Molecular and Cellular Biochemistry **153:** 167–171, 1995.

Membrane – vanadium interaction: A toxicokinetic evaluation

Raj K. Upreti

Biomembrane Division, Industrial Toxicology Research Centre, M.G. Marg, Post Box No.80, Lucknow-226 001, India

Abstract

Vanadium is an important trace metal widely distributed in environment. Interaction of vanadate with skeletal muscle sarcolemma and basement membrane has been focussed. Scatchard analysis indicated the presence of more than one binding site for vanadate. Vanadate inhibits sarcolemmal and intestinal brush border membrane enzymes in a non-competitive manner. Membrane-vanadium interaction may lead to several structural and functional changes. The binding of vanadium to basement membrane may have some protective role. (Mol Cell Biochem **153:** 167–171, 1995)

Key words: vanadium, sarcolemma, brush border, membrane enzymes

Introduction

Unlike organic pollutants, the trace metals are not biodegradable and they tend to build up the ecosystem to levels which may be toxic. Vanadium is an important trace metal that is widely distributed in environment. It is considered as an essential element but it is highly toxic when introduced in excessive doses to animals and humans [1]. It is used extensively in various types of industry, and exposure to high vanadium levels is not uncommon. Due to its wide industrial use, the biological actions of vanadium are of interest [2]. Vanadium compounds enter the body primarily through the lungs where they are absorbed slowly and excreted mainly in the urine [1]. Vanadium fumes induce inflamatory changes in the mucous membranes of the respiratory tract in exposed humans and animals. Inhalation exposure to vanadium can cause conjuctivitis, pharyngitis, rhinitis, chronic productive cough and tightness of the chest [3]. In case of severe exposure, vanadium may cause cellular necrosis to liver and kidney. Vanadium salts have been used medicinally as antiseptic, spirochetocide , antituberculotic and antianemic agents [3]. Further, vanadate has been reported to counteract glucagon effects in isolated rat hepatocytes [4]. The medicinal use may also result in gastrointestinal disorders and nervous system effects [5]. The absorption of vanadium through the gastrointestinal tract is low [6, 7]. However, vanadium has been reported in tissues and urine with in hours after a single [8] and repeated oral exposure in rats [9]. Much higher absorption of vanadium in young rats have been observed due to a greater nonselective permeability of the undeveloped intestinal barrier [10]. Vanadium is rapidly distributed in tissues after inhalation or oral exposures. There is an initial accumulation in the lungs, kidneys, liver and muscles. However, retention of vanadium occurs primarily in the bone [8, 10].

Membrane as a model for toxicity evaluation

Chemicals encountered in occupation or in environment undergo physico-chemical interactions with reactive biological entities to manifest their pathological, physiological or pharamacological effects. The prime target sites for xenobiotic toxicity are surface membranes possibly due to their exposed location and chemical reactivity. The interaction of a chemical with either protein or lipid components of the cell membrane may substantially alter membrane structure and function.

In addition to the well defined and widely worked out erythrocyte plasma membrane, the other important surface membranes which come directly in contact with xenobiotics are skeletal muscle cells sarcolemma and intestinal brush border membrane (BBM). Sarcolemma is made up of a

Address for offprints: R.K. Upreti, Biomembrane Division, Industrial Toxicology Research Centre, M.G. Marg, Post Box No. 80, Lucknow-226 001, India

plasma membrane, a felt-like electron dense basal lamina, and an overlying reticular lamina containing collagen and reticular fibrils embedded in an amorphos matrix [11,12]. Basal lamina and reticular lamina together constitute basement membrane (B.M.). Sarcolemma plays an important role in the excitation and contraction phenomenon of muscles [13]. In general, basement membrane is known to play significant role in filteration, structural organization of tissues and cell-cell adhesion [14]. The intestinal BBM is highly specialized plasma membrane responsible for digestive and absorptive functions and their closed vesicles retain the original orientation of the membrane [15].

Sarcolemmal and basement membrane interaction of vanadium

While there are many reports which describe the inhibition of membrane bound ATPase and other enzymes by vanadate, studies on skeletal muscle sarcolemmal enzymes are scanty. The preceding text is mainly focussed on frog skeletal muscle sarcolemma. Owing to the fact that bulk of the literature is available on the mechanism of action of vanadate on Na^++K^+-ATPase from a wide range of sources and the functions of this enzyme in skeletal muscle are little appreciated as compared to Ca^{2+}-ATPase. The present article, therefore, does not deal much with Na^+K^+-ATPase. We have demonstrated the presence of Ca^{2+}-ATPase, Mg^{2+}-ATPase, Ca^{2+}+Mg-ATPase, 5′-nucleotidase, alkaline phosphatase and acetyl cholinesterase in purified frog skeletal muscle sarcolemma. Most of the enzymes were inhibited by vanadate, with the prominent exception of acetyl cholinesterase (Figs 1 and 2). In frog sarcolemma, the ion transporting ATPases, 5′-nucleotidase and alkaline phosphatase behaved similarly to vanadate and can be considered in the same class due to their common feature distinguished by the formation of a phosphoenzyme intermediate during the reaction cycle. Acetyl cholinesterase is structurally and functionally different from the above enzymes and therefore its response against vanadate is different. It has been shown that vanadate stimulates rather to inhibit acetyl cholinesterase in rat ventricular strips, electric eel and also in erythrocytes [16]. However, frog sarcolemmal esterase revealed no significant effect of vanadate.

Vanadate inhibition of frog skeletal muscle sarcolemmal ATPases was non-competitive type. Inhibitor constant of Ca^{2+}-ATPase at two different substrate concentrations revealed Ki value of 4.6×10^{-5} M. Additional enzyme kinetic studies indicated that vanadate did not effect the Km (1.10 mM) of the enzyme but it reduced the Vmax (0.80 umole Pi/mg/h to 0.55 umole). It has also been shown that vanadate acts as a non-competitive inhibitor to plasma membrane ATPases of yeast and corn root [17, 18]. The mechanistic view of the action of vanadate on phosphoenzyme ion transport ATPases and other enzymes of this catagory can be understood by considering the structure of these enzyme molecules. All members of this class are inhibited by vanadate. Vanadate enters the reaction sequence by the back door and forms a stable inactive complex in a reversible reaction with the E_2 conformation of the enzyme in a stoicheometry of one vanadate bound per active site for phosphorylation [19]. Vanadate is also known to induce the formation of two-dimensional crystalline arrays of Ca^{2+}-ATPase molecule in sarcoplasmic reticulum membrane vesicles [20].

Not much is available in literature on the mechanism of action of vanadate on 5′-nucleotidase. The presence of 5′-nucleotidase is controversial in all membranes. However, we observed good activity of this enzyme in frog skeletal sarco-

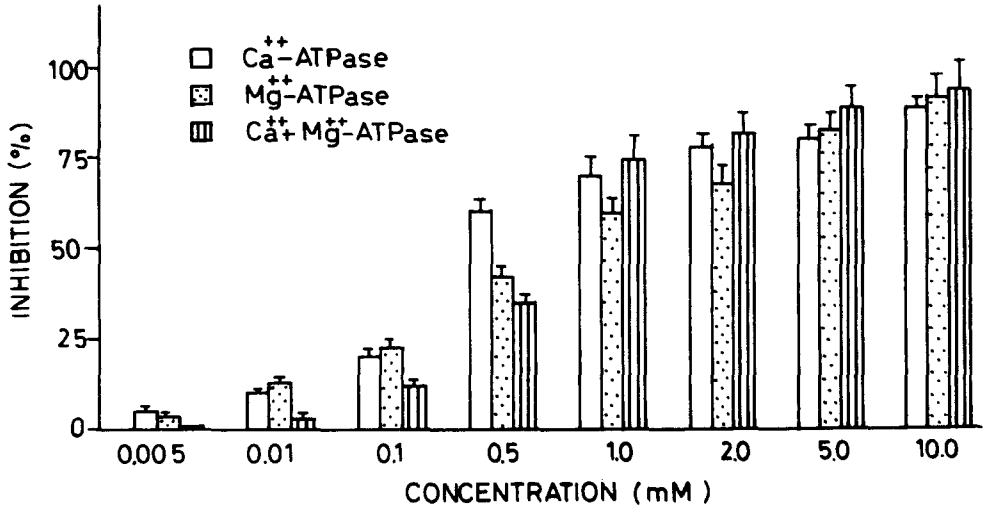

Fig. 1. Inhibition pattern of frog skeletal muscle sarcolemmal ATPases by vanadate.

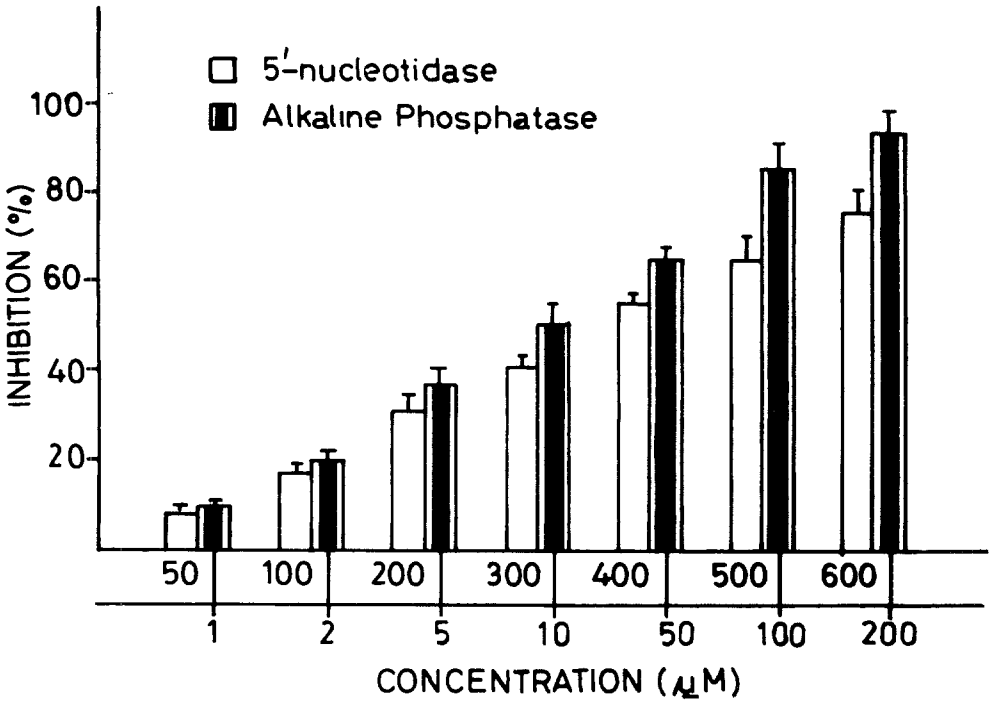

Fig. 2. Inhibition pattern of frog skeletal muscle sarcolemmal 5'-nucleotidase and alkaline phosphatase by vanadate. The upper line vanadate concentrations refer to 5'-nucleotidase and the lower line to alkaline phosphatase.

lemma. 5'-nucleotidase was also inhibited non-competitively by vanadate. Inhibitor constant (Ki) value was 6.2×10^{-5} M. Further, vanadate did not effect the Km (0.75 mM) of the enzyme but reduced the Vmax (0.066–0.038 umole). Similar inhibitory response of vanadate on 5'-nucleotidase could be due to its resemblence with ATPases in at least carrying out the reaction cycles in similar manner.

The effect of vanadate on the inhibition of alkaline phosphatase have been studied in few cases [21–23]. However, the mode of vanadate action on alkaline phosphatase in most of the studies are not well discussed. Kinetic studies on alkaline phosphatase of frog sarcolemma demonstrated that vanadate inhibits in a non-competitive manner. The inhibitor constant (Ki) was found to be 4.2 uM which is comparable with the findings on rat mesentric artery and human liver alkaline phosphatase [21, 22]. The higher sensitivity of alkaline phosphatase to vanadate may be, in part, due to that it is closely related to Na^++K^+-ATPase as is the case with K^+-activated p-nitrophenyl phosphatase. Since vanadate is a potent inhibitor of Na^++K^+-ATPase and it also inhibits K^+-dependent phosphatase approximately with the similar potential, it is likely for the vanadate to inhibit alkaline phosphatase in a similar fashion.

Vanadate binding studies with isolated frog skeletal muscle sarcolemma and basement membrane revealed a concentration-dependent binding. Scatchard analysis indicated biphasic curves with a high and low affinity components for

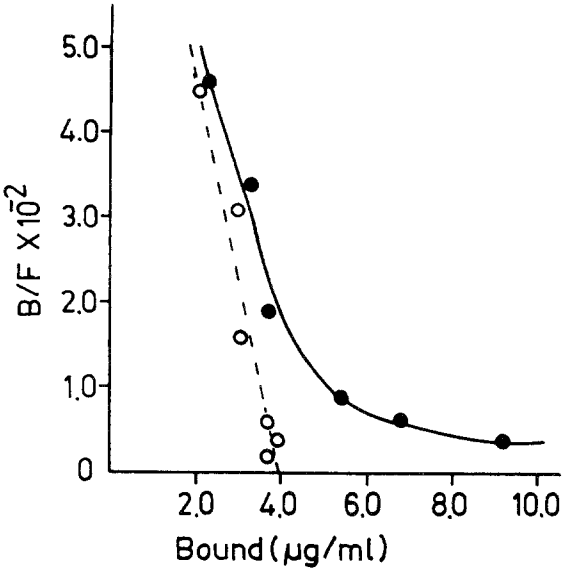

Fig. 3. Scatchard analysis of the data for the binding of ammonium metavanadate to frog skeletal muscle sarcolemma. B is the concentration of bound and F is the concentration of free vanadium. Dotted straight line was obtained after applying correction factor for non specific binding sites showing the presence of one major binding site.

both membranes (Figs 3 and 4). This suggests the existence of more than one binding site responsible for binding of vanadium. The binding constants for high affinity binding site of sarcolemma were $n_1 = 3.8$ n mole/mg protein, $k_1 = 1.57 \times$

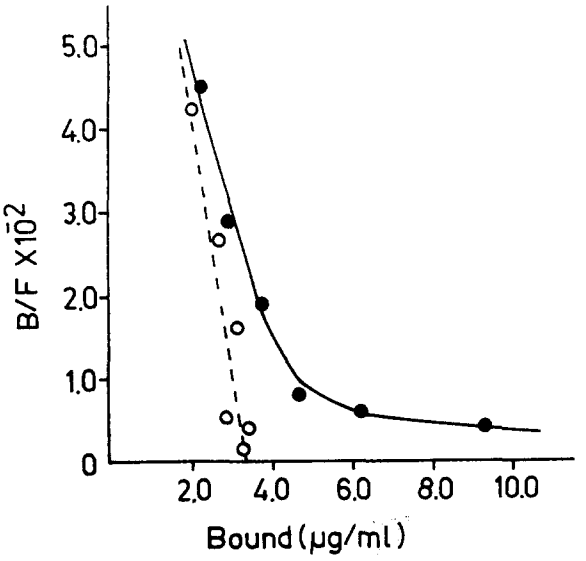

Fig. 4. Scatchard analysis of the data for the binding of ammonium metavanadate to frog skeletal muscle basement membrane. Dotted straight line was obtained after applying correction factor for non specific binding sites showing the presence of one major binding site.

10^7 M^{-1} and that of basement membrane were n_1 = 4.1 n mole/mg protein, $k_1 = 1.31 \times 10^7$ M^{-1}. Their respective low affinity binding constants were n_2 = 11.4 n mole/mg protein, $k_2 = 6.55 \times 10^5$ M^{-1} and n_2 = 12.8 n mole/mg protein, $k_2 = 4.69 \times 10^5$ M^{-1}. Applying correction factor to minimize the non-specific binding sites, the biphasic curve was converted into a straight line suggesting that there exist at least one major binding site in sarcolemma as well as in basement membrane. The binding constants obtained after correction were also similar to the high affinity constants.

Interaction of vanadium with biological entities are of great interest. Besides binding to membranous components, vanadium compounds can also bind to some endogenous phosphate, carboxyl and amino-ligands present inside the cell [24]. However, binding of vanadium with basement membrane suggest that the cells having an outer layer of basement membrane such as skeletal muscle, lens capsule and kidney cortex, could play significant protective role against its entry into the cell [25].

Intestinal brush border membrane interaction of vanadium

Significant concentration-dependent inhibition of rat intestinal brush border membrane Ca^{2+}+Mg^{2+}-ATPase and alkaline phosphatase were observed following vanadium exposure (Fig. 5). However, inhibition of disaccharidases and acetyl cholinesterase activities were not observed over the vanadium concentrations upto 40 mM. The toxicokinetic study of Ca^{2+}-Mg^{2+}-ATPase and alkaline phosphatase further indicated that the inhibition caused by vanadium was non-competitive type [26]. This is in agreement with earlier findings on sarcolemma membrane ATPase and alkaline phosphatase and further suggest a generalized mode of vanadium action. These enzymes are involved in the movement of important intermediates across the intestinal brush border membrane. Therefore, inhibition of ATPase and phophatase by vana-

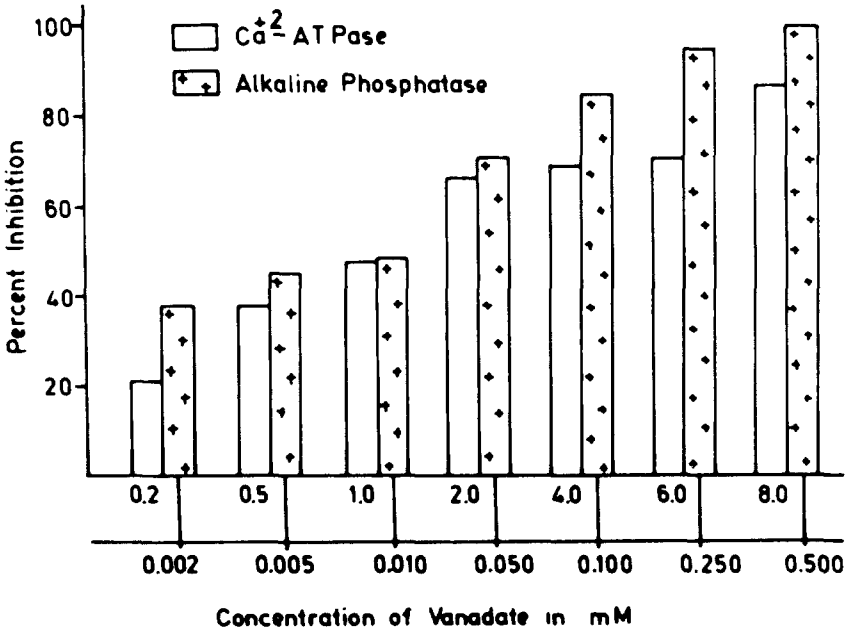

Fig. 5. Inhibition pattern of rat intestinal brush border membrane Ca^{2+}-ATPase and alkaline phosphatase by vanadate. The upper line vanadate concentrations refer to Ca^{2+}-ATPase and the lower line to alkaline phosphatase. [from Gupta K, Upreti RK, Kidwai AM: Bull Environ Contam Toxicol 52: 919–926, 1994].

dium may bring about deleterious effects at least in higher concentrations.

Conclusions

This article has focused on vanadium interaction with skeletal muscle sarcolemmal membrane and intestinal brush border membrane enzymes. Membrane enzymes inhibited by vanadate in a non-competitive manner suggests that it binds the enzyme molecules either at the active site or any other available site. Furthermore, vanadate binding with surface membranes also indicates the existance of some structural constituents (vis-a-vis membrane enzymes) responsible for its binding. The binding of vanadate to enzyme(s) could cause the conformational changes resulting into the enzymatically inactive state.

Acknowledgement

Author is grateful to Dr. A.M. Kidwai for his keen interest and valuable suggestions.

References

1. Jandhyala BS, Hom GJ: Physiological and pharmacological properties of vanadium. Life Sci 33: 1325–1340, 1983
2. Nechay BR: Mechanisms of action of vanadium. An Rev Pharmacol Toxicol 24: 501–524, 1984
3. Domingo JL, Llobet JM, Tomas JM, Corbella J: Short term toxicity studies of vanadium in rats. J Appl Toxicol 5: 418–421, 1985
4. Miralpeic M, Gil J, Rosa JL, Carreras J, Bartrons R: Vanadate counteracts glucagon effects in isolated rat hepatocytes. Life Sci 44: 1491–1497, 1989
5. Hammond PB, Beliles RP: Metals In: J. Doull, C.D. Klaassen, M.O. Amdur (eds). Toxicology, the Basic Science of Poisons, MacMillan Publishing, NY, 1980 pp 460–470
6. Roshchin AV, Ordzhonikidze EK, Shalganova IV: Vanadium-toxicity, metabolism, carrier state. J Hyg Epidemiol Microbiol Immunol 24: 377–383, 1980
7. Conklin AW, Skinner CS, Felten TL, Sanders CL: Clearance and distribution of intratracheally instilled vanadium-48 compounds in the rat. Toxicol Lett 11: 199–203, 1982
8. Edel J, Sabbioni E: Retention of intratracheally instilled and ingested tetravalent and pentavalent vanadium in the rat. J Trace Elem Electrolytes Health Dis 2: 23–30, 1988
9. Parker RD, Sharma RP: Accumulation and dipletion of vanadium in selected tissues of rats treated with vanadyl sulfate and sodium orthovanadate. J Environ Pathol Toxicol. 2 : 235–245, 1978
10. Edel J, Pietra R, Sabbioni E, Marfante E, Springer A, Ubertalli L: Disposition of vanadium in rat tissues at different age. Chemosphere 13: 87–93, 1984
11. Sanes JR, Marshall LM, McMahan UJ: Reinnervation of muscle fiber basal lamina after removal of myofibrils. J cell Biol 78: 176–198, 1978
12. Borg TK, Caulfield JB: Morphology of connective tissue in muscle. Tissue & Cell 12: 197–207, 1980
13. Weber A, Murray JM: Molecular control mechanism in muscle contraction. Physiol Rev 53: 612–673, 1980
14. Grant ME, Codfrey HJ, Orkin RW: Current concept of basement membrane structure and function. Biosci Rep 1: 819–842, 1981
15. Klip A, Grinstein S, Semenza G: Transmembrane disposition of the phlorizine binding protein of intestinal brush border. FEBS Lett 99: 91–96, 1979
16. Catalan RE, Martinez AM, Aragones MD, Godoy JE: Activation of acetylcholinesterase by vanadate. Neuropharmac 28: 1119–1122, 1985
17. Dufour JP, Boutry M, Goffeau A: Plasma membrane ATPase of yeast. Comparative inhibition studies of the purified and membrane bound enzymes. J Biol Chem 255: 5735–5741, 1980
18. Tu S-I, Sliwinski BJ: Mechanistic investigation of corn root plasma membrane ATPase. Arch Biochem Biophys 241: 348–355, 1985
19. Huang WH, Askari A: Simultaneous binding of ATP and vanadate to (Na^++K^+)-ATPase. J Biol Chem 259: 13287–13291, 1981
20. Dux L, Martonosi A: Ca^{2+}-ATPase crystals in sarcoplasmic reticulum; Effect of trypsin digestion. J Biol Chem 258: 10111–10115, 1983
21. Kwan CY: Characteristics of plasmalemma alkaline phosphatase of rat mesentric artery. Blood Vessels 20: 109–121, 1983
22. Chakraborttey A, Stinson RA: Properties of membrane bound and solubilized forms of alkaline phosphatase from human liver. Biochim Biophys Acta 839: 174–180, 1985
23. Farley JR, Baylink DJ: Skeletal alkaline phosphatase activity as a bone formation index *in vitro*. Metabol Clin Exp 35: 563–571, 1986
24. Nechay BR, Nanniuga LB, Nechay PSE: Vanadyl and vanadate binding to selected endogenous phosphate, carboxyl and amino ligands: Calculations of cellular vanadium species distribution. Arch Biochem Biophys 251: 128–138, 1986
25. Ali N, Upreti RK, Kidwai AM: Sarcolemma as model for testing toxicity of chemicals. Ind J Biochem Biophys 25: 209–214, 1988
26. Gupta K, Upreti RK, Kidwai AM : Toxicokinetic study of rat intestinal brush border membrane enzymes following in vitro exposure to lead and vanadium. Bull Environ Contam Toxicol 52: 919–926, 1994