Effect of methyltin trichloride on the activity of lactate dehydrogenase

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The effect of $MeSnCl_3$, which is a highly toxic compound, on the activity of L-lactate:NAD oxidoreductase (lactate dehydrogenase) in the extract from the liver of Russian sturgeon (*Asipenser gueldenstaedti* B.). Noncompetitive inhibition of the enzymatic reaction was discovered. This can be due to a change in the enzyme conformation caused by the action on the thiol groups, important for enzyme activity.

Key words: organotin compounds, methyltin trichloride, lactate dehydrogenase, noncompetitive inhibition.

Organotin compounds, which are either man-made or are formed in biochemical alkylation reactions in the environment, are superecotoxicants.[1,2] The toxicity of Sn compounds is normally attributed to enzyme inhibition caused by the interaction of the tin atom with the SH groups in the active sites.[3] The toxicity of alkyl derivatives of tin decreases in the series $R_3SnX > R_2SnX_2 > RSnX_3$; methyltin trichloride $MeSnCl_3$ is believed to be a low-toxicity compound.[4,5] In addition, organotin compounds, Me_nSnX_{4-n}, exhibiting clear-cut oxidative capacity, which increases in the series $Me_3SnX < Me_2SnX_2 < MeSnX_3$, can participate in biochemical redox processes, for example, in the transformations of NAD and NADH (NAD is β-nicotinamide, NADH is the reduced form of NAD)[6,7] or in the transport of electrons in the respiration cycle.[8] Thus, biological oxidation reactions or respiratory processes can serve as the targets of action of these toxicants at the cellular level. The initial stage of respiration under aerobic or anaerobic conditions is glycolysis whose terminal enzyme is L-lactate:NAD-oxidoreductase (lactate dehydrogenase (LDH), EC 1.1.1.27). Lactate dehydrogenase catalyses the reversible transformation of lactic acid into pyruvic acid.

The formation of ATP upon glycolysis would be impossible without this enzyme.[9] Normal functioning of this enzyme becomes especially significant for a living organism being exposed to a series of stress factors under extremal conditions under which glycolytic processes predominate in the tissues. This fact has been established on exposure of fish to hypoxia, some toxic agents of both natural and artificial origins (excretion of cyanobacteria, toxins, Trichlorfon, and dichlorodiphenyltrichloroethane).[10]

It is well known that LDH is inhibited by so-called SH-reagents, which can undergo alkylation or oxidation or form thiolates.[11] Lactate dehydrogenase was shown to be inhibited by the following tin compounds: inorganic tin (rat liver LDH);[12] triethyltin sulfate and diethyltin dichloride in concentrations above 10^{-3} mol L^{-1} (rat brain LDH)[13].

The purpose of this work is to study the influence of $MeSnCl_3$, possessing the highest oxidizing capacity, on the activity of lactate dehydrogenase from the Russian sturgeon liver.

Experimental

The LDH activity was assayed in the extract from the Russian sturgeon liver (*Asipenser gueldenstaedti* B.) using a standard procedure[14] on the basis of the rate of lactate oxidation. The sturgeon liver (1 g) was homogenized in 5 mL of 0.25 *M* saccharose containing 1 mmol L^{-1} of EDTA. After 30 min, the homogenate was centrifuged for 20 min at 3000 rpm at 25 °C. The supernatant containing the enzyme was used in the subsequent procedure. The protein concentration in the supernatant was determined by spectrophotometry according to the method of Warburg and Christian.[15] The incubating mixture consisted of 2.57 mL of a 0.15 *M* glycine buffer (pH 9.9—10), 0.3 mL of 5 *mM* NAD, and 0.03 mL of 0.3 *M* sodium lactate. The final concentrations in the cell were the following: glycine buffer, 0.1 mol L^{-1}; NAD^+, 0.5 mmol L^{-1}; sodium lactate, 3 mmol L^{-1}. The solution temperature was maintained at 25 °C. The reaction was initiated by adding 0.1 mL of the sturgeon liver extract to the incubating medium.

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When investigating the influence of the $MeSnCl_3$ additive on the LDH activity, the reagents were added in the following sequence: the medium of measurements (glycine buffer), NAD, sodium lactate, an aqueous solution of the toxicant, and the liver extract.

To establish the type of inhibition, the steady-state kinetics of the enzymatic reaction was measured based on the dependence of the initial reaction rate v_0 on the initial substrate S concentration ([S] = 1.5—9 mmol L^{-1}) in the absence and in the presence of $MeSnCl_3$.[16] This method of measurements was used due to the potential inhibiting effect of the reaction product (pyruvate) and partial enzyme denaturation in the case of prolonged process.

The reaction rate was expressed as the increase in the optical density of the solution at λ = 340 nm corresponding to the accumulation of NADH in 1 min. The concentration of $MeSnCl_3$ in the incubating medium was varied from 0.28 mmol L^{-1} to 1.04 mmol L^{-1}. The optical density was measured on a KFK-2MP photoelectric concentration colorimeter every 5 s over a period of 3 min. The experimental data were processed by a graphical method in the Linuoiver-Berk coordinates (the method of double reciprocals) to give the Michaelis constant K_M and the maximum reaction rate v_{max}).

Methyltin trichloride (Strem, 99%), NAD (Sigma, sodium salt), and the other reagent grade compounds were used without purification.

Results and Discussion

LDH is known to be an allosteric enzyme.[17] The hyperbolic Michaelis—Menten law does not always hold for this type of enzymes (if the active sites in the molecule of an allosteric enzyme interact). The dependence of the initial rate of the enzymatic oxidation of lactate at pH 10 on the substrate concentration (within the studied concentration range) follows the Michaelis—Menten equation (Fig. 1) both in the absence and in the presence of the toxicant.

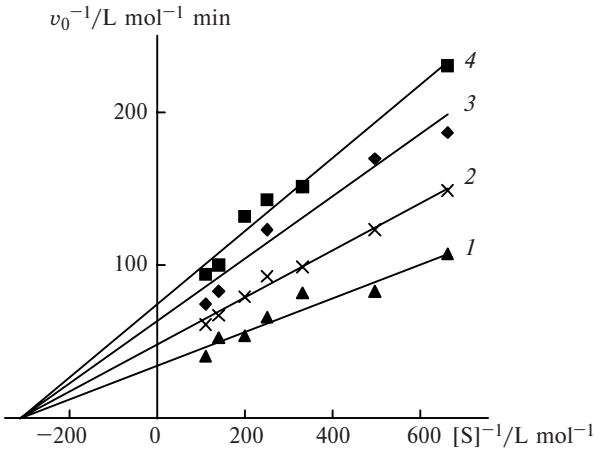

Fig. 1. Initial rate of the enzymatic oxidation of lactate *vs.* substrate concentration in the Linuoiver—Berk coordinates without the $MeSnCl_3$ additive (*1*) and in the presence of $MeSnCl_3$: 0.42 (*2*), 0.75 (*3*), and 1.04 mmol L^{-1} (*4*).

The SH groups can serve as potential chemical targets for the action of $MeSnCl_3$. Lactate dehydrogenase is a fairly well-studied SH-containing enzyme. The enzyme active site incorporates the cysteine SH groups and the histidine imidazole residue.[18] However, binding of both the substrate and the coenzyme in the enzyme active site occurs without participation of the SH groups.[19]

The experimental results indicate that $MeSnCl_3$ is responsible for a purely noncompetitive inhibiting of the enzymatic reaction. Indeed, with an increase in the $MeSnCl_3$ concentration, the v_{max} decreases, while the K_m value does not change, being equal to 4.17 mmol L^{-1}.

[$MeSnCl_3$]/mmol L^{-1}	0	0.42	0.75	1.04
v_{max}/mmol L^{-1} min^{-1}	0.028*	0.019*	0.017*	0.015*

* The standard deviation is 0.001.

Thus, methyltin trichloride does not hamper the substrate binding to the enzyme. The decrease in v_{max} might be either due to the action of the toxicant on the allosteric center of the enzyme, inducing a change in the enzyme conformation and in the spatial structure of its active site, or due to oxidizing capacity of $MeSnCl_3$.

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To make the **publication time as short as possible**, authors are requested to pay special attention to the **layout of the paper**. Issues of the current year should be consulted. The full instructions are published annually in the January issue of the Journal and are available through the Internet at **http://rcb.ioc.ac.ru**. The following points are essential.

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❑1.1. The surname, first name, and position of the person with whom the editors should communicate (postal address, telephone number, fax, e-mail address). The surname of the author to whom correspondence should be addressed should be marked by an asterisk.

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❑1.3. A structured list of words for the subject index (see clause 2.4 of the Instructions and Appendix 2).

❑1.4. A graphical abstract (in duplicate, see clause 2.6 of the Instructions and Appendix 3).

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❑4. The **Experimental** section should contain data confirming the structure and purity of all newly synthesized compounds, sources or procedures for the synthesis of the **nontrivial reagents** used, and the conditions of the **additional** preparation of reagents and solvents (see clause 3.11 of the Instructions).

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❑8. Spectra should **not** be drawn **by hand**.

❑9. All **abbreviations** and **contractions** should comply with the list presented in the Instructions for authors (see Appendix 8) or expanded when mentioned for the first time.

❑10. X-Ray diffraction data should be presented as patterns of molecules (with numbered atoms) or crystal packings and tables containing **necessary** geometric characteristics of molecules (**selected** bond lengths and bond and torsion angles). Full tables of atomic coordinates, thermal factors, and full tables of bond lengths and bond angles will be deposited at the Cambridge Structural Data Bank. For this purpose, in addition to the printed **full tables** enclosed as an appendix to the paper (*not for publication*), authors should enclose a separate floppy disk with files named **filename.res** or **filename.cif**, corresponding to the ultimate structure refinement, and comments matching particular structures in the text to particular files (see clause 3.12 of the Instructions).

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❑13. Characters for physical variables (for example, temperature T) but not units of measure (K), stereochemical descriptors (*cis*, *Z*, *R*), locants (*N*-methyl), and letters (but not numerals) in the designations of groups of symmetry should be typed in *italic* (C_{2v} but not C_{2v}).

❑14. The oxidation numbers of compounds with the names of elements should be typed in SMALL CAPS and enclosed in parentheses (iron(II)), and the oxidation numbers with the symbols of elements are to be given as superscripts (Fe^{II}).

❑15. **Each reference** mentioned in the list of references should also be mentioned in the text. **References** in the text are to be typed in **Bold** as **superscripts**; the numbering should strictly correspond to the order in which they are mentioned.

❑16. Only standard abbreviations of journal titles may be used in **the list of references** (see Appendix 11).