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Vanadium–Mediated Lipid Peroxidation in Microsomes from Human Term Placenta

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Vanadium is considered an essential element present in living organisms in trace amounts but it is toxic when introduced in excessive doses to animals and humans (see review by Jandhyala and Hom 1983). Vanadium compounds are extensively used in modern industry and occupational exposure to high doses of vanadium is quite common. Substantial amounts of vanadium are released into our environment by burning natural oil and gas as well as by incidental spilling of crude oil into the sea (Sadiq and Zaidi 1984). In US cities concentrations of vanadium in lungs increased in the aging population (Tipton and Shafer 1964). Vanadium is present in normal human tissues in concentrations ranging from 1 to 140 ng/g wet weight, and for instance, "physiological" concentration measured by photometric method in human placenta is about 3 ng/g (for review see Nechay 1984). However, as reported by Soremark and Ullberg (1962) in pregnant mice, vanadium accumulates preferentially in the placenta and to lower extent in fetal skeleton and mammary gland during exposure to radioactive vanadium.

Especially, accumulation of vanadium in fetoplacental unit may present threat to the fetus by interacting with enzymes and ion-transporting systems in membranes (Erdmann et al. 1984). It is also possible that accumulation of vanadium with its concomitant reduction to vanadyl (Bruech et al. 1984) may lead to lipid peroxidation (Donaldson et al. 1985), followed by damage to biological membranes, lysosomal enzymes release (Younes et al. 1984) and destruction of placental tissue. Moreover, superoxide anion-radical produced during vanadyl/vanadate redox cycling (Liochev and Fridovich 1987) may generate the family of reactive oxygen species which are known to act as mutagens (see review by Byczkowski and Gessner 1988).

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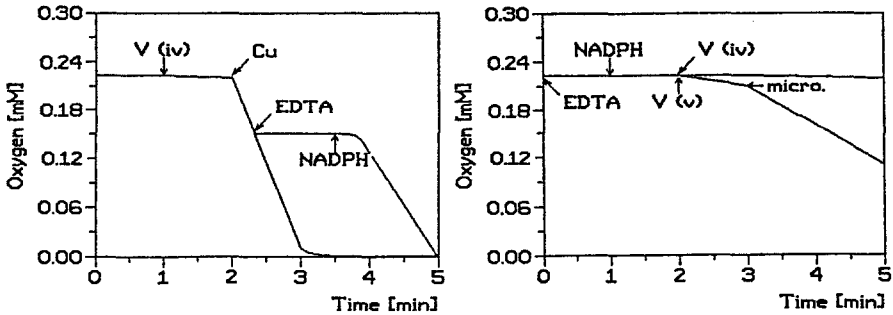
To explore some of these possibilities we decided to examine whether vanadate can undergo redox cycling in microsomes from human term placenta (HTP) that can lead to lipid peroxidation.

MATERIALS AND METHODS

Microsomes were isolated from full term human placentas obtained from healthy, non-smoking women. Blood was washed out with buffered isotonic saline plus 0.5 mM EDTA and then with 0.25 M sucrose plus 0.5 mM EDTA in 20 mM Tris-HCl buffer (pH 7.3). Scrapped cotyledon tissue was washed and homogenized in 0.25 M sucrose containing 0.2% bovine serum albumin, 0.5 mM EDTA and 20 mM Tris-HCl (pH 7.3), using a glass Potter-Elvehjem homogenizer with loosely fitted teflon pestle. The homogenate was filtered through cheese cloth and centrifuged for 5 min at 1000 g at 0-4^{o}C, the supernatant centrifuged again for 15 min at 15,000 g. Postmitochondrial supernatant was centrifuged for 1 h at 105,000 g in MSE ultracentrifuge. The microsomal pellet was suspended in 50 mM Tris-HCl buffer (pH 7.3) containing 15 mM KCl, and recentrifuged again. Incubations were carried out at 37^{o}C in Tris buffer in the presence or absence of 0.5 mM NADPH and 0.5 mg/mL of microsomal protein. The reaction was started by the addition of either vanadyl or vanadate at indicated concentrations. Malondialdehyde (MDA) content was measured as thiobarbituric acid (TBA) reactive material, essentially as described by Kulkarni and Kenel (1987). NADPH oxidation was followed spectrophotometrically at 340 nm. Spectral analysis was done using a Gilford recording spectrophotometer, and oxygen uptake was measured at 30^{o}C with a Clark-type electrode (Byczkowski et al. 1979). Vanadyl sulfate (Aldrich), sodium orthovanadate (Sigma), ammonium metavanadate (Baker) and potassium superoxide (Sigma) were used, and polyvanadate was prepared by dissolving metavanadate in 0.1 M NaOH, followed by neutralization with HCl and adjusting to pH 7.3 with Tris. The experiments were repeated at least three times and the representative results are given in the figures.

RESULTS AND DISCUSSION

Fig. 1 A shows oxygen uptake representing nonenzymatic oxidation of vanadyl (iv) in 50 mM Tris buffer at pH 7.3. The reaction was dramatically increased by Cu^{2+} and inhibited by EDTA. When NADPH was added to the system inhibited by EDTA after 36% of oxygen was already used, a rapid oxygen uptake was observed again. The oxygen uptake did not occur when EDTA was present in the medium before the addition of vanadyl (Fig. 1 B). A similar vanadyl (iv) oxidation reaction was described

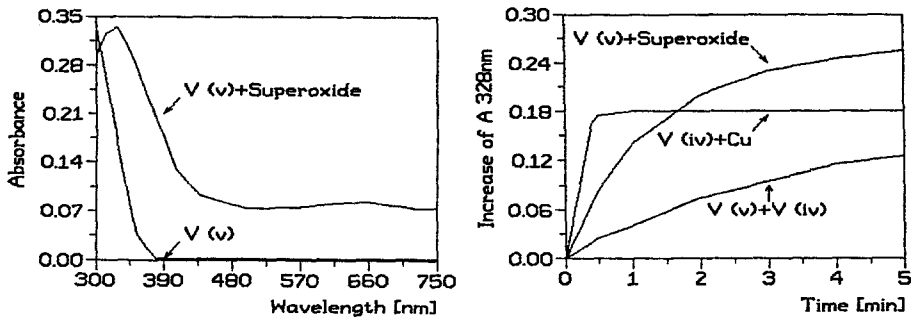


A. B.

Figure 1. Effects of 1 mM vanadyl V (iv) and 1 mM polyvanadate V (v) on oxygen uptake without or with 0.5 mM NADPH in 50 mM Tris buffer (pH 7.3). Downward line on Fig. A represents result without EDTA; branch to the right represents result with 1 mM EDTA. Two experiments shown in Fig.B were done in the presence of EDTA, NADPH and either V(iv) or V(v). All sequential additions are shown by arrows. Other abbreviations used: 50 μM $CuSO_4$ (Cu), 0.5 mg/mL of HTP microsomes (micro).

by Liochev and Fridovich (1987) as a source of $O_2^{\cdot-}$ or $^{\cdot}OH$. This reaction was suggested to initiate one-electron oxidation of NADH. However, in our system 50 mM Tris probably scavenged $^{\cdot}OH$. Moreover, in control experiments (not shown here) addition of 1 mM potassium superoxide to 0.5 mM NADPH in the same buffer did not cause any measurable oxidation of this nucleotide. It seems, therefore, that according to the suggestion of Liochev and Fridovich (1987) a peroxy-vanadyl complex, formed from superoxide and vanadate (v), is the mediator which actually initiates abstraction of hydrogen from NADPH. Addition of microsomes to the system containing EDTA, NADPH and polyvanadate V (v) resulted in increased oxygen uptake (Fig.1 B).

As may be seen in Fig. 2 A, the addition of potassium superoxide to a solution of 0.5 mM yellow polyvanadate resulted in the formation of a faint greenish complex which exhibited a distinct absorption peak at 328 nm. Addition of either vanadyl (iv) to vanadate (v), or Cu^{2+} to vanadyl (iv) resulted in a comparable increase in absorbance at 328 nm (Fig.2 B). A small but distinct peak at 328 nm appeared also in a buffered vanadyl solution which was stored overnight in air (result not shown here). It seems that the peak detected corresponds to the peroxy-vanadyl complex formed between superoxide anion-radical and vanadate (v).

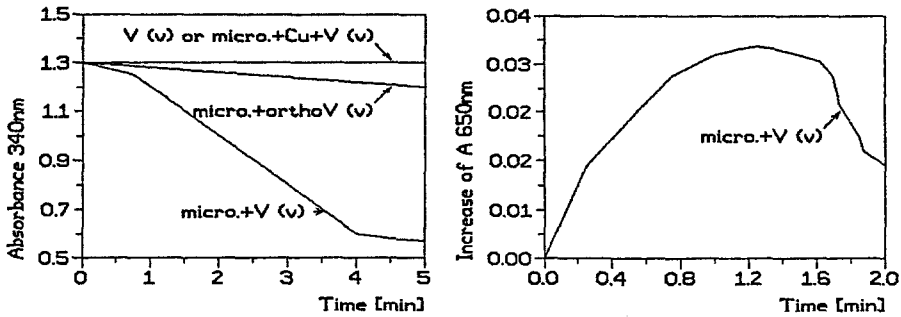


A. B.

Figure 2. A. Effects of 0.5 mM potassium superoxide on spectrum of 0.5 mM polyvanadate V (v). Fig. 2. B.Increase in absorbance at 328 nm caused by different sources of superoxide anion-radical. Other additions: 0.5 mM vanadyl V (iv), 0.1 mM $CuSO_4$ (Cu).

In contrast to the nonenzymatic reactions described above, the reduction of vanadate (v) by NADPH in our system required the presence of microsomes (Fig. 3 A). The reduction was faster and more efficient with yellow polyvanadate (v) than with white orthovanadate (v), was inhibited by the addition of 0.16 mM Cu^{2+}, and was accompanied by the appearance of a faint blue color detectable at 650 nm (Fig. 3B). Similar vanadate reduction was observed by Byczkowski et al. (1979) in rat liver submitochondrial particles and wheat seedling intact mitochondria. It was postulated that flavoprotein dehydrogenases within the inner mitochondrial membrane are responsible for NADPH-dependent reduction of vanadate (v) (for review see Byczkowski and Sorenson, 1984). In microsomes, NADPH:cytochrome P-450 reductase is a flavoprotein with redox potentials similar to that of mitochondrial NADH dehydrogenases, and moreover, its enzymatic activity was inhibited by 0.1 mM Cu^{2+} (Werringloer et al. 1979). It seems therefore, that in HTP microsomes NADPH:cytochrome P-450 reductase may catalyze NADPH-dependent reduction of vanadate (v). Of course in vivo also, nonenzymatic reductions of vanadate may occur with intracellular reductants which keep intracellular vanadium at the tetravalent state (Bruech et al. 1984). The physiological reductant, glutathione-SH was found to be able to reduce vanadate in vitro (result not shown here).

Fig.4 shows lipid peroxidation evoked in HTP microsomes following the addition of vanadyl (iv) and vanadate (v). As evidenced by the formation of TBA-reactive material vanadyl (iv) addition triggered a very rapid

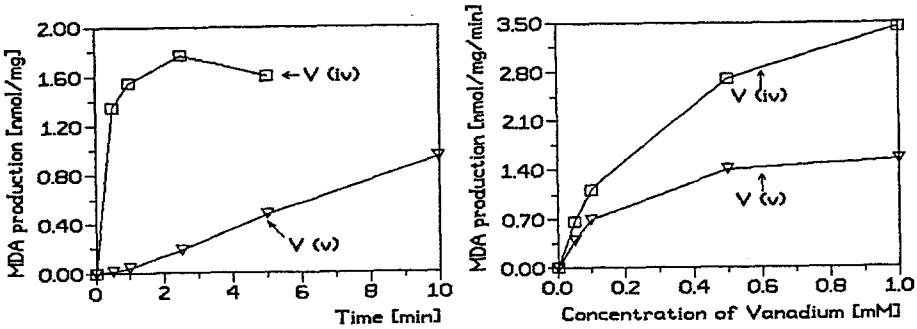


A. B.

Figure 3. Enzymatic reduction of 0.5 mM polyvanadate V(v) and 0.5 mM orthovanadate [ortho V(v)] by 0.2 mM NADPH in the presence of 0.5 mg/mL of HTP microsomes (micro). In both experiments, A. and B. the reference contained microsomes with all additions except NADPH. Top line on Fig. A was obtained without microsomes or with microsomes preincubated with 160 μM $CuSO_4$ (Cu).

lipid peroxidation. MDA accumulation was completed within 2.5 min. In contrast, the vanadate-dependent reaction was linear up to 10 min and resulted in accumulation of MDA about half of that observed for vanadyl (Fig. 4 A). From comparison of amounts of MDA produced during the first 5 min with NADPH (Fig. 4 A) and without (Fig. 4 B) it seems that NADPH actually protected microsomal lipids against peroxidation evoked by both 0.5 mM vanadyl and 0.5 mM vanadate.

This result may be explained by competition between NADPH and unsaturated lipids for the peroxy-vanadyl complex which serves as one-electron acceptor capable of abstracting hydrogen from both, NADPH and microsomal lipid. Interaction between peroxy-vanadyl and NADPH may initiate the free radical chain reaction, producing finally oxidized NADP and superoxide anion-radical (Liochev and Fridovich 1987). On the other hand, interaction between peroxy-vanadyl complex and microsomal lipid may initiate lipid peroxidation and lead to production of MDA. Lipid peroxidation initiated by peroxy-vanadyl appeared to be a nonenzymatic process which did not require NADPH. Actually, NADPH was required in the system only to reduce the vanadate (v). Cu^{2+} inhibited NADPH:cytochrome P-450 reductase and blocked the reduction of vanadate (v), whereas it dramatically accelerated the oxidation of vanadyl (iv) (compare Fig. 1 A with Fig. 3 A). This nonenzymatic oxidation of vanadyl (iv) seems to feed the system with superoxide anion-radicals necessary to initiate



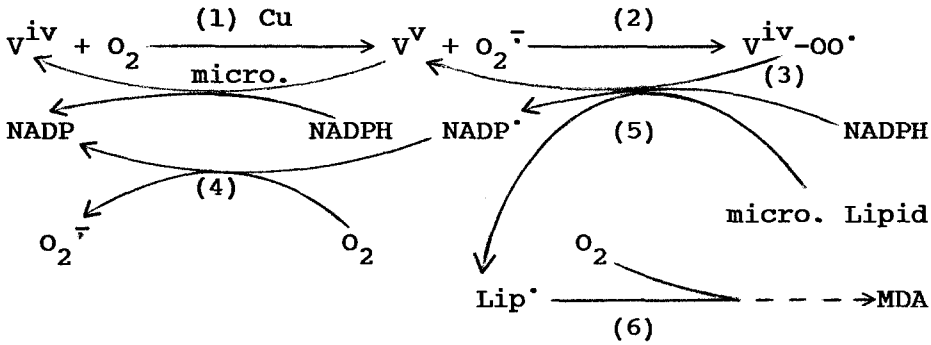
A. B.

Figure 4. Effect of vanadate V (v) and vanadyl V (iv) on lipid peroxidation in HTP microsomes (0.5 mg/mL) : A. medium supplemented with 0.5 mM NADPH; B. in the absence of NADPH. Time-dependent curves (A.) were obtained with 0.5 mM V (v) or 0.5 mM V (iv); concentration-dependent curves (B.) were obtained after 5 min incubation.

further free radical-mediated oxidative destruction of NADPH and microsomal lipids. It was demonstrated by Patole et al. (1986) that in rat liver microsomes the oxygen uptake evoked by vanadate (v) plus NADH was inhibited by superoxide dismutase (SOD) whereas SOD was without effect on polyvanadate (v) reduction and NADH disappearance. It seems, therefore, that scavenging of superoxide has no effect on vanadate redox cycling by microsomes. However, SOD breaks the events that lead to destructive chain reactions, which are known to consume oxygen (Liochev and Fridovich 1987).

Since our experiments were conducted in the presence of high concentration of Tris (50 mM), which is known to scavenge $^{\cdot}OH$, it seems that the possible formation of this radical is not essential for phenomena observed in HTP microsomes. On the other hand, HTP microsomes are known to be prone to lipid peroxidation initiated by paraquat plus iron, because of their low content of endogenous antioxidants (Kenel et al. 1987). It seems that in the system presented here, peroxy-vanadyl complex played a similar role to superoxide plus iron as an initiator of microsomal lipid peroxidation.

On the basis of our experiments as well as the evidence presented by Liochev and Fridovich (1987) the following mechanism may be postulated:



According to this scheme vanadate (v) redox cycling in HTP microsomes proceeds with concomitant NADPH consumption and oxygen uptake. Vanadyl (iv) oxidizes nonenzymatically in the presence or absence of exogenous Cu^{2+} (1) and supplies superoxide. Superoxide forms peroxy-vanadyl complex (2) which attacks NADPH, producing a free radical intermediate (3). NADP· passes its free electron to oxygen (4), producing another superoxide and oxidized NADP. Peroxy-vanadyl attacks also microsomal lipid (5), initiating lipid peroxidation processes (6), leading to oxygen uptake and production of MDA. The overall process leads to the consumption of O_2, depletion of reducing equivalents and destruction of microsomal lipids, and represents a source of reactive oxygen species.

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