carbonyls and their Properties

Source	Size	Name	Type
Synthetic	5C	glutaraldehyde	delta-dicarbonyl
	8C	phenylglyoxal	alpha-dicarbonyl
Carbohydrates	2C	glyoxal	alpha-dicarbonyl
	2C	glycolaldehyde	carbonyl (aldehyde)
	3C	dihydroxyacetone (P)	carbonyl (ketone)
	3C	methylglyoxal	alpha-dicarbonyl
	3C	glyceraldehyde (3P)	carbonyl (aldehyde)
	5C	ribose (5P)	carbonyl (aldehyde)
	6C	glucosone	alpha-dicarbonyl
	6C	deoxyglucosone	alpha-dicarbonyl
	6C	fructose (6P)	carbonyl (ketone)
	6C	glucose (6P)	carbonyl (aldehyde)
	6C	galactose (6P)	carbonyl (aldehyde)
Lipids	3C	malondialdehyde	beta-dicarbonyl
	3C	acrolein	carbonyl (aldehyde)
	9C	4-hydroxy-2-nonenal	carbonyl (aldehyde)
Alcohol	2C	acetaldehyde	carbonyl (aldehyde)
Smoking	4C	crotonaldehyde	carbonyl (aldehyde)

Diseases related to glycation include: complications of diabetes, neurodegenerative disorders of aging and osteoarthritis. Glycation-induced cross-linking of vascular wall components decreases distensibility and strength [13] contributing to the cardiovascular complications in diabetes. Furthermore, glycated LDLs prompt the formation of atherosclerotic plaques [14] and increased renal tissue levels of AGEs contribute to diabetic neuropathy [15]. Alzheimer's and Huntington's diseases are protein misfolding disorders whose degenerative properties relate to protein glycation. Affected brain areas contain AGEs, which correlate with cell apoptosis [16]. Cells with receptors for AGEs, or so-called RAGE, release cytotoxic mediators in the presence of AGEs. Expression of RAGE increases in Huntington's [17]. AGEs play a role in the progression of osteoarthritis. Accumulation of AGEs decreases cartilage turnover and reduce mechanical properties. Dogs, which were injected with ribose to promote the formation of AGEs, have more pronounced osteoarthritis as indicated by increased collagen damage and enhanced release of proteoglycans [18].

The present review focuses on the enzyme inhibition effects of carbohydrate-derived compounds (Table 1). Identification of enzymes susceptible to glycation and inactivation may lead to opportunities to design structures specific to target enzymes that contain elements of selectivity

but also have effectively placed reactive carbonyls that disable the enzyme in target cells such as those that are malignant or parasitic.

PREVENTION OF GLYCATION-INDUCED ENZYME INHIBITION

N-Acetylation appears to have a protective effect against protein glycation. Acetylsalicylic acid, or aspirin, prevents the glycation-induced inhibition of delta-aminolevulinic dehydratase and porphobilinogen deaminase in streptozotocin-induced diabetic mice [19]. These are enzymes in the heme synthesis pathway. Aspirin inhibits protein glycation by acetylation of protein amino groups. Interestingly, carnosine, which is considered an endogenous anti-glycation agent [20], is acetylated in vivo presumably to modulate its activity [3]. Further research is necessary in this area to fully understand the factors involved in controlling reversible protein acetylation and the role it plays in preserving enzyme activity.

Agents that prevent glycation can come in many forms. Aminoguanidine represents the classical AGE-inhibitor by virtue of its ability to scavenge carbonyls. Additionally, AGE-breakers such as Alton 711 (4,5-dimethyl-3-phenacylthiozolium chloride) cleave the adduct following the formation of final products. Additionally, enzymes such as amadoriase and fructosamine 3-kinase have been identified to intervene in terminating AGE formation. Furthermore, diverse anti-oxidants can prevent auto-oxidation of native sugars stopping their decomposition to dicarbonyls.

GLYCATION-INDUCED INHIBITION OF DIVERSE ENZYMES

Diverse enzymes are modified by glycation (Table 2). This table is meant to illustrate the breadth of diversity regarding cell activity and enzyme function. Studies on glycation-induced loss of enzyme activity involve in vitro conditions as well as in vivo protocols that involve harvesting and analyzing enzymes from diabetic humans and laboratory animals. While some enzymes are tissue specific like the pancreatic/liver glucokinase and the erythrocyte bisphosphoglycerate mutase, there are many that are found in all tissues.

The enzymes in Table 2 are divided into broad categories that have been selected simply based on the prevalence in the literature: bioenergetics, growth and repair, glutathione system, protection, red blood cells and blood coagulation. Of particular interest is the glutathione system, which appears quite susceptible to functional decline during "carbonyl stress". As a compensatory mechanism, glycation products such as *N*-carboxymethyl-lysine induce the expression of the gamma-glutamylcysteine synthetase gene [46], suggesting an increase in defense response and replacement of functional glutathione. It is important also to note that many enzymes do not lose their activity following exposure to glycating agents. For example, the kinetic properties of glycated phosphoglucoisomerase remains unaltered although modification of side chains occurred [47]. Additionally, enzymes like glyceraldehyde 3-phosphate dehydrogenase (GAPDH) appear to show some selectively in

Table 2. Many Enzymes with Different Cellular Roles are Affected by Carbonyl-Containing Agents

Cell Activity	Enzyme	Carbonyls	Reference
bioenergetics	glucokinase	glyceraldehyde	[21]
	2-oxoglutarate dehydrogenase	phenylglyoxal	[22]
	alanine aminotransferase	glyceraldehyde	[23]
	aspartate aminotransferase	glyceraldehyde 3P	[24]
	lactate dehydrogenase	methylglyoxal	[1]
	glyceraldehyde 3P dehydrogenase	methylglyoxal	[25]
	creatine kinase	in vivo	[26]
	Na ⁺ /K ⁺ -ATPase	glucose 6P	[27]
growth/repair	DNA polymerase	methylglyoxal	[28]
	ribonuclease A	methylglyoxal	[29]
	alkaline phosphatase	in vivo	[30]
	acylphosphatase	phenylglyoxal	[2]
glutathione system	glutathione reductase	hexoses	[31]
	glutathione peroxidase	diabetic rats	[32]
	glucose 6P dehydrogenase	methylglyoxal	[33]
	gamma-glutamyl transferase	diabetic rats	[32]
	glutathione S-transferase	diabetic mice	[34]
	gamma-glutamylcysteine synthetase	diabetic humans	[35]
protection	aldehyde reductase	in vivo	[4]
	glyoxalase	diabetic rats	[32]
	sorbitol dehydrogenase	glucose, fructose	[36]
	catalase, superoxide dismutase	ribose, fructose	[37]
	<i>p</i> -hydroxybenzoate hydroxylase	phenylglyoxal	[38]
	alcohol dehydrogenase	in vivo	[39]
red blood cells	bisphosphoglycerate mutase	diabetic humans	[40]
	delta-aminolevulinic dehydratase	glucose	[41]
	porphobilinogen deaminase	diabetic mice	[19]
	carbonic anhydrase	acrolein	[42]
blood coagulation	alpha 1-proteinase inhibitor	acrolein	[43]
	plasminogen	methylglyoxal	[44]
	antithrombin III	glucose	[45]

terms of which isoforms are susceptible to which glycation agents (see below).

CONFORMATIONAL DEPENDENCE OF SUSCEPTIBILITY TO GLYCATION

The sarcoplasmic reticulum Ca²⁺-ATPase in muscle is a member of the P-type transport ATPases, which include the Na⁺/K⁺-ATPase and gastric H⁺/K⁺-ATPase. These enzymes

share the general mechanism of catalysis and transport. They exist in two conformations: E1, cation binding sites face the cytosol; and E2, cation binding site face the inner lumen. Glycation-induced loss of activity of the cardiac Ca²⁺-ATPase is conformationally dependent [48]. During the catalytic cycle in which the protein translocates the Ca²⁺ ion and hydrolyzes ATP, the enzyme toggles between the E1 and E2 conformation [49]. In the presence of Ca²⁺ without ATP the protein can be kept in the E1 conformation, which

imparts susceptibility to glucose 6-phosphate-mediated glycation and inactivation. This conformation may allow for the accessibility of certain crucial lysine residues. Thirteen amines on the cardiac Ca^{2+} -ATPase are modified by glucose 6-phosphate [48]. The protein moves in and out of the lipid bilayer during conformational transitions [50] regulating the accessibility of target amines.

Three types of amines are identified in the Na^+/K^+ -ATPase [27]. Their susceptibility differs in the different conformations. Types B and C are more readily modified than type A amines when the enzyme is in the E1 conformation. In the E2 conformation type A amines now become more reactive than type B and C amines. The type A amines appear to be involved in nucleotide binding at the low-affinity binding site. Type C amines are at the active site and readily modified by fluorescein isothiocyanate [51]: K501 and K714. They are glycatable in the absence of the substrate ATP. As a result of glycation of these residues activity is significantly inhibited. Type A amines are those that upon glycation leads to substrate inhibition kinetics. Glycation of type B amines are associated with loss of measurable K^+ occlusion, a necessary step in the transport event.

ACTIVE SITE/BINDING SITE MODIFICATION

Glycolysis is the major pathway for carbohydrate breakdown. It occurs in all cells and thus regulates cellular and extracellular levels of glucose and its metabolites. Glycation and inhibition of the enzymes in this pathway would accelerate cell decline by allowing for the increase in levels of glycating agents. The intermediates of glycolysis are potent glycating agents and optimal enzyme function controls their steady state levels. In discussing active site modification there are several bioenergetic enzymes that exhibit susceptibility to glycation.

Glyceraldehyde inactivates pancreatic glucokinase and substrate prevents inactivation [21] suggesting active site modification. This inhibition appears linked to decreases in insulin secretion. Interestingly, under the same conditions glyceraldehyde has no effect on other glycolytic enzymes: hexokinase, glucose 6-phosphate isomerase, phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate kinase. These observations suggest that glucokinase may play a role in the pathophysiology of type II diabetes.

Fructose does inactivate GAPDH [52] suggesting that the glycolytic pathway in hexokinase-containing cells may be at risk of inhibition. Interestingly, the anti-oxidant pyruvate prevents fructose-mediated enzyme inhibition indicating that dicarbonyl derivatives from the auto-oxidation of fructose may be responsible for the inactivation of GAPDH. The primary sites of glycation are near the active site and may change the microenvironment around the crucial C149 residue [53]. The GAPDH from Ehrlich ascites carcinoma cells uses an essential lysine residue in the catalytic process [54] suggesting a difference in the nature of the active site compared with rabbit muscle GAPDH. An altered GAPDH may be related to the high glycolytic rates in malignant cells. Interestingly, methylglyoxal inhibits the GAPDH from malignant tissues but not from normal tissues or benign

tumors [33]. This difference suggests an opportunity for developing diagnostics tools and therapeutic interventions.

The bisphosphoglycerate mutase in human erythrocytes acts as a shunt from glycolysis and controls the levels of 2,3-diphosphoglycerate, which plays a role in the dissociation of oxygen from hemoglobin. Diabetics exhibit a glycated enzyme that is less active [40]. In vitro glycation of bisphosphoglycerate mutase results in the modification of K2, K4, K17, K42, K158 and K196, although it appears that glycation site K158 is primarily responsible for loss of activity as it is close to the substrate-binding site.

Phenylglyoxal, which is a synthetic dicarbonyl, is useful as an arginine reagent. The coenzyme thiamine pyrophosphate prevents pyridoxal 5-phosphate-mediated but not phenylglyoxal-mediated inhibition of α -ketoglutarate dehydrogenase, suggesting that the coenzyme binding site likely carries a lysyl residue but not an arginine residue [22]. A crucial arginine residue resides in an active site of this multi-enzyme complex. Phenylglyoxal also reacts with the active site arginine in *p*-hydroxybenzoate hydrolase rapidly inactivating the enzyme [38]. Additionally, phenylglyoxal inhibits acylphosphatase with an apparent first order rate constant reacting with a single arginine residue, which is protected in the presence of inorganic phosphate or ATP [2]. These studies suggest that carbonyl-containing agents may be useful probes in examining active sites particularly in identifying the role of specific residues. Additionally, carbonyl-containing agents are also valuable in studying enzyme-substrate interactions.

Diverse mono-aldehydic compounds (acrolein, glutaraldehyde and crotonaldehyde) produce DNA-histone crosslinks [55]. Methylglyoxal, which is an α -dicarbonyl, crosslinks DNA polymerase I to its substrate DNA [28]. The 2'-deoxyguanosine of template DNA becomes covalently linked to a lysine or cysteine residue of the active site of the DNA polymerase. The authors observed that *N*-acetyllysine and *N*-acetylcysteine are the only amino acid derivatives that cross-link to 2'-deoxyguanosine in the presence of methylglyoxal. Interestingly, the arginine derivative does not favor cross-linking. While methylglyoxal readily reacts with arginine residues [56, 57], it's likely that this reaction creates an irreversible imidazolone that is stable [58] and not likely to form cross-links.

Calmodulin, which has been glycated, exhibits a decreased ability to activate target enzymes such as adenylate cyclase, phosphodiesterase and protein kinase [59]. Glycated calmodulin has a decreased Ca^{2+} -binding capacity, but the Ca^{2+} -induced change in alpha helical content and the radius of gyration is the same for glycated and unmodified calmodulin. Hence, only two of the four Ca^{2+} binding sites – at domains II and IV – need to bind Ca^{2+} in order to promote conformational change, which is not hindered by glycation. While minimal structural effects occurred due to glycation, the presence of lysine-bound AGEs interfere with the binding site of the target enzymes.

DECREASED PEPTIDE CHAIN FLEXIBILITY

The extent to which conformational change occurs during the enzymatic event does vary considerably. Almost

universally, however, there is at least some molecular movement in proteins during catalysis: firstly, in order to accommodate substrate or cofactors/coenzymes; next, in achieving the transitional state; and lastly, in the release of product(s). A decrease in peptide chain mobility or flexibility would certainly have an effect on enzyme kinetics.

Extensive glycation (5 mM ribose 5-phosphate for 64hr at 37°C) of aspartate aminotransferase decreases 1-anilinonaphthalene 8-sulfonate (ANS) binding [60]. One plausible interpretation of this observation is that the molecular movement necessary for ANS binding to the hydrophobic regions of the protein is restricted due to chemical modification. Additionally, acrolein (2 mM; 17.5hr; 37°C) [61] and glyceraldehyde (0.5 mM; 72hr; 37°C) [62] increase T_m , which is the temperature at 50% denaturation. The observation that carbonyl modification suppresses thermal-induced protein unfolding/aggregation is consistent with the model of decreased flexibility. Loss of enzyme activity correlates with increased T_m when a concentration range of glycation agent was tested [60], suggesting that enzyme inhibition may be due to the proposed decreased flexibility of the protein. NMR and fluorometry may provide further insight into this proposed phenomenon of carbonyl-induced rigidification. Formaldehyde and glutaraldehyde are common fixatives, and it is likely that other carbonyl-containing agents (Table 1) may act to some degree accordingly.

Interestingly, under mild glyceraldehyde-induced glycation (500 μ M; 210min at 37°C and 125 μ M; 180min at 37°C) ANS binding increases and enzyme activity increases, respectively, suggesting that mild glycation conditions may promote protein flexibility and thus increase catalytic activity [60].

The reaction K_m for glycated glutathione peroxidase increases 3-fold compared to the non-glycated enzyme [63]. The glycation site, K110, is a lysine residue at the surface of the protein about 15 angstrom from the active site. Modification may be decreasing the conformational flexibility of the enzyme needed to accommodate substrate.

The rigidification of glycated proteins may be in part attributed to cross-linking both intra- and inter-molecular. Free amino groups from the N-terminus or from lysine residues are required for protein cross-linking as evidenced by incubation of α -dicarbonyls (methylglyoxal, glyoxal) with lysine-free peptides (that contain arginine) and arginine-free peptides (that contain lysine) [29]. Protein engineering studies demonstrate that there is a stabilizing effect following lysine to arginine substitutions as evidenced by the K253R substitution in glucose isomerase [64] and the K9R substitution in superoxide dismutase [65]. These substitutions may minimize the chance of protein cross-linking by glycation agents. Protein cross-linking typically accompanies glycation-induced loss of enzyme activity [1, 24, 61] and anti-crosslinking agents such as carnosine [20] also prevent inhibition [66]. However, dicarbonyl-initiated cross-linking can be stopped via aminoguanidine or 3,5-dimethylpyrazole without concomitant preservation of enzyme function [29], which would suggest that the target site involves the active site.

Interestingly, glycation of HDL modifies apolipoprotein conformation and solvation causing a decrease in fluidity and

hence decreases its affinity to the cholesteryl ester transfer protein [67] and decreases enzyme activity [68]. Similarly, glycation of aldehyde reductase at K67, K84 and K140 decreases catalytic efficiency (k_{cat}/K_m) [4], suggesting that conformational transitions may be slowed by glycation-induced molecular rigidification.

Tissue flexibility represents the fluidity of the attendant molecules. The fluidity of brush border membrane, for example, from streptozotocin-induced diabetic rats decreases relative to controls and the rigidification correlates with the appearance of AGEs [32]. In addition to diabetes, aging is a good model for AGE production. One of the effects of aging include a decrease in the speed of actin filaments propelled by type I myosin in humans [69]. This observation suggests that glycation may play a role. As an evidence for this hypothesis, glucose cross-links myosin, which correlated with a decrease in motility speed [70].

The alkaline phosphatase from the renal cortical brush border membrane of diabetic rats exhibits an 81% increase in K_m with no change in V_{max} [32]. This observation suggests that the glycation-induced chemical events decrease the conformational mobility in catalysis at low substrate concentration.

PROTEIN COMPACTION

One novel proposal is that glycation (or carbonyl-induced modification) causes protein compaction, a concept that provides exciting opportunities for creative experimental approaches. Open pockets or crevices in enzymes are determined in part by the internalization of hydrophobic regions, which fold into substructures that exclude water. The open spaces are occupied by water and can represent binding sites for cofactors and substrates. The surface topology largely includes hydrophilic residues that help maintain the native structure of the enzyme and define areas for protein-protein interaction. As a result of intra-molecular cross-linking by AGEs, progressive localized compaction may result that is likely to be dysfunctional. Furthermore, introducing AGEs, which are hydrophobic, would also promote a local condensation of substructures. Evidence for this hypothesis is the observation that methylglyoxal (500 μ M; 0-50min at 37°C) causes a redshift (max from 388nm to 417nm) in protein fluorescence (ex. 280nm) [71] due to increased solvent access to interior fluorophores. Localized compaction may create surface to interior channels and may impair conformational transitions during enzyme catalysis. The thermodynamic constraints associated with the creation of local condensation of newly formed hydrophobic AGEs [62] may inhibit the necessary domain movement during catalysis.

Circular dichroism, which assesses secondary structure, is a useful tool for examining the effects of protein glycation. Glycated GAPDH exhibits a change in its circular dichroism spectrum indicating a significant change in secondary structure and that kinetic inactivation of glycated GAPDH is biphasic [72] suggesting a localized compaction of the protein. The circular dichroism spectrum of acylphosphatase changes following phenylglyoxal modification of non-essential arginine residues [2] suggesting that changes in secondary structure could lead to compaction.

At high concentrations of glycation agent (2 mM methylglyoxal at 37°C) the emission intensity of intrinsic (or tryptophan) fluorescence (ex. 280nm; em. 388nm) of the aspartate aminotransferase decreases to approximately 50% in 25min [71]. Additionally, acrolein (2.5 mM at 37°C) decreases intrinsic fluorescence of aspartate aminotransferase by 50% in 19hr [61]. These observations indicate that carbonyl-induced modification decreases the distances between the intrinsic fluorophores (tryptophans) the quenching fluorophore, which is the bound pyridoxal 5-phosphate, suggesting that global compaction of the protein occurred. Interestingly, the decrease in the emission intensity correlates with the decrease in enzyme activity [71] suggesting that the glycation-induced change in protein topography contributes to enzyme inhibition.

PROTEIN AGGREGATION

Sites of glycation on proteins act as trapping agents that bind transition metals [73] and proteins [74], which may provide a nucleation center for extra-cellular plaque or intra-cellular inclusion body formation. Glycation of albumin promotes the formation of cross-beta structures that lead to aggregation [75]. Methylglyoxal-induced glycation of α -crystallin causes aggregation as evidenced by increased 90° light scattering [76]. Incubation of acrolein and albumin can result in large structures, which are visible under light microscopy, suggesting that they may be polyacrolein-albumin aggregates [77]. Carnosine, which is an endogenous dipeptide, may act as a disaggregating agent [62,76], and α -alanine reactivates heat-denatured lactate dehydrogenase [78], suggesting some promising areas of future research for small molecular weight chemical chaperones that may exhibit preventive, disaggregating, stabilizing, refolding and even reactivating properties.

SUMMARY

This review covered many of the current observations of carbonyl-induced enzyme inhibition with particular emphasis on glycation. A vast array of glycation agents exist that may be useful probes for examining specific changes in the catalytic function of enzymes. Modification of functional groups may identify the crucial residues involved in catalysis as well as the molecular motion involved. This review described the effects of glycation on enzyme inhibition discussing new perspectives, which included glycation-induced compaction and loss of protein flexibility. Protein aggregation, which is due in part by glycation-induced misfolding, was also described. Elucidation of the mechanism of enzyme glycation and inactivation may lead to opportunities of designing glycation agents specifically engineered to target enzymes and that contain elements of selectivity with strategically placed reactive carbonyls that disable enzymes in target cells such as those that are malignant or parasitic.

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