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Metal Cu(II) and Zn(II) bipyridyls as inhibitors of lactate dehydrogenase

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Abstract Metal complex–protein interaction is an evolving concept for determining cellular targets of metallodrugs. Lacatate dehydrogenase (LDH) is critically implicated in tumor growth and therefore, considered to be an important target protein for anti-tumor metal complexes. Due to efficient biocompatibility of copper (Cu^{2+}) and zinc (Zn^{2+}), we synthesized $CubpyAc_2 \cdot H_2O$ (Cu-bpy) and $ZnbpyAc_2 \cdot H_2O$ (Zn-bpy; where bpy = 2,2′ bipyridine, Ac = CH_3COO^-) complexes and evaluated their interaction with and modulation of LDH in mouse tissues. The increasing concentration of both the complexes showed a significant shift in UV–Vis spectra of LDH. The binding constant data (Kc = 1×10^3 M^{-1} for Cu-bpy and 7×10^6 M^{-1} for Zn-bpy) suggested that Zn-bpy-LDH interaction is stronger than that of Cu-bpy-LDH. LDH modulating potential of the complexes were monitored by perfusing the mice tissues with non-toxic doses of Cu-bpy and Zn-bpy followed by activity measurement and analysis of LDH isozymes on non-denaturing polyacrylamide gel electrophoresis (PAGE). As compared to the control sets, Cu-bpy caused a significant decline ($P < 0.05$–0.001) in the activity of LDH in all the tissues studied. However, Zn-bpy showed inhibition of LDH only in liver ($P < 0.01$), kidney ($P < 0.001$) and heart ($P < 0.01$), but with no effect in spleen, brain and skeletal muscle tissues. PAGE analysis suggested that all the five LDH isozymes are equally sensitive to both the complexes in the respective tissues. The results suggest that Cu- and Zn-bpy are able to interact with and inhibit LDH, a tumor growth supportive target protein at tissue level.

Keywords Lactate dehydrogenase · Metal bipyridyl complexes · Glycolysis · Tumor growth · LDH isozymes

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Introduction

Metal ions and metal coordination compounds are known to affect cellular processes in a dramatic way (Bertini et al. 1994, 2001). During recent past, a number of metal complexes have been formulated and evaluated for their anti-tumor properties (Clarke 2003; Keppler et al. 1993). Cytotoxicity of most of the metal complexes has been mainly correlated with their ability to bind with and damage DNA (Keppler et al. 1993; Novakova et al. 2003). However, the dictum that DNA is the primary target for metallo-

drugs is rapidly declining (Dyson and Sava 2006), and therefore, defining additional pharmacological targets for metal complexes at cellular level is of much current interest.

Metal–protein interaction is of prime importance for the biodistribution, mechanism of action and for the toxic effects of anti-tumor metal complexes (Trimerbaev 2005; Dyson and Sava 2006). In this respect, formulating complexes of biologically important metal ions is of special merit as they are likely to be better biocompatible and hence, can be delivered to the cells easily. In addition to iron, copper (Cu), and zinc (Zn) are the most abundant metal ions implicated in a number of biochemical reactions and as structural determinant of several proteins (Theophanides and Anastassopoulou 2002; Dudev 2003).

Increased level of Cu in cells is known to bind DNA (Bar-Or et al. 2001; Theophanides and Anastassopoupou 2002) and also to certain proteins (Halliwell and Gutteridge 1990). However, DNA damage potential of Cu is attributed to Cu dependent production of reactive oxygen species (ROS) in the cells (Theophanides and Anastassopoupou 2002). The relevance of Cu-complexes as anti-neoplastic agent got attention after a Cu-phenanthroline complex was found to show nuclease activity (Downey et al. 1980; Marshall et al. 1981). Later, Cu-bipyridyl [$Cu(bpy)_2^{2+}$] complexes were found more effective, as they showed strong affinity for DNA (Yang et al. 2004). Recently, a Cu-1,10-phenanthroline has been reported to induce apoptosis by increasing ROS load in a liver carcinoma (Cai et al. 2007). However, little is known about modulation of metabolic enzymes by exogenous Cu-complexes except inhibition of certain enzymes that do not require Cu for their catalytic functions, like NADH oxidase and lactate dehydrogenase by Cu(I) & Cu(II) ions (Zwart et al. 1991).

Zinc is the second most abundant transition metals in animal tissues implicated in a number of physiological functions (Berg and Shi 1996; Prasad 1998; Frederickson et al. 2000; Kikuchi et al. 2004). It shows strong interaction with and modifies a variety of cellular proteins (Cox and McLendon 2000) including certain key enzymes (Trigun and Singh 1989) and several transcription factors (Prasad 1998). A Zn-thiosemicarbazone complex has also been shown to act as anti-tumor agent in vitro and is as cytotoxic as cisplatin (Beraldo and Gambino 2004).

However, information is scanty on protein based anti-neoplastic activity of Cu- and Zn-complexes except some reports, wherein, a 8-hydroxyquinoline-Cu(II) and 5,7-dichloro-8-hydroxyquinoline-Cu(II) have been shown to inhibit proteasome and to induce tumor cell apoptosis (Daniel et al. 2004a, b) and certain Zn metals that inhibit some tumor associated extracellular enzymes (Cox and McLendon 2000; Berg and Shi 1996; Jiang and Guo 2004). Moreover, most of these findings are based on in vitro studies on cell lines and thus, it is important to define pharmacological targets of Cu- and Zn-complexes at cellular level in animal system. Therefore, examining and defining tumor associated protein targets for exogenous Cu- and Zn-complexes at tissue level in animal models are of much relevance.

The clinical use of ^{18}fluorodeoxyglucose positron-emission tomography has demonstrated that the glycolytic phenotype is observed in most human cancers (Gatenby and Gillies 2004). The cells genetically engineered to become cancerous have also shown significant increase in its glycolytic pathway (Ramanathan et al. 2005). This is mainly due to the increased energy demand for angiogenesis (neovascularization) in the cancerous cells (Palmer et al. 1990; Biskupiak and Krohn 1993). There are some reports on direct involvement of glycolytic enzymes in cellular immortalization (Kim et al. 2005; Kondoh et al. 2005), including the increased expression of hexokinase and lactate dehydrogenase, the first and the last enzyme of glycolytic pathway, respectively (Mathupala et al. 1997; Dang et al. 1997).

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a tetrameric protein consisting of two types of subunits, the M type (pre-dominantly expressed in skeletal muscle) and the H type (pre-dominantly expressed in heart) contributed by the two genes (Cahn et al. 1962; Markert 1963). Combination of these two subunits in different ratio gives rise five isozymic forms (M4, M3H, M2H2, MH3 & H4), which are expressed in a tissue specific manner in most of the mammalian tissues. The differential expressions of LDH isozymes in different types of tumors have been reported (Koukourakis et al. 2003), and therefore, inhibition of LDH mediated step is now considered to be one of the effective mechanisms to control tumor growth (Gaber 2006). Abnormally high LDH activity in cancerous tissues vis-a-vis decreased LDH activity

with tumor regression have been demonstrated in animal models (Koukourakis et al. 2003; Niakan 2001; Pathak and Vinayak 2005). There is a report on designing active site inhibitors of human LDH for therapeutic applications also (Yu et al. 2001). Recently, down regulation of LDH-A activity has been reported to affect mitochondrial bioenergetics and to retard proliferation of Neu 4145 tumor cells in culture (Fantin et al. 2006). And thus, LDH seems to be a relevant and suitable candidate to study anti-cancer potential of newly formulated metallodrugs.

We have demonstrated that a cytotoxic Ru(II) complex containing flavones inhibits LDH reversibly when administered orally to mice in a tissue specific manner (Mishra et al. 2004b). A recent report from our lab described that a cytotoxic Ru(II) complex containing 4-carboxy N-ethylbenzamide [Ru(II)-CNEB] strongly interacts with and inhibits LDH non-competitively (Trigun et al. 2007). We could also formulate cytotoxic Cu- and Zn-bipyridyl complexes with the potential to establish weak interactions with the proteins. In order to evaluate protein based therapeutic potential of these complexes, the present study deals with whether complexes Cu-bpy and Zn-bpy are able to interact with and modulate LDH in different tissues of mouse.

Materials and methods:

Chemicals

Cu(II) acetate dihydrate and Zn(II) acetate dihydrate and 2,2′ bipyridine, purified LDH, β-NADH (β-nicotinamide adeninedinucleotide, reduced) and sodium pyruvate were purchased from Sigma-Aldrich Corporation, USA. Other chemicals and solvents used were purchased from SISCO Research Laboratory, Mumbai, India.

Synthesis and characterization of Cu- and Zn-bpy

Following the method reported earlier (Mishra et al. 2004a), Cu(II) acetate dihydrate (2.17 g, 1 mmol) and Zn(II) acetate dihydrate (2.2 g, 1 mmol) were added separately to the solution of 2,2′ bipyridine (1.56 g, 1 mmol) in 20 ml methanol with constant stirring up to 4 h. The resultant solids were filtered, washed with methanol and analyzed on IR and ^{1}H-NMR (for Zn only).

IR of both complexes (KBr pelletes) showed $\upsilon_{\text{pyridyl}}$ 750 cm^{-1} $\upsilon_{\text{c = o}}$ 1417 cm^{-1} where as ^{1}H-NMR (DMSO d_6) of corresponding Zn(II) compound showed a signal at δ = 7.65(2H, s), 8.32(2H, s), 8.67(4H, s). The structure of both the complexes has been presented in Fig. 1.

Binding of complexes Cu-bpy and Zn-bpy with LDH

Binding study of Cu-bpy and Zn-bpy complexes with LDH was done using UV–Vis spectral analysis. To a fixed concentration of LDH (1×10^{-6} M) prepared in methanol:water (1:1) mixture, increasing concentration of both the complexes (1×10^{-6}–1×10^{-8} M), prepared in the same solvent, were incubated separately for 30 min at room temperature followed by measurement of optical density at 200–800 nm. To calculate association constant (Kc) between the complexes and LDH, differences in OD observed between the untreated and treated samples at 280 nm were tested on Benesi–Hilde Brand (BH) equation (Mishra et al. 2004a). The formulae adopted was

$$[\mathrm{A}] \cdot [\mathrm{B}]_0/\mathrm{d}' = [B]_0/\acute{\epsilon} + 1/\mathrm{Kc}\acute{\epsilon} \quad (1)$$

with

$$\mathrm{d}' = \mathrm{d} - \mathrm{d}^0\mathrm{A} - \mathrm{d}^0\mathrm{B} \quad (2)$$

Here, $[\mathrm{A}]_0$ and $[\mathrm{B}]_0$ are the initial concentrations of the complex and LDH, respectively, d is the absorbance of the mixture at 270 nm and d^0A & d^0B are those of complex and LDH at the same wavelength. Linear plot according to Eq. 1 was obtained for each complex and association constant (Kc) was calculated accordingly.

Animals

Adult male mice (AKR strain) of same age group (18 weeks old) were used for the experiments. Animals were maintained under a constant 12 h light and dark cycle at 25–30°C. They were provided standard mice feed and water ad libitum. The mice were maintained as per the guidelines of institutional (BHU) animal ethical committee.

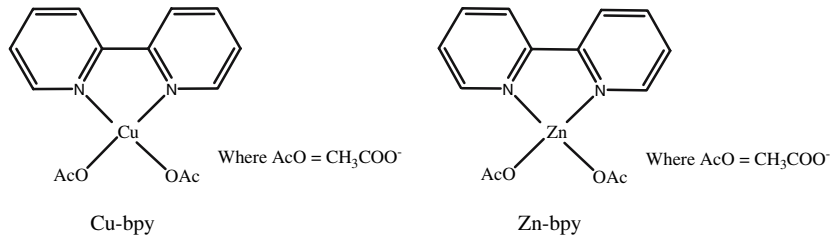

Fig. 1 Structural representation of Cu-bpy and Zn-bpy complexes

Treatment of mice with Cu-bpy and Zn-bpy complexes

Pilot experiments showed that both Cu- and Zn-bpy complexes are moderately cytotoxic to Dalton's lymphoma cells (unpublished results) in vitro, however, they are non-toxic/lethal to adult mice even at a concentration of 2 mg/kg body weight. Accordingly, Cu-bpy and Zn-bpy (2 mg each) were first dissolved in 500 µl of methanol and the final volume was made 10 ml with Krebs–Ringer Buffer (KRB: pH 7.2) so that final concentration of the solution becomes 0.2 mg/ml (non-toxic/lethal dose). Perfusion experiment was done according to Clémence et al. (2002) with some modifications as reported recently (Trigun et al. 2007). Briefly, mice were anesthetized by thiopentone injection and a canula was implanted between ventricle and atrium in the heart so that it feeds the ventral aorta and perfusate reaches to tissues directly through systemic circulation. Perfusion was done at a rate of 0.5 ml/min and at constant pressure by keeping the solution at 1 m height above the heart for 20 min. Experimental group mice were perfused with KRB containing 0.2 mg/ml of the complexes, whereas, control group of mice were perfused similarly with KRB alone. 3–4 mice were treated in each group.

Preparation of tissue extracts

Mice were sacrificed, tissues were dissected out and washed in ice-cold mammalian saline. Tissue extracts (10% w/v) were prepared in 0.02 M Tris–Cl buffer (pH 7.4) containing protease inhibitors as described earlier from this lab (Pandey et al. 2005). Extracts were centrifuged at 40,000*g* and the supernatant collected were used for non-denaturing PAGE and other enzymatic studies.

Assay of lactate dehydrogenase (LDH)

LDH activity was measured spectrophotometrically following the method of Kornberg (1955) and as described in our earlier report (Trigun et al. 2006). The reaction mixture (3 ml) was composed of 20 mM Tris–Cl (pH 7.4), 6 mM NADH, suitably diluted tissue extract and 1 mM sodium-pyruvate. The decrease in absorbance at 340 nm was recorded up to 10 min. The oxidation of 1 µmol of NADH per min at 25°C was defined as 1 unit of the enzyme and values were presented as unit mg^{-1} protein.

Protein content in all the samples was measured using Folin method of Lowry et al. (1951). Statistical analysis, wherever required, was done following Bruning and Kintz (1977). Student '*t*' test was performed to find the level of significant changes between the control and the experimental groups.

Non-denaturing polyacrylamide gel electrophoresis (PAGE)

LDH isozymes were analyzed on non-denaturing 10% PAGE following the method described in our earlier report (Trigun et al. 2006). Briefly, polymerization of gel and preparation of samples were done in a non-SDS buffer. Suitable amount (30 µg) of protein samples were loaded in each lane and electrophoresis was performed using non-SDS running buffer (Tris–Glycine pH 8.3). After electrophoresis was over, gels were subjected to LDH specific activity stain. Staining solution consisted of 0.125 M Tris–Cl (pH 7.4), 0.5 mM $MgCl_2$, 0.1 mM Li-Lactate, 1 mg/ml NAD, 0.01 M NaCl, 0.25 mg/ml nitro blue tetrazolium (NBT), and 0.025 mg/ml phenazine methosulfate (PMS). After development of LDH bands, gels were washed, LDH bands were scanned and quantitated by gel spectrometry using alpha

imager 2200 gel documentation software. The isozymic bands in the gel were characterized by comparing their migration with the pattern shown by the tissue specific characteristic LDH bands as described earlier (Mishra et al. 2004b; Trigun et al. 2006, 2007).

Results

Interaction of Cu-bpy and Zn-bpy with LDH

In order to assess whether Cu-bpy and Zn-bpy complexes are able to bind with LDH protein, UV–Vis spectral studies of purified LDH was performed in the presence of increasing concentration of both the complexes separately. According to Fig. 2A and B, as compared to the untreated LDH sample, there is a significant leftward shift in λ_{max} (from 280 nm to 270 nm) of LDH treated with the increasing concentration of both the complexes. A linear increase in the OD due to treatment with both these complexes also suggests the possibility of significant charge transfer between LDH protein and both the complexes. The treatment of absorption data on Benesi–Hilde Brand equation produced linear plots for both the complexes and the association constant calculated was 1×10^3 M^{-1} for Cu-bpy-LDH and 7×10^6 M^{-1} for that of Zn-bpy–LDH interactions.

Effect on LDH activity

According to Figs. 3–5A, in comparison to the control group of mice, perfusion of non-toxic dose of Cu-bpy caused a significant decline in the activity of LDH in liver ($P < 0.001$), kidney ($P < 0.001$), heart ($P < 0.001$), spleen ($P < 0.001$), brain ($P < 0.05$) and skeletal muscle ($P < 0.01$). However, perfusion of Zn-bpy though significantly inhibited LDH in liver ($P < 0.01$), kidney ($P < 0.001$) and heart ($P < 0.001$), but with no effect on the activity of LDH in spleen, brain and skeletal muscle tissues.

Effect on LDH isozyme

To ascertain whether the effects of Cu-bpy and Zn-bpy are LDH isozyme specific, the untreated and treated tissue extracts were subjected to PAGE analysis of LDH isozymes. Results in Figs. 3–5B and C re-confirm that liver, spleen and skeletal muscle express mainly M4-LDH (LDH-5) whereas kidney, heart and brain tissues show the expression of all the five isozymes. Nonetheless, a comparison of intensities of LDH bands from untreated and the corresponding treated tissues apparently corroborate the activity measurements data (Figs. 3–5A) in the respective tissues. Also, it was observed that wherever there was inhibition in LDH activity due to Cu-bpy and Zn-bpy, all the isozymes expressed in the concerned tissue were equally sensitive to both these complexes.

Discussion

A mechanistic understanding of how metal complexes act at cellular level is crucial to their clinical success. Metal centers are positively charged and therefore, likely to bind with negatively charged biomolecules like nucleic acids and proteins, and thus offer a predictable mechanism for their use as pharmacological agents. Since, Cu and Zn metal ions are known to modulate the catalytic functions of several enzymatic proteins, it is speculated that the complexes containing such biocompatible metal ions

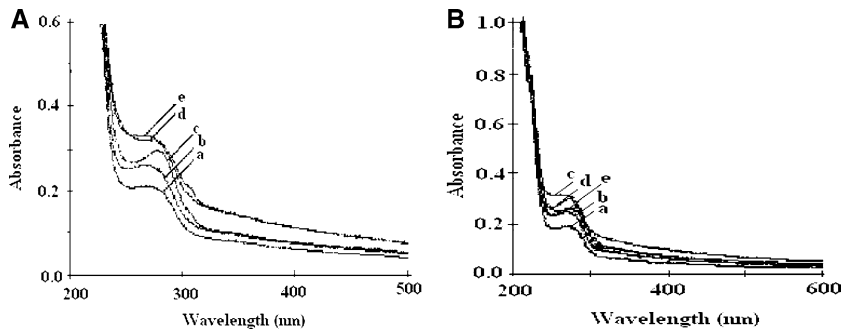

Fig. 2 UV–Vis spectral pattern of purified LDH (1×10^{-6} M) at 280 nm in the presence of different concentration (a = LDH alone, b = 1×10^{-6} M, c = 2×10^{-6} M, d = 4×10^{-6} M, e = 8×10^{-6} M) of Cu-bpy (**A**) and Zn-bpy (**B**) complexes

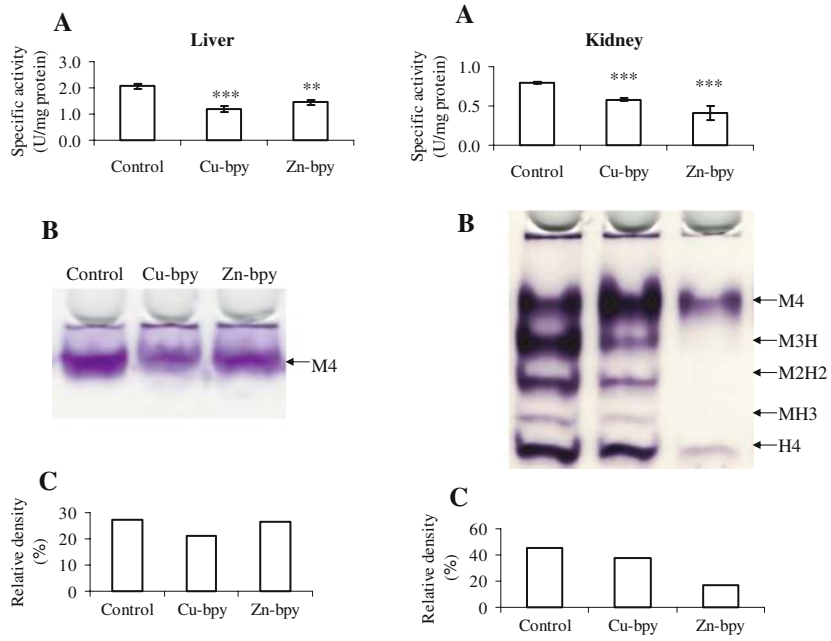

Fig. 3 Effect of Cu-bpy and Zn-bpy complexes on the activity (**A**) and isozymic pattern (**B**) of LDH in liver and kidney. Data in panel **A** represents mean ± SD from 3 to 4 observations. ** $P < 0.01$, *** $P < 0.001$ (Control versus treated samples). In panel **B**, 30 μg protein from the pooled tissue extracts from 3 to 4 mice from the untreated and treated groups were loaded in the corresponding lanes. Panel **C** represents relative intensity of LDH band taking total of the three lanes as 100%)

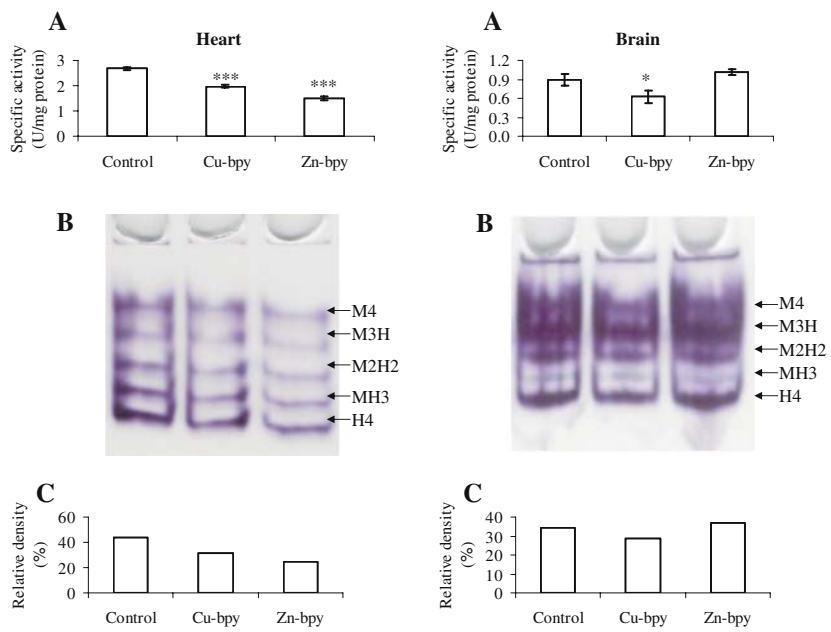

Fig. 4 Effect of Cu-bpy and Zn-bpy complexes on the activity (**A**) and isozymic pattern (**B**) of LDH in heart and brain. Data in panel **A** represents mean ± SD from 3 to 4 observations. * $P < 0.05$, *** $P < 0.001$ (Control versus treated samples). In panel **B**, 30 μg protein from the pooled tissue extracts from 3 to 4 mice from the untreated and treated groups were loaded in the corresponding lanes. Panel **C** represents relative intensity of LDH band taking total of the three lanes as 100%)

could be of much use for protein based therapeutic applications. LDH, critically implicated in tumor growth (Koukourakis et al. 2003; Niakan 2001; Pathak and Vinayak 2005), though does not need any metal ion for its activity, however, the enzyme has been described to be a potential target for certain metal complexes in vivo and in vitro (Mishra et al. 2004b; Trigun et al. 2007). We describe here that Cu-bpy and Zn-bpy complexes, synthesized and characterized in our laboratory, could be the other candidates that can interact with and inhibit LDH at tissue level in mice.

A shift in the absorption pattern of a protein at 280 nm in the presence of a metal complex suggests

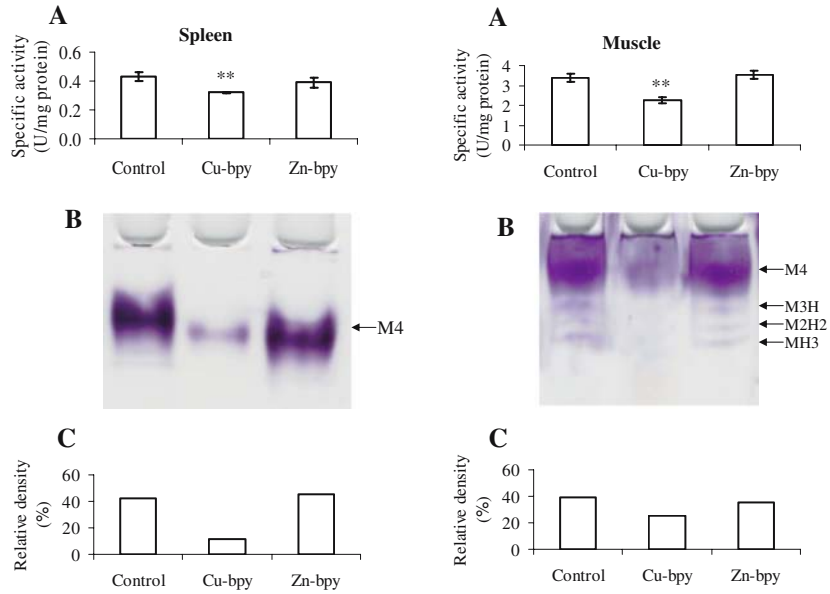

Fig. 5 Effect of Cu-bpy and Zn-bpy complexes on the activity (**A**) and isozymic pattern (**B**) of LDH in spleen and muscle. Data in panel **A** represents mean ± SD from 3 to 4 observations. ** $P < 0.01$ (Control versus treated samples). In panel **B**, 30 μg protein from the pooled tissue extracts from 3 to 4 mice from the untreated and treated groups were loaded in the corresponding lanes. Panel **C** represents relative intensity of LDH band taking total of the three lanes as 100%)

for protein–metal interaction. Such an approach has been applied to study the interaction of certain metal-complexes with serum proteins (Trynda-Lemiesz et al. 2000; Piccioli et al. 2004; Kartz et al. 1994) and also with LDH (Trigun et al. 2007). Using the similar parameter, results in Fig. 2A and B suggest significant binding of both these complexes with LDH. Nonetheless, binding constant data indicates that Zn-bpy–LDH interaction is stronger than the Cu-bpy–LDH.

The binding study data presented may be interpreted in terms of electrostatic interaction and/or H-bonding or a direct metal coordination of the complex to certain surface residues of the protein. Based on the absorption and spectrofluorometric data, the interaction of gold (III) with serum albumin has been interpreted in similar way (Marcon et al. 2003). Cu and Zn metals have been found to bind with the cellular proteins (Halliwell and Gutteridge 1990) and suggested to interact at His, Cys, Asp, and Glu side chains of most of the proteins studied in this context (Jernigan et al. 1994; Maynard and Covell 2001; Dudev and Lim 2002; Dudev et al. 2003). These amino acid residues could be the putative sites on LDH also for the interaction of Cu- and Zn-bpy complexes. Moreover, with a pharmacological viewpoint, it was important to define whether these complexes after binding to LDH modulate the activity of this enzyme at tissue level.

Based on in vitro studies using cell lines, it has been reported that certain Cu-complexes act as anti-tumor (Gokhle et al. 2001), as anti-proliferative (Collins et al. 2000) and pro-apototic (Daniel et al. 2004a, b; Cai et al. 2007) agents in selected tumor cells. However, cellular targets of such complexes in vivo largely remain undefined. Enzymatic proteins are highly sensitive to ligand dependent allosteric modulations and therefore, considered to be the most suitable targets for pharmacological agents (Groebe 2006). An exogenous factor can modulate the enzyme activity in two ways. First, by altering the synthesis of the enzyme at gene level and second, by direct interaction with the enzymatic proteins. Since, both the complexes have shown their strong interaction with LDH in vitro, it was worth to first work out with the second possibility in animal system. In order to assess specificity of both these complexes with respect to the different isozymes of LDH, different mice tissues, including those who express all the five isozymes, were selected for the present study. Liver, skeletal muscle and spleen express M4-LDH, whereas, kidney, brain and heart are known to contain all the five LDH isozymes in different ratio. The results in Figs. 3–5A, B, and C suggested that Cu-bpy inhibits LDH in all the tissues studied, however, Zn-bpy could inhibit this enzyme only in liver, kidney, and heart. Nonetheless, wherever LDH was inhibited, all the five isozymes were found to be

sensitive (PAGE results: panel B and C) for both these complexes, suggesting that Cu-bpy and Zn-bpy are able to inhibit all the five isozymes of LDH.

It is hard to explain why Zn-bpy is able to inhibit LDH only in selected tissues. Nonetheless, it has been described that tissue specific factors present in the cellular milieu generally determine cell responses to the endogenously developed conditions and the exogenous factors (Singh and Kanungo 1968; Trigun and Singh 1989). This could also be the reason in case of non-responsiveness of Zn-bpy to LDH in certain tissues. The argument gets support from a significant inhibition of purified LDH (out side the tissue) by Zn-bpy (unpublished data) and also from the greater sensitivity of LDH for Zn-bpy than the Cu-bpy in some of the tissues like kidney and heart (Figs. 3, 4). It has also been reported that DNA cleavage potential of a Cu-bpy is not uniform and depends on the concentration of porphyrin and DTT (Laine et al. 2004).

In general, binding of a ligand to an enzyme is known to induce subtle changes in the enzyme conformation resulting into alterations in the enzyme activity (Proud 1984; Trigun and Singh 1989; Groebe 2006). So far modulations of enzymatic proteins by metal complexes are concerned, inhibition of cytochrome c activity by a Ru(III) complex (Trynda-Lemiesz 2004) and that of LDH by Ru(II)-CNEB complex (Trigun et al. 2007) have been tested on this parameter. The data presented here provide evidence that Cu-bpy & Zn-bpy complexes are also able to inhibit LDH by their interaction at protein level. Keeping in account of biocompatibility of both these complexes, our findings provide a relevant biochemical basis for therapeutic assessment of such complexes. This is important because till recent, the enzyme based chemotherapeutic intervention by Cu- and Zn-complexes has been relatively less focused baring some isolated examples (Berg and Shi 1996; Cox and McLendon 2000; Daniel et al. 2004a, b).

The importance of coupling between cellular energy metabolism and development of abnormal cell behavior such as apoptosis and cancer is just beginning to be appreciated (Matoba et al. 2006; Robey and Hay 2005). Energy production in cancer cells has been reported to be over 400-fold higher (Newsholme and Board 1991) than the normal cells and in turn, growing cancer cells up regulate anaerobic glycolysis vis-a-vis LDH activity (Koukourakis et al. 2003; Niakan 2001; Pathak and Vinayak 2005) even in the presence of oxygen (Kim et al. 2005). The use of anti-metabolite 2-deoxy-D-glucose to antagonize the high glucose dependency of tumors has been evaluated in vitro as a novel chemotherapeutic approach against cancer (Aft et al. 2002). The findings presented here, in addition, provide a metal-based strategy to inhibit LDH whose activation is critically implicated in meeting the increased energy need of the tumor cells.

It has been demonstrated that many cancer cells accumulate high amount of copper (Kuo et al. 2002; Chan et al. 1993; Geraki et al. 2002; Jayadeep et al. 1997). While the precise role of copper in tumors is not yet clear, it is suggested to be required for angiogenesis (Daniel et al. 2004a, b) and can also generate ROS in tumor cells (Theophanides and Anastassopoupou 2002). In the present context, we speculate that property of Cu to get accumulated in tumor cells would be helpful in delivering exogenously given Cu-complexes preferentially to the tumor cells than the normal cells. Based up on the similar approach, an organic Cu-complex has been predicted to act as an anti-tumor agent in human cancer cells (Daniel et al. 2004a, b). Thus, capability of Cu-bpy and Zn-bpy complexes to inhibit all the five isozymes of LDH assumes great significance with a viewpoint of their therapeutic applications.

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