Letters

Ni(II), Cu(II), and Zn(II) Diethyldithiocarbamate Complexes Show Various Activities Against the Proteasome in Breast Cancer Cells

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Abstract: A series of three complexes with diethyldithiocarbamate ligand and three different metals (Ni, Cu, Zn) was prepared, confirmed by X-ray crystallography, and tested in human breast cancer MDA-MB-231 cells. Zinc and copper complexes, but not nickel complex, were found to be more active against cellular 26S proteasome than against purified 20S proteasome core particle. One of the possible explanations is inhibition of JAMM domain in the 19S proteasome lid.

Since FDA approval of cisplatin 30 years ago, thousands of coordination compounds have been synthesized and screened. However, cisplatin and its analogues (carboplatin and oxaliplatin), ranked among "alkylating agents", are the only metal-based anticancer drugs in common use.[1] Because many metal-based compounds are effective in vitro,[2] it is a great challenge to find an in vivo active metal drug sufficiently safe for clinical use. One of the promising strategies to accelerate drug discovery and development research seems to be in finding new uses for old drugs. Once approved, these drugs can quickly enter phase II clinical trials and save a lot of time and money.[3−5]

Along that line, it deserves attention that an old anti-alcoholic drug disulfiram (tetraethylthiuram disulfide), in combination with zinc gluconate, has been reported to reduce hepatic metastases and produce clinical remission in a patient with ocular melanoma.[6] Furthermore, disulfiram mixed with copper was found to selectively destroy melanoma cells in vitro.[7] The antimelanoma activity of disulfiram was suggested to be attributed to diethyldithiocarbamate (EtDTC[a]) complex with copper because disulfiram can be converted to EtDTC in the body.[8] Also, few other metal−dithiocarbamate complexes were shown to be effective against many types of cancer cells in vitro and in vivo.[9−13] However, the involved molecular mechanism has not been completely defined.

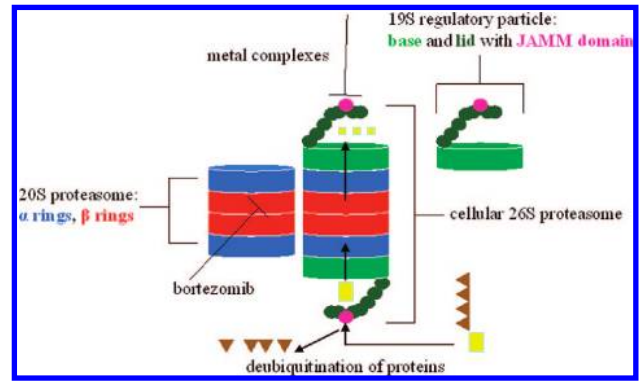

Figure 1. Mechanism for inhibition of 20S proteasome by bortezomib and a suggested mechanism for metal−dithiocarbamate complexes to inhibit 26S proteasome in intact cells.

The proteasome inhibition is a new and viable strategy in cancer therapy with the first-in-class approved drug bortezomib (dipeptide boronic acid analogue) as a successfule example.[14] A giant protease responsible for degradation of about 90% cellular proteins, the proteasome is usually targeted at its proteolytically active β-rings (Figure 1). However, there is an increasing demand for new approaches to inhibit the proteasome, e.g., by targeting its so-called 19S particle (Figure 1).[15] This component of the proteasome is responsible for recognizing ubiquitinated target proteins and their further processing (cleavage of ubiquitin chain) before the degradation.[16] The deubiquitinating activity of 19S particle is dependent on a metalloisopeptidase with a coordinated zinc ion,[17,18] and this structural motif in 19S particle (JAB1/MPN/Mov34 domain or JAMM domain) has been suggested as a perspective target for anticancer drugs.[19]

In our current work, we show that synthetic and well-characterized complexes of three metals (Cu, Zn, and Ni) with EtDTC have different abilities to inhibit purified 20S proteasome (without 19S particles containing JAMM domain) and cellular 26S proteasome (with JAMM domain-containing 19S particles).

Regarding the metals, we selected zinc and copper as their EtDTC complexes were reported to suppress cancer growth in vivo, while nickel was used to create stable complexes with a coordination sphere similar to that of Cu(II) and Zn(II).

These complexes were prepared as described previously,[20] and their molecular structures were confirmed using X-ray single-crystal diffraction (for details, see Supporting Information).

In view of the fact that disulfiram was reported to be able to inhibit the proteasome activity in MDA-MB-231 breast cancer xenografts with elevated copper levels, we decided to test the complexes in this highly malignant and metastatic cell line (experimental procedures are summarized in Supporting Information).[21] We determined: (a) toxicity of synthetic compounds, (b) cellular morphological changes after treatment, (c) activity of the proteasome in the treated cells, and finally, (d) ability of the three complexes to inhibit purified 20S proteasome.

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[a] Abbreviations: EtDTC, diethyldithiocarbamate; PyDTC, pyrrolidinedithiocarbamate; CT-like, chymotrypsin-like; JAMM, JAB1/MPN/Mov34 domain; ATF/CREB, activating transcription factor/cAMP response element-binding protein

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Table 1. Inhibitory Effect of Diethyldithiocarbamate Complexes on the Chymotrypsin (CT)-like Activity of Purified 20S Proteasome (without JAMM Domain) and Cellular 26S Proteasome (with JAMM Domain)

	inhibition of proteasome CT-like activity	
compd	20S (50 μM) (%)	intact cells (20 μM) (%)
$Cu(EtDTC)_2$	35	>90
$Zn(EtDTC)_2$	35	>90
$Ni(EtDTC)_2$	3	0

We found that Ni(II) complex was quite inactive to MDA-MB-231 cells in all the experiments. In a sharp contrast, both $Zn(EtDTC)_2$ (the putative active compound in disulfiram/zinc gluconate treatment of metastatic melanoma) and $Cu(EtDTC)_2$ were toxic to these breast cancer cells, associated with inhibition of cellular 26S proteasome (Table 1). When a purified 20S proteasome was used, both $Zn(EtDTC)_2$ and $Cu(EtDTC)_2$ showed much less inhibitory activity than against cellular 26S proteasome (Table 1).

Our previous work with different ligands (including dithiocarbamates) and their metal complexes suggested that the proteasome-inhibitory effects were strictly attributed to the complexes with specific metals. Ligands alone were active only against the tumor cells grown in copper-enriched medium, which increased the level of cellular copper available for reacting with the ligand within the cells,[22] or against the tumors in vivo (that have elevated copper levels as well).[23]

The stability of dithiocarbamate complexes with metals within the cells has not been directly proved yet, although some of these complexes, such as $Cu(EtDTC)_2$, seem to be stable in brain tissue.[24] However, the putative (in)activity of our currently reported compounds toward the proteasome could be explained by their molecular structures. Until now, almost nothing is known about interactions between metal complexes and JAMM domain (except for, e.g., JAMM domain protein inhibition by zinc acetate[25]). In recent years, we have published a number of metal complexes that inhibit the proteasome, specifically its chymotrypsin-like (CT-like) activity, and induce apoptosis in human cancer cells.[10,13,22,23] On the basis of the published work on metal complexes, the proteasome and JAMM domain, we have recently suggested that dithiocarbamate complexes with metals could inhibit JAMM domain proteins.[26] Here, in the light of new data generated with these three metal complexes against the purified and cellular proteasome, we are proposing that inhibition of JAMM domain in the 19S particle of the proteasome is responsible for the effect of the tested metal complexes observed in the breast cancer cells. Following arguments support our hypothesis.

The JAMM domain is very similar to the metal-binding domain of carboxypeptidase A because both of them comprise glutamine and two histidines, a water molecule and glutamine, or aspartate in nearly the same arrangement. Carboxypeptidase A was shown to be efficiently inhibited by Zn(OH)Cl, creating a binuclear complex with the zinc from the protein by displacing the water molecule[27] (Figure 2).

To bridge the JAMM zinc by dative bond, the diethyldithiocarbamate ligand requires rich electronic density on the sulfur atom (Figure 2, the sulfur in pink). Recently, electronic densities on atoms and bonds within $Ni(EtDTC)_2$, $Cu(EtDTC)_2$, and $Zn(EtDTC)_2$ were analyzed. The greatest σ-repulsion (that moves electronic densities from the bond to the atoms) was found between Zn and S, while the lowest σ-repulsion was found between Ni and S.[28] Therefore, a sulfur atom within a zinc complex has higher electronic density compared to the sulfur atoms in copper and nickel complexes. Moreover, $Zn(EtDTC)_2$ and $Cu(EtDTC)_2$ are, in solid phase, actually dimers (for figures, see Supporting Information) with sulfur atom bridging two metals; the bond length of the Zn−S(bridge) is shorter than that of the Cu−S(bridge) (2.391 vs 2.779 Å), suggesting the larger electronic density on the sulfur atom within $Zn(EtDTC)_2$ compared to the one within the $Cu(EtDTC)_2$ complex. Nickel complex, however, is not able to create dimers (according to our data).

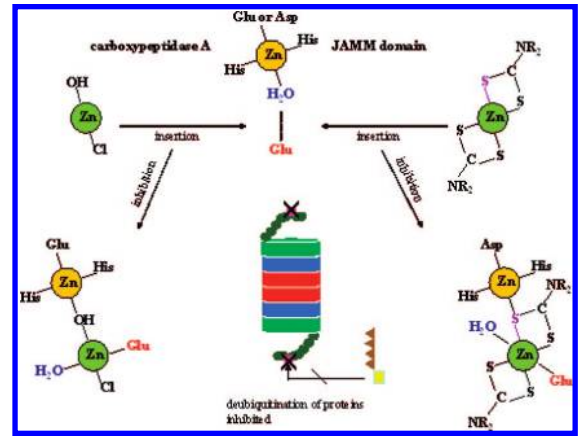

Figure 2. Probable analogy among published molecular mechanism of carboxypeptidase A domain inhibition by a zinc coordination particle and proposed inhibition of JAMM domain of 19S particle by zinc−diethyldithiocarbamate complex.

From this point of view, $Zn(EtDTC)_2$ should be most active against the JAMM domain of the 26S proteasome, $Cu(EtDTC)_2$ activity would be less potent against the JAMM domain, and nickel complex would not be active at all. However, this needs to be confirmed by showing direct interactions between the JAMM domain and diethyldithiocarbamate complexes and consequent inhibition of JAMM activity.

We have also recently reported[29] that another group of dithiocarbamate complexes with copper and zinc, $Cu(PyDTC)_2$ and $Zn(PyDTC)_2$ (Py = pyrrolidinedithiocarbamate), could inhibit CT-like activity of cellular 26S but not purified 20S proteasome. It is possible that the overall effect of metal−dithiocarbamates within the cell is a result of their different effects on CT-like activity located on 20S catalytic core and deubiquitinating activity located on the 19S particle (e.g., JAMM domain) within the 26S proteasome. Further electronic density analysis of different dithiocarbamate complexes would help in answering this question.

Moreover, the hypothesis that metal complexes might inhibit JAMM domain could also, in some way, explain several previously observed effects of dithiocarbamate complexes, including blockage of ATF/CREB DNA binding[6] and inhibition of H4 histone acetylation[30] that requires ATF/CREB DNA binding.[31] According to our hypothesis, JAMM domain acts as a deubiquitinase that coordinates histone acetylation and deubiquitination[32] and by targeting this deubiquitinase, dithiocarbamate complexes would be able to regulate acetylation and biological activity of H4 and other players.

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Supporting Information Available: Basic information and reaction yields, crystal structure determination, crystal data and structure refinement, molecular structures of monomer complexes, molecular structure of dimers, inhibition of MBA-MD-231 cell line

proliferation, apoptotic morphological changes in MBA-MD-231 cell line, proteasomal activity in intact cells, activity of purified 20S proteasome. This material is available free of charge via the Internet at http://pubs.acs.org.

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