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Cobalt Derivatives as Promising Therapeutic Agents

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Abstract

Inorganic complexes are versatile platforms for the development of potent and selective pharmaceutical agents. Cobalt possesses a diverse array of properties that can be manipulated to yield promising drug candidates. Investigations into the mechanism of cobalt therapeutic agents can provide valuable insight into the physicochemical properties that can be harnessed for drug development. This review presents examples of bioactive cobalt complexes with special attention to their mechanisms of action. Specifically, cobalt complexes that elicit biological effects through protein inhibition, modification of drug activity, and bioreductive activation are discussed. Insights gained from these examples reveal features of cobalt that can be rationally tuned to produce therapeutics with high specificity and improved efficacy for the biomolecule or pathway of interest.

Introduction

The clinical success of inorganic drugs, such as platinum (II) chemotherapeutics and gold-containing antiarthritic agents, has significantly advanced the use of transition metals in medicine in recent years [1,2]. Numerous transition metals (including cobalt) can adopt a wide variety of coordination numbers, geometries, oxidation states, and ligand binding affinities that can be exploited in the development of innovative therapeutic drugs [2,3,4**]. Despite their well-known versatility, cobalt derivatives have not been studied extensively as inorganic pharmaceuticals as compared to other metals. To date, the only cobalt-based therapeutic that has reached clinical trials is Doxovir, a Co(III) Schiff base complex effective against drug-resistant herpes simplex virus 1 [5]. The mechanism of action of Doxovir, however, is not fully understood.

A substantial amount of literature on bioactive cobalt derivatives has been published in the last decade, demonstrating its rich potential in medicinal applications [6,7**]. However, the rationale behind the design and mechanisms of many of these agents has not been clearly elucidated. An understanding of how the unique properties of cobalt complexes interplay with biology to elicit therapeutic effects is clearly necessary for the development of cobalt-based drugs. To this end, we highlight recent articles that address the mechanisms of action of specific cobalt coordination complexes rather than surveying all the bioactive cobalt

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agents reported. Particular attention has been given to published examples wherein the effects of the physicochemical properties of cobalt are investigated to emphasize and understand the advantages of its use in therapeutic applications (Figure 1).

Cobalt Complexes Reactive to Ligand Exchange

The promising antiviral activity of Doxovir is attributed to the direct interaction of the Co(III) Schiff base complex with proteins involved in viral penetration [5]. While the exact mechanism of action of Doxovir is unknown, studies have suggested that this class of Co(III) complexes, $[\text{Co}(\text{acacen})(\text{L})_2]^+$, interacts with proteins by coordinating histidine (His) residues through a dissociative exchange of the labile axial ligands, L (e.g. L = 2-methylimidazole [2MeIm] in Doxovir) (Figure 2) [8,9].

Our lab has been investigating the application and mechanism of $[\text{Co}(\text{acacen})(\text{L})_2]^+$ as selective inhibitors of His-containing proteins wherein the axial ligands are labile amines (L = NH_3) [8–10,11*,12]. Conjugation of targeting molecules such as peptides or oligonucleotides to the ancillary acacen chelate improves the specificity of the cobalt complexes for His residues in the proteins of interest. Such conjugates have been recently shown to inhibit Cys₂His₂ zinc finger transcription factors with remarkable specificity, namely the Snail and Gli family of transcription factors associated with embryonic development and cancer progression [10,11*,12]. Aberrant signaling by transcription factors has been implicated in the pathogenesis of diseases including cancer and inflammation and thus, such proteins are attractive drug targets [13,14]. In these examples, DNA sequences mimicking the native binding partners of the respective transcription factors were tethered to $[\text{Co}(\text{acacen})(\text{NH}_3)_2]^+$ for target selectivity. The conjugates were found to inhibit relevant pathways in *in vivo* embryo models and cancer cell lines [11*,12].

The mechanism of protein inhibition by $[\text{Co}(\text{acacen})(\text{NH}_3)_2]^+$ has been investigated spectroscopically by employing model peptides of the zinc finger proteins (MC Heffern *et al.*, unpublished and [8]). Significant changes in NMR ^1H resonances (>1 ppm) of His residues in the peptides were observed upon addition of $[\text{Co}(\text{acacen})(\text{NH}_3)_2]^+$. Furthermore, CD, NMR and electronic absorption studies correlated axial Co(III)-His coordination to structural perturbation of the zinc finger motif, likely due to displacement of zinc in the tetrahedral binding site by the octahedral Co(III) complex. These results demonstrated that His is preferentially coordinated to $[\text{Co}(\text{acacen})(\text{NH}_3)_2]^+$ and consequent perturbation of protein structure is likely to be responsible for the loss of activity.

In addition to zinc finger transcription factors, His-containing enzymes including thermolysin, α -thrombin and MMP-2 can be inhibited by $[\text{Co}(\text{acacen})(\text{L})_2]^+$ complexes [9,15,16]. Enzyme inhibition studies revealed that complexes with the most labile axial ligands (L = NH_3 or 2MeIm) exhibited the highest degree of inhibition [9]. Interestingly, analogous complexes with His mimics (such as imidazole and N-methylimidazole) as the axial ligands showed significantly lower inhibition [9]. We have recently correlated these observations with ligand exchange dynamics through spectroscopic and computational methods (LM Manus *et al.*, unpublished; LM Matosziuk *et al.*, unpublished). The lability of NH_3 and 2MeIm was attributed to a combination of pK_a of the N-donor and the steric interactions between the axial ligand and the acacen ancillary ligand. The relationship between enzyme inhibition and axial ligand lability validates the proposed dissociative ligand exchange mechanism for His coordination to Co(III).

This current understanding of Co(III) Schiff base complexes demonstrates their ability to directly affect protein function through ligand exchange. The properties of the complexes can be adjusted depending on the oxidation state and ancillary ligands, affording a versatile scaffold to target biomolecules of therapeutic relevance [4**].

Cobalt Complexes with Bioactive Ligands

Bioactive small molecules that elicit therapeutic action *in vivo*, such as non-steroidal anti-inflammatory drugs (NSAIDs) [17–19], antibacterial agents [20,21], antiprotozoal agents [22,23], antifungal agents [24,25], and antihelmintic agents [26], have been attached to cobalt complexes to improve or alter their therapeutic efficacy. Although mechanisms for many of these agents are not understood, complexation to cobalt scaffolds is thought to influence the physicochemical properties of the bioactive ligand [2,6,27,28*]. For example, therapeutic agents have been tethered to the naturally occurring Co(III) complex, cobalamin (Vitamin B₁₂) for drug delivery. This concept has been reviewed extensively elsewhere [28*,29]. In brief, peptides, proteins and other small molecules such as Rh-based CO-releasing molecules [30] and cisplatin analogues [31] have been tethered to cobalamin to improve the uptake, biocompatibility and stability of the agents.

The dinuclear hexacarbonyl cobalt cluster Co₂(CO)₆ is known to modify the bioactivity of small molecules that are coordinated to the cluster via an alkyne bond ([alkyne]-Co₂(CO)₆). A series of mechanistic studies have evaluated the effect of [alkyne]-Co₂(CO)₆ on the anticancer activity of the NSAID aspirin (Figure 3A). Aspirin displays modest pro-apoptotic activity through inhibition of cyclooxygenase (COX) enzymes [27,32*]. Coordination of aspirin to [alkyne]-Co₂(CO)₆ was shown to significantly enhance COX inhibition and the resulting antiproliferation levels were comparable to cisplatin. By systematically altering the alkyne bond, the bioactive ligand and the Co₂(CO)₆ cluster, Ott *et al.* and Rubner *et al.* demonstrated that while the NSAID may be involved in directing the complex to the COX enzymes, all these components were necessary for the remarkable potency [33,34].

Enhancement of activity of [alkyne]-Co₂(CO)₆ was initially attributed to improved cell uptake due to increased lipophilicity. However, comparison of the aspirin-[alkyne] ligand with more hydrophobic derivatives showed no conclusive correlation between lipophilicity and efficacy, implicating that there were additional factors that affected bioactivity. Ott *et al.* identified a potential difference in the mode of action between aspirin and aspirin-[alkyne]-Co₂(CO)₆ using Trypsin-digest MS studies of the purified COX enzyme (Figure 3B) [32*]. Both aspirin and its alkyne derivative acetylated serines in the active site, while the cobalt complex acetylated lysines throughout the enzyme. Although the exact mechanism by which the cobalt cluster modifies the NSAID mode of action remains unclear, this example highlights the importance of a systematic approach to understanding the activity of cobalt agents in biological systems.

Bioreductive Cobalt Complexes

Co(III) complexes can be used as prodrugs that are capable of undergoing bioreduction, a process whereby intracellular reduction produces a bioactive agent [7**,35**,36**]. Reduction of inert *d*⁶ Co(III) complexes yields labile *d*⁷ high-spin Co(II) complexes which rapidly undergo ligand substitution [2]. The reduction potentials of Co(III) complexes can be tuned to the eukaryotic cytosolic reduction potential (–0.2 V to –0.4 V) [37] by ligand modification. The significant difference in lability of the accessible oxidation states and the large range of the reduction potential makes cobalt an ideal candidate for use in redox-activated prodrugs [7**]. An appropriately designed complex can undergo bioreductive activation to yield a cobalt species that acts as an effector *or* release an effector ligand that was deactivated by coordination to the cobalt. In this context, an effector is defined as a molecule that can alter biological activity [38,39].

Cobalt Complexes as Redox-Activated Effectors

Redox activation of Co(III) to generate complexes that act as effectors of biological activity was demonstrated by Tomco *et al.* who studied $\text{Co}(\text{L}^{\text{NN}'\text{O}})_x$ complexes ($\text{L}^{\text{NN}'\text{O}} = 2,4$ -diiodo-6-((pyridine-2-ylmethylamino)methyl)phenol) [40,41*]. These complexes are thought to inhibit proteasomes through ligand exchange with amino acid residues in the active site. $\text{Co(III)(L}^{\text{NN}'\text{O}})_2$ was found to be far more inert to substitution than $\text{Co(II)(L}^{\text{NN}'\text{O}})_2$. However, the Co(III) complex exhibited higher inhibition of chymotrypsin-like activity in purified proteasomes as well as improved apoptotic induction in PC-3 cancer cells [40].

The difference in activity between the Co(III) and Co(II) complexes was attributed to complex stability. The authors postulated that the higher lability of the Co(II) complex results in rapid degradation in biological environments whereas the stability of the inert Co(III) complex offers improved bioavailability. Consequently, the substitutional inertness facilitates intracellular activation of the Co(III) complex following reduction [41*]. The feasibility of this concept was tested by examining the LMCT absorption band of the Co(III) complex. In the presence of a cellular reductant, a decrease in the LMCT band was observed, suggesting a change in oxidation state and ligand binding. The formation of a labile cobalt species upon reduction followed by solvent exchange was observed by mass spectroscopy (Figure 4A), and the experimental results were corroborated with DFT calculations. These findings show that bioreductive activation is a viable mechanism for ligand release and pharmacophore formation in Co(III) prodrugs. The ability to modulate the stability of the agent and to generate an active species with this strategy shows potential for adding a level of control to the efficacy of cobalt therapeutics.

Cobalt Complexes as Redox-Responsive Drug Carriers

The principle of bioreductive activation can be applied to the selective release of bioactive ligands from Co(III) complexes. Coordination to Co(III) can deactivate antitumor cytotoxins such as nitrogen mustards [42–45], DNA intercalators [38,46,47], and the MMP-inhibitor marimastat [48]. These agents take advantage of the hypoxic nature of the core of solid tumors. Bioreductive conversion of these complexes in hypoxic environments leads to selective release of the cytotoxins for antitumor activity [7**,35**,36**,49].

Fluorophores have been employed as visualization tools to probe the site and timescale of activation of redox-responsive Co(III) complexes in biological systems. The fluorescence of fluorophores such as coumarin-343 is quenched upon coordination to Co(III) but returns upon ligand release following reduction [50]. Kim *et al.* developed a 3-dimensional system of fluorescently labeled hypoxic cells within an avascular solid tumor model to determine if bioreductively activated Co(III) complexes effectively target the hypoxic regions of the model (Figure 4B) [51*]. Fluorescence return from a Co(III) /cyclam complex of coumarin-343 (c343) was observed in the same region as the fluorescently labeled hypoxic cells located within the core of the tumor models. This suggests that the release of the fluorophore payload is occurring at the correct site, validating the use of bioreductive Co(III) complexes for delivering anticancer agents to the target site [50,51*].

Future Prospects and Conclusions

The cobalt complexes highlighted in this review illustrate the diverse mechanisms by which cobalt complexes can act as potential pharmaceutical agents. Evaluations of the physicochemical properties of different cobalt materials in biological systems reveal intricate functions beyond indiscriminate binding of bio-macromolecules. The examples discussed here include selective protein inhibition, bioactivity enhancement, and bioreductive prodrug activation. However, the versatility of cobalt has been further

exemplified in other materials that have not been discussed here, such as the cobalt-based metallacarborane HIV protease inhibitor [52,53] and cobalt nanoparticles [54,55] for magnetic hyperthermia applications. In addition to these strategies, the flexible cobalt platform can be enhanced with further levels of control. For example, the low-energy *d-d* transitions and MLCT bands of cobalt complexes allows for the incorporation of radiation-[38,46,47] or photo-responsive elements [56–60]. Complexes that respond to such exogenous energy sources will permit spatial and temporal control of therapeutic activity.

The diverse potential of cobalt materials in disease treatment can be further realized by the rational employment of its properties. The overall stability, compatibility and activity of cobalt agents are directly influenced by the reduction potential, overall charge, coordination environment, solubility and hydro- or lipophilicity. These factors can be rationally tuned to effectively target the pathway or biomolecule of interest. Mechanistic insight into the diverse bioactivities of cobalt materials may inform the design of such well-controlled and selective cobalt-based drugs. Understanding the mode of action can thus guide the progression of promising cobalt agents to the clinic.

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Highlights

- Cobalt is a versatile transition metal for drug development.
- The tunable physicochemical properties can be exploited by understanding the mechanism of bioactive cobalt complexes.
- The review highlights examples of mechanistic investigations of cobalt therapeutics.

Figure 1. Schematic representation of the different modes of action of bioactive cobalt complexes. Three classes of cobalt complexes are discussed: 1) complexes that directly act on biomolecules through ligand exchange, 2) complexes that modify the activity of ligated drugs and 3) complexes that are activated by bioreduction to either (I) yield a cobalt effector species or (II) release a small molecule drug.

Figure 2.

A schematic showing the Co(III) Schiff base complex, $[\text{Co}(\text{acacen})(\text{L})_2]^+$, that undergoes a dissociative exchange of the labile axial ligands and coordinates His residues [8,9]. Attachment of a targeting molecule, R, on the ancillary acacen chelate allows for selective inhibition of the protein of interest [8,11,12].

Figure 3.

Cobalt complexes with bioactive ligands. A) Structure of the bioactive molecule aspirin complexed to $\text{Co}_2(\text{CO})_6$ through an alkyne bond [32*,33]. B) Interaction of aspirin-[alkyne]- $\text{Co}_2(\text{CO})_6$ with the human COX-2 enzyme with residues relevant to catalytic activity (green) and acetylation by cobalt complex (orange) is highlighted [32*]. Aspirin induces anti-inflammatory effects through acetylation of Ser-516 in the catalytic site of the COX enzyme. In contrast, conjugation of aspirin to the cobalt scaffold alters the pharmacology of the NSAID, as lysine residues are acetylated throughout the enzyme. In particular, acetylation of Lys166 and Lys432 near the heme prosthetic group and Lys346 near the entrance channel of the active site is suspected to result in potent COX inhibition and antiproliferative effects. Figure adapted from ref [32*] with permission.

Figure 4.

Cobalt complexes that undergo bioreductive activation. A) $\text{Co(III)(L}^{\text{NN'O}}\text{)}_2$ undergoes reduction to the more labile Co(II) counterpart to yield an active proteasome inhibitor [40,41*]. In the presence of the reducing agent ascorbic acid, one of the $\text{L}^{\text{NN'O}}$ ligands of $\text{Co(III)(L}^{\text{NN'O}}\text{)}_2$ is lost and replaced by a solvent (DMF) molecule, as identified by mass spectrometry. The activated complex is thought to bind to the active site of the proteasome to elicit inhibition. Scheme adapted from ref [31] with permission. B) The fluorescence of coumarin-343 (c343) is quenched upon coordination to Co(III)/cyclam . When the complex encounters the reducing environment of a hypoxic spheroid core, the complex undergoes reduction, releasing the fluorophores and restoring fluorescence. The process is monitored by fluorescence confocal microscopy [51*].