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TETRAVALENT VANADIUM RELEASES FERRITIN IRON WHICH STIMULATES VANADIUM-DEPENDENT LIPID PEROXIDATION

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The iron storage protein, ferritin, represents a possible source of iron for oxidative reactions in biological systems. It has been shown that superoxide and several xenobiotic free radicals can release iron from ferritin by a reductive mechanism. Tetravalent vanadium (vanadyl) reacts with oxygen to generate superoxide and pentavalent vanadium (vanadate). This led to the hypothesis that vanadyl causes the release of iron from ferritin. Therefore, the ability of vanadyl and vanadate to release iron from ferritin was investigated. Iron release was measured by monitoring the generation of the Fe^{2+}-ferrozine complex. It was found that vanadyl but not vanadate was able to mobilize ferritin iron in a concentration dependent fashion. Initial rates, and iron release over 30 minutes, were unaffected by the addition of superoxide dismutase. Glutathione or vanadate added in relative excess to the concentration of vanadyl, inhibited iron release up to 45%. Addition of ferritin at the concentration used for measuring iron release prevented vanadyl-induced NADH oxidation. Vanadyl promoted lipid peroxidation in phospholipid liposomes. Addition of ferritin to the system stimulated lipid peroxidation up to 50% above that with vanadyl alone. Ferritin alone did not promote significant levels of lipid peroxidation.

KEY WORDS: Vanadium, ferritin, iron, lipid peroxidation.

INTRODUCTION

Nearly 20% of the total body iron is stored as ferritin. Iron is deposited within the central core of the protein as a ferric oxyhydroxide polymer and is protected from oxidative reactions occurring in the surroundings.[1,2] It has been shown that O_2^- and several radical generating xenobiotics can reductively release iron from ferritin.[3-7] Thus ferritin could be a source of iron for catalysing oxidative reactions of these radicals.

Vanadium is an essential element. The chemistry of this element is complex and characterized by multiple oxidation states.[8] In biological systems, vanadium is normally found in the +4 (vanadyl, VO^{2+}) and +5 (vanadate, VO_4^{3-}) states. It is present in all mammalian tissues at low concentrations ($< 10\,\mu M$). At these concentrations vanadyl and/or vanadate are able to inhibit certain ATPases, phosphotransferases and kinases.[8] Vanadium-induced nephrotoxicity and changes in brain catecholamine levels were observed in rats injected with the metal.[9]

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In neutral solution, vanadyl reacts with oxygen to generate vanadate and O_2^-.[10] This led us to hypothesize that vanadyl could release iron from ferritin and that this could contribute to vanadium toxicity. In this paper we show that vanadyl releases ferritin iron and that iron release is accompanied by enhanced lipid peroxidation. However, it is vanadyl itself rather than O_2^- produced on autoxidation that is responsible for the release.

MATERIALS AND METHODS

Ferritin (from horse spleen), ferrozine, human transferrin, SOD, catalase, glutathione, NADH and phospholipids from bovine brain, Type I- Folch Fraction were purchased from Sigma Chemical Co., St. Louis, MO. Vanadyl sulfate and sodium orthovanadate were obtained from Aldrich Chemical Co., Milwaukee, WI. All other reagents were of analytical grade and purchased from Fisher Scientific Co., Springfield, NJ. Solutions were prepared in distilled deionized water (Milli Q System) and buffers were stirred with Chelex 100 resin (Bio-Rad Laboratories, Richmond, CA) to decrease contamination by transition metals. Vanadyl stock solutions (5 or 10 mM) were prepared daily in deionized waters. Stock solutions of vanadyl were adjusted to pH 3.0 to protect against air oxidation.

Iron release

Two batches of ferritin containing 0.25 nmol iron/μg protein (batch 1) and 0.45 nmol/μg proein (batch 2) as measured by the method of Hoy *et al.*,[11] were used. To standardize the conditions we used different ferritin protein concentrations to achieve approximately the same final concentration of ferritin iron (50 μM). There were no significant differences either in initial rates or in total amounts of iron mobilized over 30 min. by the same vanadyl concentrations between ferritin solutions prepared from batch 1 and batch 2.

Iron release from ferritin (50 μM iron) was performed at 37°C in phosphate buffered saline (PBS), pH 7.3, containing ferrozine (200 μM), the desired concentrations of vanadyl (10–200 μM) and other additives, when required. The appearance of the Fe^{2+}-ferrozine complex was monitored at 562 nm. Rates measured during the first 3 minutes of the reaction were calculated using $\xi_{562} = 27{,}900\,M^{-1}cm^{-1}$.[12] Total changes in Δ_{562} over 30 min incubation times were also determined.

NADH oxidation was followed at 340 nm at 37°C using $\Delta\,\xi_{340} = 6{,}200\,M^{-1}cm^{-1}$.

Lipid peroxidation

Multilamellar liposomes were prepared daily by shaking a suspension of phospholipids in Chelex-treated 0.15 M NaCl, pH 7.0 (10 mg/ml), with glass beads. Liposomes (1 ml of phospholipid in 1 ml of PBS) were incubated at 37°C in air for 30 min. with ferritin and vanadyl at concentrations given in Table II. Lipid peroxidation was determined according to the method described by Buege and Aust.[13] Butylated hydroxytoluene was added to the thiobarbituric acid (TBA) reagent to prevent further lipid peroxidation during the assay procedure.

RESULTS AND DISCUSSION

Increasing concentrations of a Fe^{2+}-ferrozine complex were detected when increasing concentrations of vanadyl were incubated with horse spleen ferritin (Figure 1). Vanadate (200 μM) did not mobilise iron from ferritin. A 5-fold excess of vanadate inhibited iron release by vanadyl, whether measured as an initial rate or after 30 min (Table I).

Vanadyl salts autoxidise in neutral aqueous solution generating O_2^- and vanadate.[10] Although O_2^- is known to mobilize iron from ferritin,[3,4] SOD did not affect the rate of iron release by vanadyl (Table I). Addition of increasing concentrations of catalase gave a progressive decrease in the initial rates of Fe^{2+}-ferrozine complex accumulation, although differences were less with longer incubation. It is unlikely that the decrease was due to H_2O_2 scavenging since it was still apparent in the presence of 500 μM cyanide which fully inhibited the catalase. Nonspecific protein binding of vanadyl may account for this effect. GSH reduces vanadate and thus decreases the net oxidation of vanadyl.[8,14] It did not alter the initial rate of ferritin iron release but inhibited release over longer periods (Table I). GSH can bind to vanadyl[8,14] and form GSH: vanadyl complexes, thus the inhibition may reflect this binding.

An interaction between ferritin and vanadyl was also apparent when ferritin was added to vanadyl (250 μM) and NADH (150 μM). Consistent with previous studies,[10] we observed that vanadyl promoted NADH oxidation at a rate of 40 μM/min. This process was 90% inhibited when ferritin at the same concentration as used in the iron release experiments (50 μM iron) was present.

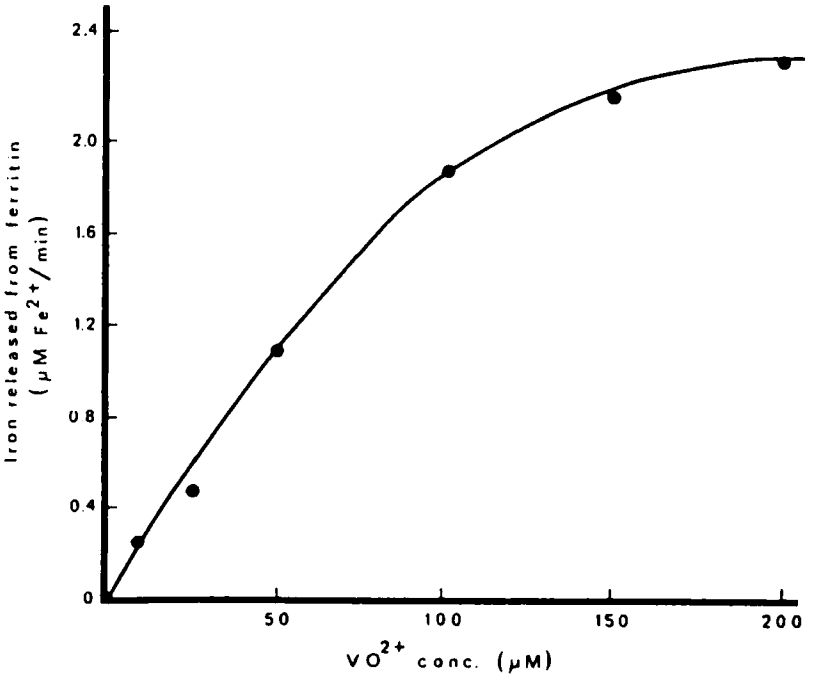

FIGURE 1 Initial rate of iron release from ferritin in the presence of vanadyl. Incubations are in PBS pH 7.3 at 37°C, ferritin iron (50 μM), ferrozine (200 μM).

TABLE I

Effect of SOD, catalase, glutathione and vanadate on the release of iron from ferritin by vanadyl under aerobic conditions

Additions	Iron release	
	Initial rates (μM/min)	Amount released in 30 min (μM Fe^{2+})
None	2.44	27.8
VO_4^{3-}	2.46	17.9
SOD	2.54	27.8
CAT_1	2.03	27.4
CAT_2	1.07	24.1
CAT_2 + NaCN	0.90	
NaCN	2.39	
GSH_1	2.44	20.2
GSH_2	2.44	18.6

Reaction mixtures contained in a final volume of 1.0 ml. VO^{2+} (200 μM) ferritin (50 μM iron) and ferrozine (200 μM) in PBS with VO_4^{3-} (1 mM), SOD (10 μg/ml), CAT_1 (60 μg/ml), CAT_2 (120 μg/ml), NaCN (500 μM), GSH_1 (500 μM) and GSH_2 (1 mM) added where indicated. Measurements of Fe^{2+}-ferrozine complex were made in PBS, pH 7.3 at 37°C. Results are means of duplicates which agree within 10%.

Our results indicate that vanadyl can reduce and mobilize iron from ferritin. Although vanadyl produces O_2^- on autoxidation, vanadyl itself appears responsible for the release. Vanadyl has been shown previously to interact with specific iron binding sites in proteins including ferritin.[15,16] It is possible, therefore, that it could reduce and displace Fe^{3+} bound either in the core or to the apoferritin shell. The observed inhibition by vanadate may indicate competition for these sites or the possible formation of a vandyl:vanadate complex.[17]

Vanadyl alone is capable of promoting lipid peroxidation.[18] By measuring the formation of TBA-reactive substances, we confirmed this observation, and also found that adding Fe^{2+} or ferritin substantially enhanced vanadyl-dependent lipid peroxidation (Table II). Iron or ferritin alone gave only basal levels of TBA-reactive substances. With ferritin this was decreased to the level of the blank by adding desferrioxamine immediately before heating with TBA indicating that the reaction occurred during heating and not from catalysis of peroxidation by ferritin.

Vanadyl (25 μM) should have released 8.4 μM iron from the ferritin present in the lipid peroxidation system. The enhancement of lipid peroxidation seen with this concentration of iron present from the start of the incubation is only slightly more

TABLE II

Lipid peroxidation in phospholipid liposomes mediated by vanadyl and ferritin

Additions to Liposomes	A_{535}
none	0.000 ± 0.000
Ferritin	0.030 ± 0.001
VO^{2+}	0.112 ± 0.003
Ferritin + VO^{2+}	0.187 ± 0.003
Fe^{2+}	0.020 ± 0.000
Fe^{2+} + VO^{2+}	0.257 ± 0.003
Ferritin + VO^{2+} + transferrin	0.120 ± 0.000
VO^{2+} + transferrin	0.117 ± 0.003

Reaction mixtures contained liposomes (1 mg/ml), ferritin (50 μM iron), transferrin (500 μg/ml), VO^{2+} (25 μM) and Fe^{2+} (8.4 μM) and were incubated for 30 min in PBS, pH 7.3 at 37°C. TBA reactive substances were determined as described in Material and Methods. Results are the means of duplicates ± SD.

than that seen in the presence of ferritin, and is consistent with iron release being responsible for the observation. This is supported by the ability of apotransferrin to decrease lipid peroxidation in the presence of ferritin and vanadyl to that seen with vanadyl alone (Table II). Apotransferrin complexes iron so that it is catalytically inactive in lipid peroxidation. The enhancement of iron-dependent lipid peroxidation by vanadyl probably reflects its ability to reduce Fe^{3+}.

We conclude, therefore, that vanadyl can release iron from ferritin directly, and that radical-mediated processes involving interactions between vanadyl and iron, possibly derived from ferritin, could contribute to vanadium toxicity.

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