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OXIDATIVE ACTIVITY OF ORGANOMETALLIC COMPOUNDS IN RELATION TO THE COENZYMES NADH AND NADPH

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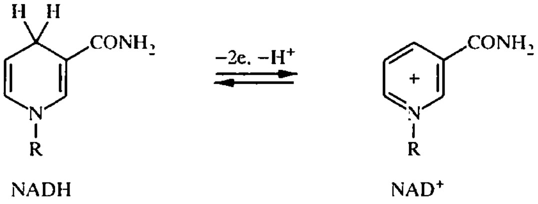
Kinetic investigations of the interaction of Hg and Sn-containing organometallic compounds with NADH and NADPH using spectro-photometric methods have shown that these compounds may act as oxidizing agents in relation to the coenzyme. Their oxidative activity depends on the nature and number of organic groups in the molecule. Comparison of the kinetic data for the activity of Hg and Sn compounds with those for Fe porphyrins imitating the active centers of redox enzymes indicates the competitiveness of the organometallic compounds and models of natural electron acceptors.

The coenzymes NAD and NADP are participants in a large number of oxidation–reduction enzymic processes occurring in living cells. Their function includes the reversible transport of electrons and protons by the following scheme.



R is a residue of adenine dinucleotide (NAD);
adenine dinucleotide phosphate (NADP)

Active centers of redox enzymes, such as iron porphyrins [1], may serve as acceptors of electrons. In addition the reduced forms of the coenzymes, NADH and NADPH, may be oxidized by exogenous ecotoxicants such as organometallic compounds of heavy metals, occurring anthropogenically or formed as a result of biochemical alkylation reactions of the corresponding inorganic salts [2,3]. A hypothesis was expressed previously on the oxidation of NADH to NAD$^+$ in the presence of methylmercury chloride [4].

A comparative investigation has been carried out in the present work on the oxidative activity of model iron porphyrins and organomercury and organotin compounds in relation to NADH and NADPH by absorption spectroscopic methods under conditions maximally close to those *in vivo*.

Metal complexes used in this study, to model the active centers of redox enzymes, were iron porphyrins with various substituents on the periphery of the macrocycle, including etioporphyrin (Ia), protoporphyrin IX (hemin) (Ib), tetraphenylporphyrin (Ic), and iron porphyrin (Id) containing 2,6-di-*tert*-butylphenol in a *meso* position.

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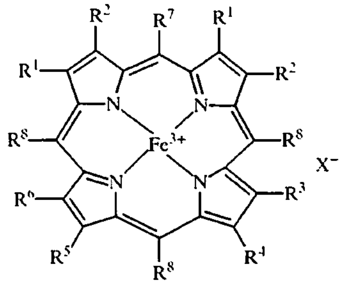
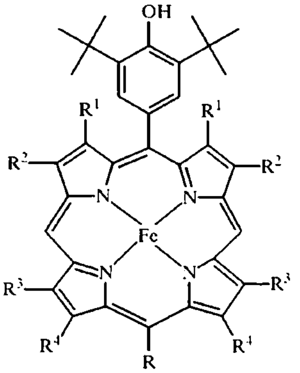
Organometallic compounds considered as ecotoxicants were of general formula RnMXm; R = Me, Et, *i*-Pr, *n*-Bu, *i*-Bu, *n*-C_7H_{15}, *n*-C_8H_{17}, Ph, *p*-MeC_6H_4; M = Hg, Sn; X = Cl, Br, I; n = 0-4; m = 0-4. In addition a comparison was made with inorganic salts regarded as typical oxidizing agents, $HgCl_2$, $Hg(OAc)_2$, $SnCl_4$, and $Pb(OAc)_4$.



Ia R^1, R^3, R^5 = Me, R^2, R^4, R^6 = Et, X = Cl;
Ib R^1, R^3, R^6 = Me, R^2 = CH=CH$_2$, R^4, R^5 = CH_2CH_2COOMe, X = Cl;
Ic R^1–R^6 = H, R^7, R^8 = Ph, X = Cl



Id R^1–R^4 = H, X = Cl

In view of the relative ease of the redox transition in the pair NADH NAD$^+$ (~0.4 V at the first rate-determining step of oxidation to the corresponding cation radical [5]), it might be thought that not only inorganic salts [such as $Pb(OAc)_4$] but also their organic derivatives will display oxidative activity. In reality all the organometallic compounds listed are oxidizing agents in relation to NADH and NADPH. In all cases the character of the NADH spectrum, as for NADPH, on adding an oxidizing agent is changed similarly to that given in Fig. 1a (b is a typical kinetic curve). With an excess of oxidizing agent the reaction is first order with respect to substrate. Rate constants are given in Table 1 for the oxidation of NADH in the presence of various Hg and Sn compounds. The rate constant of 0.942 sec^{-1} for oxidation with $Pb(OAc)_4$ was the maximum.

The effect of the additional phosphate group in NADP on the course and rate of its reaction with certain oxidizing agents compared with NAD was studied with the aim of clarifying the difference between coenzymes NAD and NADP. Oxidizing agents used were $HgCl_2$, MeHgI, and *n*-PrHgBr. The kinetic parameters of the reactions, carried out under identical conditions, are given in Table 2. It is evident that in the reactions investigated the behavior of both coenzymes was the same, which enables it to be concluded that they are identical. A comparative investigation was caused by assuming possible complex formation, as a result the heavy metal ion coordinates with fragments of the coenzyme molecule. The heterocyclic rings or phosphate groups may act as chelating agents. In the latter case the formation of insoluble phosphates might be expected, which is expressed in a sharp reduction of the coenzyme concentration in solution. However no precipitation of solid was observed during the course of the reaction which indicates the absence of similar conversions linked with low values of reaction parameters. Consequently the presence of the additional phosphate group in NADP has no effect on the overall mechanism of oxidation, which is a one-electron transfer from the coenzyme molecule to the organometallic compound [6].

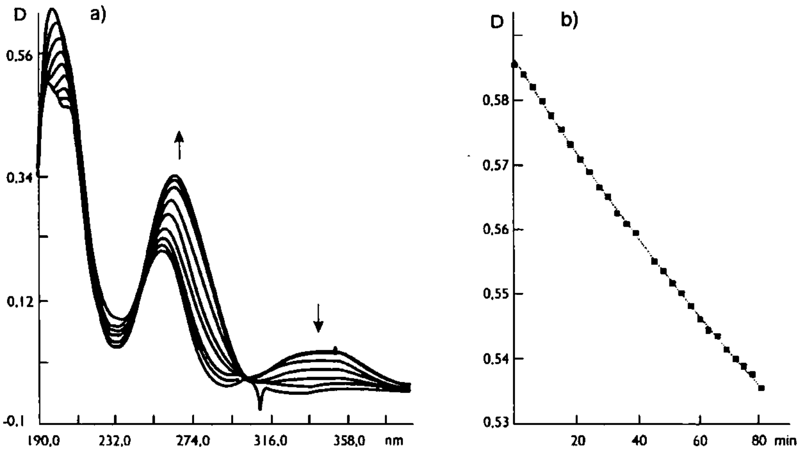


Fig. 1. a) Spectral changes (decrease of D, λ_{max} 340 nm) on oxidizing NADH with MeHgI; b) kinetic curve for the oxidation of NADH (2×10^{-3} M) in the presence of MeHgI (6×10^{-3} M) (pH 7.5, MOPS, H_2O, 20°C).

The experimental data obtained indicate that the lead and tin compounds may act as oxidizing agents in relation to NADH and NADPH, though a higher oxidation rate was observed for the inorganic salts than for the organometallic compounds with a difference in rate constant of 2-3 orders of magnitude. On the whole the reaction proceeds somewhat more slowly with organotin compounds than with organomercury derivatives. Using the methyltin derivatives it was clearly shown that the oxidation rate ofNADH was reduced on increasing the number of organic groups in the molecule of oxidizing agent, which may be connected with similar changes of their reduction potentials [7,8].

The rate constants for the oxidation of NADH in the presence of iron porphyrins (Ia-d) are given in Table 3. In this case the oxidative activity of the electron acceptors is linked with the change in the degree of oxidation of Fe ion in the porphyrin ring. This process is characterized by higher values of the reduction potentials (-0.11 to -0.17 V [9]) compared to the lead and tin organometallic compounds (-0.4 to -1.2 V [7,8]), which may not be reflected in the oxidation rate of the coenzymes.

TABLE 1. Rate Constants for the Oxidation of NADH in the Presence of Hg and Sn Compounds (pH 7.4, H_2O, 20°C, concn. [R_nMX_m] $4.2\text{-}42 \times 10^{-4}$ M, conc. [NADH] $3.5\text{-}3.9 \times 10^{-4}$ M)

RHgX	$k \times 10^{-4}$, sec^{-1}	R_nSnX_{4-n}	$k \times 10^{-4}$, sec^{-1}
No additions	0.004 ± 0.0006		
$HgCl_2$	2080 ± 0.9	$SnCl_4$	510 ± 0.5
$Hg(OAc)_2$	2250 ± 1.9		
MeHgI	11 ± 0.04	$MeSnCl_3$	4 ± 0.2
		Me_2SnCl_2	0.2 ± 0.04
		Me_3SnCl	0.06 ± 0.0085
n-PrHgBr	1 ± 0.65	$EtSnCl_3$	1 ± 0.45
i-PrHgBr	0.8 ± 0.09	Et_2SnCl_2	1 ± 0.35
		Et_3SnCl	0.2 ± 0.04
i-BuHgCl	0.7 ± 0.04	Bu_2SnCl_2	0.3 ± 0.07
n-$C_7H_{15}HgBr$	0.6 ± 0.085	Bu_3SnCl	0.3 ± 0.05
n-$C_8H_{17}HgBr$			
p-MeC_6H_4HgBr	1 ± 0.7	$PhSnCl_3$	67 ± 0.075
(*p*-$MeC_6H_4)_2Hg$	0.07 ± 0.01	Ph_2SnCl_2	0.3 ± 0.04
		Ph_3SnCl	0.2 ± 0.01

TABLE 2. Comparative Kinetic Parameters of the Oxidation of NADH and NADPH in the Presence of Mercury Compounds (pH 7.4, H_2O, 20°C)

Compound	$C_i \times 10^{-4}$ M		k, sec^{-1}	
	NADH	NADPH	NADH	NADPH
$HgCl_2$	4.7	4.8	0.208 ± 0.0005	0.197 ± 0.0008
MeHgI	3.8	4.0	0.0011 ± 0.0004	0.0015 ± 0.0008
n-PrHgBr	4.28	4.1	0.0001 ± 0.0007	0.0001 ± 0.0006

TABLE 3. Rate Constants for the Oxidation of NADH in the Presence of Iron Porphyrins (Ia-d) (pH 7.4, H_2O, 20°C, concn. $[R_nMX_m]$ 2.1-17 × 10^{-4} M, concn. [NADH] 1.4-1.9 × 10^{-4} M)

Compound	Ia	Ib	Ic	Id
$k \times 10^{-4}$, sec^{-1}	1.1 ± 0.1	1.9 ± 0.3	1.1 ± 0.4	1.4 ± 0.2

Analysis of the kinetic data given in Tables 1 and 3 shows that in the majority of cases the organometallic compounds proved to be weaker oxidizing agents. However methylmercury iodide for example, which possesses an extremely high toxicity [1], is also characterized by a high rate for oxidation of the coenzymes.

The detailed kinetic investigations, the data of which are given in this study, enable a conclusion to be made on the possible competing effect of heavy metal toxic compounds, when penetrated into a cell, on the process of oxidizing the coenzymes NADH and NADPH. This may lead to another pathway for the transport of electrons and general disturbance of metabolism.

EXPERIMENTAL

The electronic absorption spectra were recorded on a Varian DMS 100s instrument in quartz cuvettes at room temperature. The NADH, NADPH, NAD^+, $NADP^+$, and protoporphyrin IX (hemin) were used as prepared samples (Sigma) without further purification. Mercury and tin compounds, and also porphyrins Ia,c,d were obtained by known procedures [10-13].

Solutions of the organometallic compounds and porphyrins Ia-d were prepared in buffer directly before an experiment and were stored in closed cuvettes while spectral experiments were being carried out. The preparation of solutions was carried out by calculation using a combination of the amount of reagent being used and an acceptable value of the optical density of the substrate used. When a compound being investigated was insufficiently soluble a solution was first prepared in EtOH or DMF and then diluted with buffer solution (potassium phosphate buffer or MOPS). The kinetic investigations were carried out using the fall in intensity of the absorption band at 340 nm (ε 6.22 × 103 $M^{-1} \cdot cm^{-1}$) corresponding to the reduced form of NAD (dihydronicotinamide part of the molecule). In each case a comparison was made of the obtained spectrum after oxidation of NADH with the spectrum of ready-made NAD^+ of appropriate concentration.

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