INDUCTION OF A MENADIONE-DEPENDENT RESPIRATORY SHUNT BY A PLATINUM COMPLEX

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On treatment of submitochondrial particles (SMP) from bovine heart with the platinum complex K $[C_2H_4PtCl_3]$ (Zeize's salt) a menadione-dependent shunt is formed in them, and is manifested as stimulation of the oxygen uptake by menadione when electron transport is blocked by rotenone. The effect is observed only when NADH and not succinate is used as substrate. Menadione-dependent respiration induced by Zeize's salt is sensitive to dicoumarin but is not inhibited by antimycin or cyanide, which distinguishes it from the menadione-reductase shunt in intact mitochondria. KEY WORDS: menadione; respiratory shunt; platinum complexes.

Electron transport between NADH and cytochrome b in liver mitochondria can be shunted by vitamin K_3 (menadione) on account of the presence of a special enzyme (menadione reductase) catalyzing the reduction of vitamin K_3 from NADH, in them. The shunting effect of vitamin K_3 on liver mitochondria is manifested by the fact that it restores respiration when blocked by rotenone, for the site on the respiratory chain at which reduction of vitamin K_3 takes place with the participation of menadione reductase is on the substrate side of the rotenone block. However, in bovine heart mitochondria there is no menadione reductase, and vitamin K_3 has no shunting action [5].

The object of the present investigation was to study the sensitivity of mitochondrial respiration in the heart and liver to platinum and palladium complexes which, as has been shown previously [1, 4], possess high activity in relation to energy-converting membrane systems.

EXPERIMENTAL METHOD

Experiments were carried out on rat liver mitochondria and submitochondrial particles (SMP) from bovine heart, isolated by methods described previously [3, 6]. The incubation medium contained 0.22 M sucrose, 50 mM Tris-HCl (pH 7.6), 10 mM KH_2PO_4, 5 mM $MgSO_4$, 20 mM KCl, and 0.25 mM EDTA. The concentration of mitochondrial protein in the incubation medium was 5 mg/ml and that of SMP was 1 mg/ml. The respiratory substrates for liver mitochondria were a mixture of glutamate and malate (5 mM of each) and succinate (7 mM), and those for heart SMP were NADH (1 mM) and succinate (7 mM). To convert the mitochondria and SMP into the uncoupled state (state 3p) the uncoupling agent chloromethoxycarbonyl cyanidephenylhydrazone was added to the incubation medium in a concentration of $5 \cdot 10^{-7}$ M. The rate of respiration was measured polarographically with a closed Clark's platinum electrode. The kinetics of oxidation and reduction of cytochrome b was recorded on a Hitachi-356 spectrophotometer, using a two-wave scheme with $\lambda_1 = 562$ nm and $\lambda = 575$ nm. The combined contribution of cytochromes b_K and b_T to the absorption was recorded.

EXPERIMENTAL RESULTS

Data showing the effect of the platinum complex K $[C_2H_4PtCl_3]$ (Zeize's salt) on respiration of rat liver mitochondria and bovine heart SMP are given in Table 1. In state 3p, Zeize's salt depressed respiration in low concentrations (0.06-0.55 mM). The inhibitory effect of Zeize's salt increases with time. The lag period (the time between addition of the salt and the beginning of manifestation of its inhibitory effect) on mitochondria and SMP was 0.5-1 min whatever substrate was used. Incubation of Zeize's salt with mitochondria but without substrate for 25 min (or with SMP for 2 min) led to disappearance of the lag-period, i.e., the kinetics of inhibition was connected with penetration of Zeize's salt inside the mitochondria to the functional groups of

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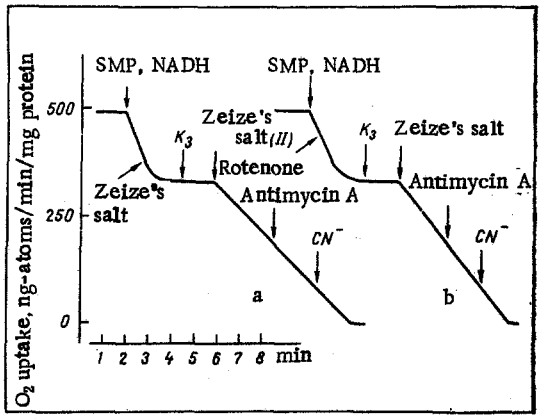

Fig. 1

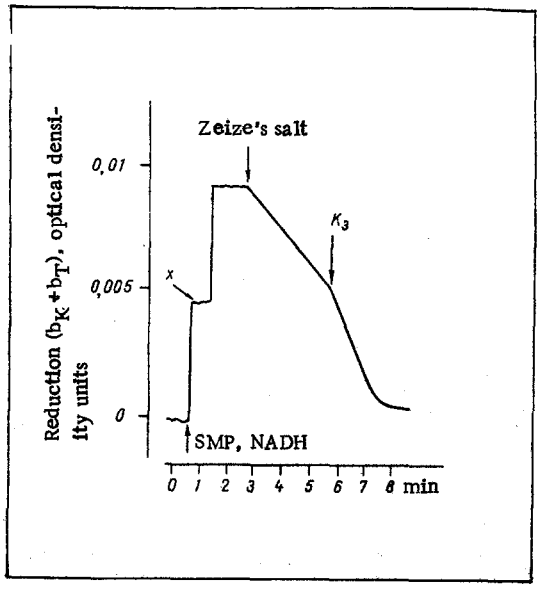

Fig. 2

Fig. 1. Effect of Zeize's salt and vitamin K_3 on oxidation of NADH by submitochondrial particles of bovine heart in state 3p. a) Zeize's salt (I) 1.7×10^{-4} M, Zeize's salt (II) 5×10^{-4}, vitamin K_3 5×10^{-5}, antimycin A 5×10^{-6} M, NaCN 10^{-3} M; b) rotenone 5×10^{-5} M, vitamin K_3 5×10^{-6} M, Zeize's salt 5×10^{-4} M, antimycin A 5×10^{-6} M, NaCN 10^{-3} M.

Fig. 2. Effect of Zeize's salt and vitamin K_3 on oxidation–reduction state of cytochrome b. Zeize's salt 5×10^{-4} M; vitamin K_3 5×10^{-5} M. New steady state of cytochrome b determined by rate of diffusion of oxygen through membrane.

the enzyme with which it interacts. In the case of incubation of mitochondria with Zeize's salt, the concentrations causing 50% inhibition of substrate oxidation were close to those on SMP without incubation.

Zeize's salt in a concentration of 0.17 mM completely inhibited respiration of SMP when NADH was used as substrate. The addition of vitamin K_3 gave no effect, but further addition of the salt (0.5 mM) led to restoration of respiration by 30-35% (Fig. 1a).

As Fig. 1b shows, addition of rotenone (2×10^{-5} M) to SMP completely inhibited respiration on NADH as substrate. Vitamin K_3 gave no effect. However, subsequent addition of Zeize's salt in a concentration of 0.5 mM restored respiration by almost 50%. The same effect also was observed if Zeize's salt was added first, and vitamin K_3 later. Without menadione, Zeize's salt did not stimulate the uptake of oxygen.

Oxidation of NADH in the presence of Zeize's salt and vitamin K_3 did not take place without SMP or if SMP preparations which had lost their activity were used. Hence it follows that the observed effect is an enzymic process and not chemical interaction of NADH with vitamin K_3 and Zeize's salt. The effect was specific for Zeize's salt. Of 12 other complexes of platinum and palladium which were tested, only two compounds – $PtC_7H_8Cl_2$ and $Pd(NH_3)_2(NO_2)_2$ – could induce menadione-dependent respiration, but the rate of oxygen uptake (with optimal concentrations of the complexes) was only 25-50% of that found when Zeize's salt was used.

Menadione-dependent respiration of SMP treated with Zeize's salt was recorded only when NADH was used as the substrate for SMP, or both substrates for liver mitochondria, Zeize's salt had only an inhibitory action (Table 1) which was independent of the presence of menadione in the medium.

Menadione-dependent respiration of SMP treated with Zeize's salt was inhibited by dicoumarin (50% inhibition at 8×10^{-5} M) but was resistant to antimycin and cyanide. This significantly distinguishes the shunt induced in bovine heart mitochondrial membranes by Zeize's salt from the "natural" menadione-reductase shunt in the liver mitochondria, where vitamin K_3 transports electrons from the NADH-dehydrogenase site on cytochrome b and menadione-dependent respiration is inhibited by antimycin and cyanide. The absence of an inhibitory action of antimycin and cyanide in the case of the shunt developing in SMP under the influence of Zeize's salt suggests that in this case menadione transports electrons from the NADH-dehydrogenase site of the respiratory chain not to cytochrome b, but directly to oxygen.

TABLE 1. Effect of Zeize's Salt on Respiration of Rat Liver Mitochondria and Bovine Heart SMP in State 3p

Experimental conditions	Substrate	Concen. (in M) causing 50% inhibition of oxidation of substrate	
		rat liver mitochondria	bovine heart SMP
Incubation of mitochondria with inhibitor for 25 min	Glutamate and malate	$7 \cdot 10^{-5}$	—
Without incubation	"	$2{,}5 \cdot 10^{-4}$	—
" "	NADH	—	$6 \cdot 10^{-5}$
Incubation of mitochondria with inhibitor for 25 min	Succinate	$1{,}2 \cdot 10^{-4}$	—
Without incubation	"	$5{,}5 \cdot 10^{-4}$	$7 \cdot 10^{-5}$

This hypothesis was confirmed by the changes observed in the oxidation–reduction state of cytochrome b under the influence of Zeize's salt and menadione (Fig. 2). If cytochrome b was reduced by NADH and, after establishment of the steady state, Zeize's salt was added (0.5 mM), reoxidation of the cytochrome b was observed. Subsequent addition of vitamin K_3 accelerated the reoxidation of cytochrome b once it had begun, hence it follows that vitamin K_3 catalyzes electron transport from the NADH-dehydrogenase site not to cytochrome b, but via some other pathway. Otherwise the rate of oxidation of cytochrome b under the influence of menadione should have been reduced. Vitamin K_3 in the absence of Zeize's salt, incidentally, causes additional reduction of cytochrome b by NADH.

Zeize's salt in a concentration of 0.4-0.5 mM was found to inhibit the reduction of cytochrome b by NADH and to act after the same lag phase as on respiration. In concentrations below 0.3 mM Zeize's salt did not affect the reduction of cytochrome b by NADH and did not change the established steady state of cytochrome b. This fact, and also the agreement between the concentrations causing 50% inhibition of oxidation of NADH and succinate, is evidence that inhibition of respiration by low concentrations of Zeize's salt takes place at the stage after cytochrome b. With an increase in the concentration of Zeize's salt to 0.4-0.5 mM the NADH-dehydrogenase site also was inhibited. The menadione-dependent shunt in SMP was induced by Zeize's salt only in concentrations (0.5 mM) at which it interacts with the NADH-dehydrogenase site.

Reduction of menadione by the NADH-dehydrogenase complex, modified by Zeize's salt, may be connected with the formation of a relatively high steady-state concentration of the semiquinone form of menadione, which enables it to interact with molecular oxygen. Evidence was obtained previously to show that reduction of quinoid components of the respiratory chain is carried out by a system of two single-electron carriers [2]. Under the influence of Zeize's salt the structure of this system may be disturbed so that one of its electron-transporting components is separated from the other, and so becomes accessible for menadione present in the medium. Interaction between the single-electron carrier and menadione in the absence of the second single-electron donor can take place (in the presence of a sufficiently high concentration of menadione, when reduction of the single-electron carrier is the limiting state) to the creation of a relatively high concentration of the semireduced semiquinone form of menadione, which enables it to interact with molecular oxygen.

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