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Metal-based methods for protein inactivation

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Metal complexes are efficient catalysts for protein inactivation. Because of their special reactivity, metal-based molecules can achieve results that are difficult, if not impossible to obtain with more traditional, organic-based inhibitors. This article summarizes the chemistry of hydrolytic and oxidative catalysts for protein inactivation.

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Introduction

Protein inhibition is an indispensable tool in biological research and medicine. To achieve inhibition, molecules are designed to bind selectively to their target. Inhibition can be reversible or irreversible. In the case of reversible inhibition, the inhibitor binds to its target using non-covalent interactions. The downfall of this strategy is that it is reversible and can require high concentrations of inhibitor to achieve the desired effect. Irreversible inactivation, also known as suicide inhibition, involves irreversible formation of a covalent bond between the inhibitor and protein. Suicide inhibition is less common in pharmaceuticals because of concerns over toxicity and immunogenicity, although successful examples in medicine still exist [1]. Importantly, both strategies require a stoichiometric amount of inhibitor. In contrast, the catalytic inactivation of proteins represents an attractive approach for inhibition, because it is irreversible and can occur with low concentrations of inhibitor (Figure 1a). In some cases catalytic inhibition can even be carried out with substoichiometric amounts of inhibitor relative to the target protein. Because of these attributes, catalytic inactivation may represent the next logical step for method development in creation of highly effective biological tools and chemotherapeutic agents.

This article summarizes catalytic, metal-based methods for protein inactivation. Although a number of attractive methods are available for using metal-based compounds as stoichiometric inhibitors, only catalytic methods are described herein. Metal-based stoichiometric inhibitors are described elsewhere, in several comprehensive review articles [2••,3••]. Likewise, this article will not cover applications of organic-based chromophores or fluorophores in protein inactivation. Together with their inorganic counterparts, organic compounds for chromophore-assisted light inactivation (CALI) are powerful tools for inactivating proteins selectively [4–15].

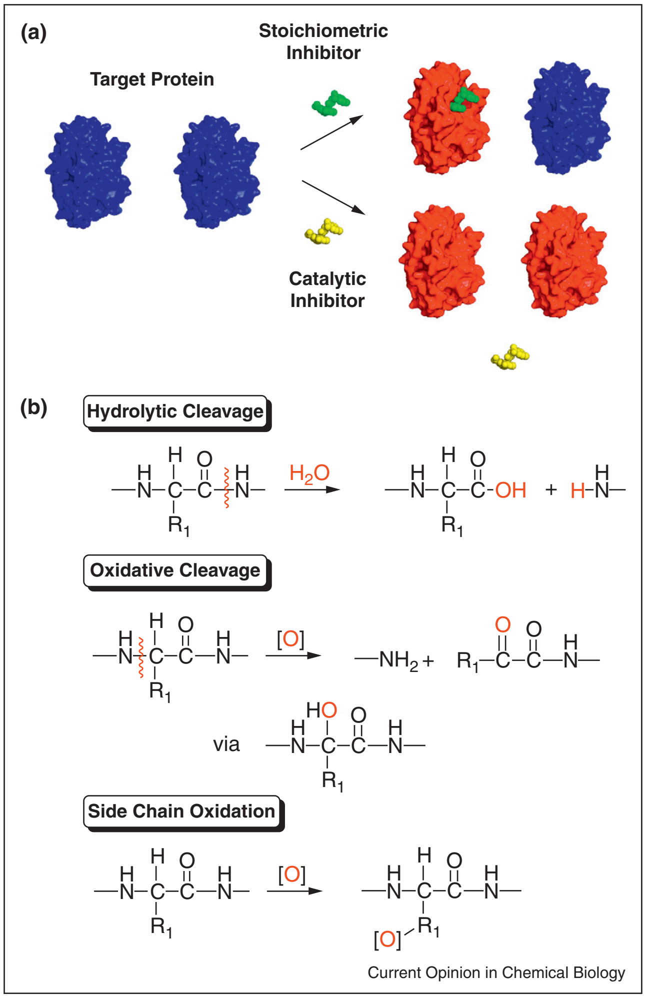
Overview

Metal catalysts are ideal for protein inactivation because of their reactivity. These catalysts can be classified as hydrolytic complexes, which attack the amide bonds of the peptide backbone, or oxidative, which cleave the peptide backbone or oxidize amino acid side chains (Figure 1b). Both hydrolytic and oxidative methods have proven to be effective in peptide and protein inactivation. In either case, the strategy to achieve selectivity is the same. Catalytically active metal complexes are tethered to protein-affinity ligands. This creates a higher effective molarity of the complex in the presence of the target protein relative to other non-target proteins, which often garners selectivity for inactivation.

Hydrolytic methods

Complexes of copper [16–18], cobalt [19•,20,21•] and palladium [22–24] have shown efficacy for catalyzing amide bond hydrolysis near physiological pH [17,25]. In particular, Co^{III} and Cu^{II} complexes of cyclen have proven to be effective for cleaving peptides and proteins [26,27]. For example, selective destruction of myoglobin was observed in the presence of other proteins, which proves that selectivity can be achieved when a catalyst is attached to the appropriate protein-affinity ligand [19•]. Cleavage of peptide deformylase was demonstrated with the Co^{III} catalyst **1** (Figure 2) [21•]. The cleavage site was identified to be between residues Gln(152)-Arg(153), which agrees well with data from docking simulations. In this case the putative nucleophile, a Co^{III}–OH species that attacks the amide bond, is situated close to the carbonyl of the Gln(152). In a related approach, pendant aldehydes were designed into these cyclen-derived catalysts. Aldehydes anchor reversibly to the target proteins through the formation of a Schiff base with lysine side chains exposed on target protein surfaces [28,29]. Beyond proteins, these catalysts cleave numerous peptides including β-amyloid peptide [20] and human islet amyloid polypeptide [30,31], which are associated with Alzheimer's disease and type 2 diabetes mellitus, respectively.

Figure 1



(a) The concept of stoichiometric vs. catalytic inhibition. Active protein is shown in blue, inactive in red. **(b)** Methods for protein inactivation; proteins can be inactivated by hydrolysis of the amide backbone or by oxidation. Oxidative cleavage can occur through hydroxylation of the α-CH group. Alternatively, proteins can be inactivated through oxidation of their side chains.

Oxidative methods

When peptides or proteins are exposed to reactive oxygen species (ROS) including superoxide, hydroxyl radical and singlet oxygen, irreversible oxidation events can occur. These events include oxidation of amino acid side chains, formation of protein–protein cross-linkages, and oxidation of the polypeptide backbone, which can lead to protein fragmentation. Because of its significance in the progression of aging and neurodegenerative diseases, the oxidation of proteins by ROS has been thoroughly investigated and is well understood today [32–34]. Because ROS can permanently damage proteins, catalysts that generate ROS have applications in catalytic protein inactivation. In contrast to ROS, very little is known about the oxidation of proteins by metal-based oxidants, where the reactive oxygen atom is bound to the metal center and does not diffuse away from the catalyst. The following sections are separated according to catalysts that generate ROS, and those where metal-based oxidants have been implicated.

Protein inactivation by ROS

Concerning oxidizing metal complexes, early examples showed that metal catalysts could be delivered to proteins by tethering a metal-binding domain to a protein-affinity ligand. Importantly, these ligand–catalyst conjugates exhibited enhanced cleavage of the protein backbone when compared with the free catalyst (Figure 3). Examples include the Cu^I complex **3** based on 1,10-phenanthroline for targeting carbonic anhydrase (**3**) [35], as well as Fe^{III} EDTA-based reagents for targeting calmodulin (**4**) [36] and streptavidin (**5**) [37]. These catalysts cleaved proteins by generating ROS, whose reactivity with proteins is well understood. In each of these cases, dioxygen was used in the presence of reductants such as ascorbate or reducing thiols.

More recently, catalytic inactivation of several target proteins was achieved using metal-based inhibitors and CALI. Rather than using reductants in the presence of dioxygen, this strategy utilizes a photocatalyst that generates singlet oxygen, which is a known inactivator of proteins. Most notably, selective inactivation of extracellular and intracellular protein targets was demonstrated [38••]. By taking advantage of parallel synthesis and a one-bead one-compound screening approach, peptoids were discovered that selectively bind to the vascular endothelial growth factor receptor 2 (VEGFR2) and the ATPase protein Rpt4, which interacts with the 26S proteasome. The efficient photosensitizer Ru^{II}(tris-bipyridyl)$^{2+}$ was attached to these peptoids. In the presence of light, the peptoid–ruthenium conjugates (ex. **6** for VEGFR2, Figure 3) inactivated VEGFR2 and the 26S proteasome selectively in cultured cells, at up to three orders of magnitude lower concentration than under dark conditions. Importantly, the metal-based photosensitizer $Ru^{II}(bpy)_3^{2+}$ showed advantages over competing organic photocatalysts by being more resistant to photobleaching [39].

Metal-based oxidants

Apart from previous examples that generate ROS, one early report was consistent with protein cleavage occurring by a metal-based oxidant. The Ni^{II}-bound tripeptide Gly-Gly-His was tethered to trifluoroperazine in a similar fashion to the EDTA conjugate **4**. In the presence of oxidant, site-specific cleavage of calmodulin was observed [40]. Cleavage occurred in the presence of catalase and was not affected by O_2, which is consistent with a metal-based oxidant being at play. Despite these attributes, the identity of the metal-based oxidant was not established.