

Ion pumps as biological targets for decavanadate

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The putative applications of poly-, oligo- and mono-oxometalates in biochemistry, biology, pharmacology and medicine are rapidly attracting interest. In particular, these compounds may act as potent ion pump inhibitors and have the potential to play a role in the treatment of e.g. ulcers, cancer and ischemic heart disease. However, the mechanism of action is not completely understood in most cases, and even remains largely unknown in other cases. In the present review we discuss the most recent insights into the interaction between mono- and polyoxometalate ions with ion pumps, with particular focus on the interaction of decavanadate with Ca^{2+} -ATPase. We also compare the proposed mode of action with those of established ion pump inhibitors which are currently in therapeutic use. Of the 18 classes of compounds which are known to act as ion pump inhibitors, the complete mechanism of inhibition is only known for a handful. It has, however, been established that most ion pump inhibitors bind mainly to the E2 ion pump conformation within the membrane domain from the extracellular side and block the cation release. Polyoxometalates such as decavanadate, in contrast, interact with Ca^{2+} -ATPase near the nucleotide binding site domain or at a pocket involving several cytoplasmic domains, and therefore need to cross through the membrane bilayer. In contrast to monomeric vanadate, which only binds to the E2 conformation, decavanadate binds to all protein conformations, i.e. E1, E1P, E2 and E2P. Moreover, the specific interaction of decavanadate with sarcoplasmic reticulum Ca^{2+} -ATPase has been shown to be non-competitive with respect to ATP and induces protein cysteine oxidation with concomitant vanadium reduction which might explain the high inhibitory capacity of V_{10} ($\text{IC}_{50} = 15 \mu\text{M}$) which is quite similar to the majority of the established therapeutic drugs.

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1. Introduction

The serendipitous discovery three decades ago of vanadium as an inhibitor of ion pumps such as Na^+/K^+ -ATPase ($\text{IC}_{50} = 40 \text{ nM}$)¹ has encouraged the investigation of mono and polyoxometalates as putative drugs in the treatment of several diseases in which the molecular target has been established to be an ion pump such as Na^+/K^+ -ATPase, H^+/K^+ -ATPase, or Ca^{2+} -ATPase.² These enzymes are all classified as P-type, or E1-E2, ATPases, in which the ion pump process is coupled to ATP hydrolysis. The mechanism is well-known and involves at least four steps and two distinct protein conformations, E1 and E2.³ These conformations are usually highly selective towards specific substrates, where the E1 state has a greater affinity for the exported ion and ATP, and a low affinity for the imported ion, while the E2 conformation has a higher affinity for the imported ion and a low affinity for the exported ion, thus releasing it into the extracellular environment. ATP provides the driving force in this process, by inducing the structural changes through the phosphorylation of an aspartate residue.

The E1–E2 cycle consists of four steps. For instance, in the process of calcium transport across the membrane, the E2 form of the calcium ATPase binds two calcium ions, thus forming the E1 conformation. This conformation is phosphorylated by ATP forming an E1P intermediate, where the P indicates that the enzyme is phosphorylated, which leads to ion translocation and release of the calcium. This is followed by the formation of the calcium-free proton-carrying E2P conformation which undergoes hydrolysis of the phosphate–aspartate bond to regenerate E2 and release the protons, and the cycle is complete.³ It is in fact the phosphorylation step that gives the P-type ATPases their name, and they present a common target for inhibitors which can interfere with this particular step.

In terms of structural features these P-type ATPases each possess a nucleotide binding domain and a phosphorylation binding site on the cytoplasmic side, and a cation binding site located inside the membrane region.^{3,4} These three distinct domains (Fig. 1) can all serve as potential targets for different types of E1–E2 ATPase inhibitors.



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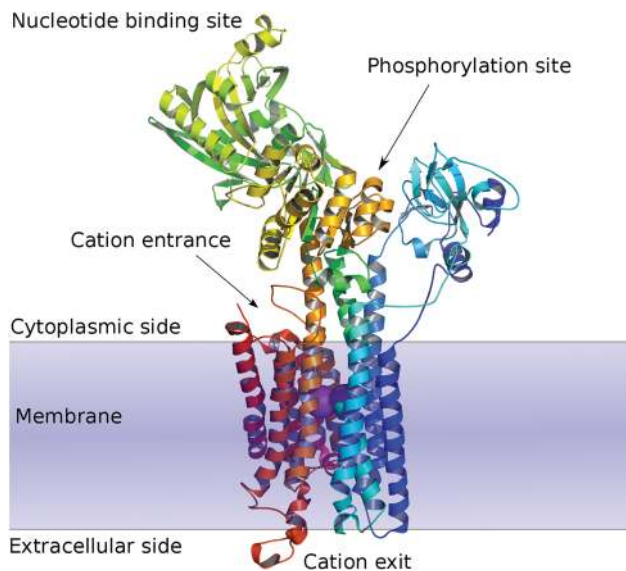


Fig. 1 Schematic view of the E1 form of Ca^{2+} SR ATPase. Note the two calcium ions (purple) in the cation channel.⁴ Figure based on RCSB deposition 1KJU.

A range of established drugs, such as ouabain, omeprazole and thapsigargin, are known to act as ion pump inhibitors, but the mechanism and protein binding sites, or even main target enzyme, have only been clearly established for a few of these compounds (described in Tables 1–3).² For example, the cardiotonic steroids (CTS) mainly inhibit Na^+/K^+ -ATPase, whereas the proton pump inhibitors (PPI) and potassium blockers (also known as potassium competitive acid blockers; PCAB) are named for their inhibition of H^+/K^+ -ATPase. Thapsigargin (TG) and cyclopiazonic acid (CPA) are examples of Ca^{2+} -ATPase inhibitors.

The principal role of the sarcoplasmic reticulum (SR) Ca^{2+} -ATPase is to remove cellular Ca^{2+} from the cytoplasm to the SR, which it does as part of the process of muscle relaxation. In addition, SR Ca^{2+} -ATPase is an important actor in the process of calcium homeostasis.^{3,5} This makes it a particularly attractive target as it may play important roles in the control of heart disease and cancer.

It is well-established that the process of calcium transport and ATP hydrolysis can be inhibited by monomeric vanadate (V_1), VO_4^{3-} , particularly under conditions where the E2 conformation is favoured, leading to the formation of a conformer analogous to E2P, but involving vanadate instead of phosphate.^{6,7} Therefore, the vanadate mode of action may involve formation of an E2V analogue after condensation of vanadate to the enzyme phosphorylation site, at aspartate residue 351. Analysis of the E2 conformation clearly shows structural changes relative to conformation E1 (Fig. 1). In particular, the phosphorylation site is now sterically hindered.^{2,4}

This is not very surprising given the similarities and differences between the aqueous chemistry phosphorous and vanadium, which may help explain some of the observed toxicity of the latter, in particular compared with other group 5 and 6 metals. In particular, both vanadate and phosphate

Table 1 IC₅₀ values and/or dissociation constants and therapeutical applications of known Na⁺/K⁺-ATPase inhibitors

Class of inhibitor	Compounds	IC ₅₀ [μM]	K _d [nM]	Therapeutical applications	References	
Na ⁺ /K ⁺ -ATPase inhibitors						
Cardiotonic steroids	Ouabain	>10		Treatment of congestive heart failure and cardiac arrhythmia	10	
		0.01–0.5			10	
		0.600			11	
		2.2			12	
		0.08–0.32			12	
		1.8			13	
		1–100			14	
		60			15	
			27.7		16	
			3.62		16	
			1		17	
			>1000		15	
			Digoxin		0.910	11
			20		15	
			Digoxigenin		>100	15
	Strophanthidin	160	15			
	Ouabagenin	>1000	15			
Chlorpromazine	Chlorpromazine (CPZ)		K _{d1} = 32 500	Phototoxic antipsychotic drug	18	
			K _{d2} = 142 500		18	
		62.5			20	
Chloroquine	Chloroquine (CLQ)		K _{d1} = 20 000	Anti-malarial drug	18	
			K _{d2} = 115 000		18	
Procaine and derivatives	Procaine	17 700		Local anesthetics	20	
	Chlorprocaine	13 000			20	
	Bupivacaine	6700			20	
	Mepivacaine	>10 000			20	
	Lidocaine	30 400			20	
Palytoxin	Palytoxin (PTX)	0.8		Tumor promoter	12	
		0.2–0.45			12	
		0.0035			16	
		0.4–3.1	20 000		21–23	
Gramicidin A	Gramicidin A	8.1		Antibiotic	24	
Tungsten compounds	(W ₁₂) 12-tungstosilicic acid	3.4–4.3		Insulin-mimetic properties	25	
	H ₄ SiW ₁₂ O ₄₀ (WSiA)					
	(W ₁₂) 12-tungstophosphoric acid,	2.9–3.1			25	
	H ₃ PW ₁₂ O ₄₀ (WPA)					
	(W ₁) sodium tungstate	1300–1500			25	

Table 2 IC₅₀ values and/or dissociation constants and therapeutical applications of known H⁺/K⁺-ATPase inhibitors

Class of inhibitor	Compounds	IC ₅₀ [μM]	K _d [nM]	Therapeutical applications	References
H ⁺ ,K ⁺ -ATPase inhibitors					
PPIs (proton pump inhibitors)	Omeprazole	30		Anti-ulcer agents (duodenal and gastric)	26
		50			27
		2.4			28
		3.9			29
		36			29
		6.8			28
PCABs (potassium-competitive acid pump antagonists, formerly APA)	SCH 28080 (2-methyl-8-(phenylmethoxy)imidazo[1,2- <i>a</i>]-pyridine-3-acetonitrile)	14.3		Anti-ulcer agents	30
Diterpenoids	Soraprazan	0.1	26.4	Antiviral agent	31
	Scopadulcic acid B (SA-B)	54			32
		20–30			33
Prodigiosins	Diacetyl scopadol (DAS)	17		Antibiotic	32
	Prodigiosin 25-C	0.03			34
	Prodigiosin	1.5			26
	Metacycloprodigiosin	3.0			26

readily form linear oligomers in the tetrahedral state, in contrast with *e.g.* molybdate and tungstate. However, condensation occurs spontaneously for vanadates in acidic solution

and has several easily accessible oxidation states, whereas phosphate condensation is thermally driven in the solid phase and phosphorous is most likely to be found in the +V state

Table 3 IC₅₀ values and/or dissociation constants and therapeutical applications of known Ca²⁺-ATPase (SERCA) inhibitors

Class of inhibitor	Compounds	IC ₅₀ [μM]	K _d [nM]	Therapeutical applications	References		
SERCA inhibitors	Thapsigargin	0.029		Prodrugs for prostate cancer therapy	35		
		0.001			36,37		
Cyclopiazonic acid	Cyclopiazonic acid (CPA)	0.01674	7	Cardioprotective action in myocardial ischemia	38		
		0.2			39		
		1.0			40		
		3.0			40		
Macrocyclic lactones	Cyclosporin A	62		Immunosuppressant agent,	41		
		Ivermectin			3.54	antihelminthic agent (ivermectin)	42
					14.9	41	
Curcuminoids	Curcumin	77		Antitumoral, anti-oxidant, antiarthritic, antiamyloid and anti-inflammatory agents	41		
		15.0			43		
2,5-Di-(<i>t</i> -butyl)-dihydroxybenzene	BHQ	7.0–17.0	K _{d1} = 550 K _{d2} = 10 000	Anti-oxidant agent	44		
					200	45	
Celecoxib	Celecoxib	35		Anti-inflammatory drug	46		
		35			35		
Vanadium compounds	Vanadate monomeric (V ₁)	50	1000	Insulin-mimetic properties	8		
	Decavanadate (V ₁₀)	15			8		
	PDC-V(v) – pyridine-2,6-dicarboxylatodioxovanadium(v)	25			47		
	BMOV – bis(maltolato)oxovanadium(IV)	40			47		
	Vanadate (V ₁)	80			47		
	HAIDA-V(IV) – bis(N-hydroxylamidoiminodiacetato)-vanadium(IV)	325			47		
Tungsten compounds	(W ₁) sodium tungstate	400			8		

under physiological conditions. There is also no shortage of polyoxovanadates consisting of octahedral building blocks while phosphates typically are found as tetrahedral units. In addition, vanadium is considerably more electrophilic than phosphorous and V–O bonds are less covalent than P–O bonds.

The situation is, however, rendered more complex by the availability of three distinct binding domains in the P-ATPases, only one of which is involved in the phosphorylation step. For example, decavanadate (V₁₀), [V₁₀O₂₈]⁶⁻, is a more potent SR Ca²⁺-ATPase inhibitor than monomeric vanadate^{6,8} and interacts with other conformations besides E2. In this case the binding site is not the same as the ATP binding site.⁸ Moreover, recently it was shown that during the mechanism of decavanadate-mediated SR Ca²⁺-ATPase inhibition, a protein cysteine unit is oxidized through reduction of the vanadate, although the full implications of this process are not yet clear.⁹ Therefore, a better understanding of the mechanism of decavanadate calcium pump inhibition will help in evaluating the potential of other members of the rich polyoxometalate family as therapeutic agents for a range of diseases.

In this Perspective we aim to analyse and compare several ion pump inhibitors, their putative mode of action and protein binding sites, with particular focus on the role of vanadium and other transition metal oxide ions. The majority of these ion pump inhibitors are in current use as therapeutic drugs and we compare their inhibitory capacity with mono- and polyoxometalates, which have activities of similar magnitude.

Decavanadate has already been established as a potent ion pump inhibitor and has several attractive properties. It is a non-competitive inhibitor with respect to ATP, it has a high inhibitory capacity (IC₅₀ = 15 μM), and it is not selective towards a specific protein interaction and can interact with the calcium-induced E1 conformation rather than just the E2 conformation. V₁₀ also binds to a cytoplasmic pocket involving several domains and it induces cysteine oxidation and vanadium reduction to yield V(IV) species. In contrast, the majority of the established drugs bind to the protein only from the extracellular side within the membrane domain, and block the cation release, without interfering with the nucleotide binding site.

2. Na⁺/K⁺-ATPase inhibitors

Na⁺/K⁺-ATPase is a target in the treatment of a wide range of conditions, and inhibitors of this target include compounds used to treat diseases such as heart failure, psychosis and malaria. In addition, this pump is also a target for anaesthetics, antibiotics and insulin mimetic agents (Table 1).

Cardiotonic steroids such as ouabain are strong inhibitors of Na⁺/K⁺-ATPase, with IC₅₀ values below 1 μM (Table 1). Ouabain inhibits the pump through binding to the extracellular domain of the pump near the cation entrance site when the pump is in the E2P conformation, thereby inducing an increase of intracellular Na⁺ concentration that leads to an

increase of intracellular calcium concentration through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. This in turn leads to an increase in muscle contraction which is the origin of the classification of ouabain as a cardiotonic drug. That it is also being used in the treatment of breast cancer emphasises the importance of the ion pumps in the regulation of calcium homeostasis and cell survival.^{10–17} It is not clear if different-sized CTS also bind to the same site or as strongly, and whether they block the translocation of intracellular sodium with the same potency.²

Other Na^+/K^+ -ATPase inhibitors include the anti-psychotic drug chlorpromazine, the anti-malarial agent chloroquine and the antibiotic gramicidine A ($\text{IC}_{50} = 8 \mu\text{M}$) (Table 1).^{10–25} These drugs also affect other proteins, however, and the effects on the Na^+/K^+ -ATPase are only observed for higher concentrations.^{18,19}

Monomeric vanadate is a well-known Na^+/K^+ -ATPase inhibitor which is selective towards the E2P conformation. Besides vanadate, tungstate also is known to inhibit the Na^+/K^+ -ATPase, with dodecatungstate (W_{12}) exhibiting a higher capacity of inhibition (IC_{50} ca. $3 \mu\text{M}$) than the tungstate ion, WO_4^{2-} ($\text{IC}_{50} = 1.5 \text{ mM}$).²⁵ However, monomeric tungstate has been demonstrated to be a more potent inhibitor than dodecatungstate towards SR Ca^{2+} -ATPase ($\text{IC}_{50} = 400 \mu\text{M}$).⁸ Moreover, it has been suggested that for Na^+/K^+ -ATPase tungstate presents a competitive type of inhibition towards ATP, and blocks the enzyme at the nucleotide domain.⁸

3. H^+/K^+ -ATPase inhibitors

H^+/K^+ -ATPase inhibitors include anti-ulceral, anti-viral and antibiotic agents (Table 2). H^+/K^+ -inhibition is normally a strategy in preventing acidic segregation since gastric diseases are very painful and can lead to hepatic ulceration. Proton pump inhibitors (PPI), such as the drug omeprazole (IC_{50} ca. $3\text{--}4 \mu\text{M}$), present lower inhibitory capacity than the potassium competitive acid blockers (PCAB; $\text{IC}_{50} = 0.1 \mu\text{M}$) or prodigiosin ($\text{IC}_{50} = 0.03 \mu\text{M}$).^{26–34}

Omeprazole irreversibly inhibits H^+/K^+ -ATPase through the formation of a metabolite which reacts with the cysteine residues of the protein and causes pump inhibition. It was shown that cysteine residue 813 is oxidized upon PPI interaction, but other cysteine residues may also be involved such as cysteine residues 321, 822 or 892, which are part of several transmembranal helices.

Vanadate is shown to be a potent H^+/K^+ -ATPase inhibitor as it is for the Na^+/K^+ -ATPase.⁶² It reacts with the E2 form of the enzyme and when the E1 form is favoured the inhibition was not observed.⁶³

4. Ca^{2+} -ATPase inhibitors

The Ca^{2+} -ATPase inhibitors include compounds used for cancer therapy, cardioprotection, immunosuppression and as antitumoral agents (Table 3). Thapsigargin (TG) is currently

the most potent calcium pump inhibitor known, and presents an inhibitory value in the nM range ($\text{IC}_{50} = 1 \text{ nM}$)^{8,35,45–47} (Table 3). The inhibition leads to increasing cytoplasmic calcium levels, which leads to the activation of apoptotic factors which trigger cell death, making TG an active anti-tumoral agent.

Both TG and cyclopiazonic acid (CPA; IC_{50} ca. $1 \mu\text{M}$) bind to the E2 conformation of the enzyme, but at different binding sites.² As described for other P-type inhibitors, these compounds bind to the protein in the membrane domain, at the entrance of the calcium release channel close to the membrane surface. However, TG and CPA do not bind to the same exact site, and therefore the protein can bind simultaneously to both inhibitors.² Benzoquinone (BHQ) inhibitors bind to the same binding site as CPA.

Whereas TG blocks the transmembranal helices TM3, TM5 and TM7, CPA moves TM1, TM2 towards to TM4. In both types of interaction the drugs prevent the release of calcium. Although the majority of the Ca^{2+} -ATPase inhibitors in Table 3 seem to block the E2 conformation of the enzyme, a few, such as curcumin, also interact with the E1P conformation.²

Vanadate species, in particular decavanadate (V_{10}), are potent inhibitors ($\text{IC}_{50} = 15 \mu\text{M}$) of the hydrolytic activity of the sarcoplasmic reticulum Ca^{2+} -ATPase, with activities similar to that observed for decaniobate, $[\text{Nb}_{10}\text{O}_{28}]^{6-}$, and higher than for WO_4^{2-} and MO_4^{2-} .⁸ SR Ca^{2+} -ATPase activity is also inhibited by vanadium complexes such as vanadium-citrate and bis(maltolato)oxovanadium(IV) (BMOV) (Table 1).^{47,48} These and other vanadium compounds have been described in the treatment of diabetes mellitus.⁴⁹ Furthermore, decavanadate,⁵⁰ and compounds containing decavanadate⁵¹ have been reported to lower glycemic levels,^{50,51} and to normalize plasma lipid profiles.⁵¹

Due to the ability of V_{10} to interfere with several biological processes, such as calcium homeostasis, muscle contraction and oxidative stress,^{8,54,55} not only *in vitro* but also *in vivo*,^{52,53} decavanadate has recently been associated with the treatment of *Leishmaniosis*.⁵⁶ In this and probably in many other cases the mechanism of action of decavanadate progresses *via* the inhibition of the E1–E2 Ca^{2+} -ATPases. However, mitochondria may be an additional target as several mitochondrial processes have been shown to be affected by decavanadate at nanomolar concentrations.^{57,58}

Decavanadate can also induce protein cysteine oxidation with concomitant $\text{V}(\text{v})$ reduction to $\text{V}(\text{IV})$. Thus we cannot exclude the potential activity of $\text{V}(\text{IV})$ species in inducing changes in the Ca^{2+} -ATPase structure and function.^{8,9} Both decaniobate and decavanadate have recently been shown to interact non-competitively with SR Ca^{2+} -ATPases with respect to ATP. The V_{10} binding site on the E2 conformer is located at the intersection of the three cytoplasmic protein domains that includes the nucleotide, the phosphorylation and the β -strand domains.⁴ Twenty years ago decavanadate was demonstrated to be specific in inducing crystallization of SR Ca^{2+} -ATPase.⁵⁹ These and other recent studies emphasize that decavanadate interactions with the calcium pump are clearly different from

those of monomeric vanadate by inducing different changes in protein structure and function, and indicate different biological roles for the two oxovanadate species. These differences may explain the different biological effects described in several studies. For instance, at near-physiological conditions with calcium and without a calcium ionophore only decavanadate inhibits calcium translocation whereas vanadate does not.⁶ Still, although there has been some progress in improving the understanding of the interaction and the effect of V_{10} on the structure and function of the Ca^{2+} -ATPases, the mechanism of action on the Ca^{2+} -ATPase is still not fully understood, particularly under physiological conditions.

Given that the decavanadate-specific Ca^{2+} -ATPase binding site is located on the cytoplasmic side, one would expect that V_{10} needs to cross the cell membrane in order to be active. Decavanadate can, however, also affect the cell membrane itself,⁶⁰ for example by decreasing lipid packing which may induce changes in the composition of proteins in membrane microdomains.⁶¹ This offers an indirect mode of action which does not require transmembranal transport. By changing membrane structure and dynamics, alterations on the structure and function of the E1–E2 ATPases can also occur. Finally, we cannot exclude that V_{10} might interact with the ion pumps from the extracellular space, as is the case with many drugs (Fig. 1).

Polyoxotungstates have also been shown to inhibit not only ion pumps such as Na^+/K^+ -ATPase, H^+/K^+ -ATPase, and Ca^{2+} -ATPase but also ecto-ATPases.²⁵ In fact, polyoxotungstates are 10–100 times more potent as inhibitors²⁵ than monovanadium coordination complexes,^{47,48} while decavanadate IC_{50} values are one to two orders of magnitude lower than those of the polyoxotungstates.^{8,9}

The mechanism of inhibition and interaction of decavanadate on the sarcoplasmic reticulum calcium pump ATPase is clearly different from that of other polyoxometalates and oxometalates. It involves protein cysteine oxidation and vanadate reduction processes, which lead to non-competitive ATPase inhibition. This inhibition is not prevented by antioxidant agents that can reverse the cysteine oxidation.⁹ Moreover, decavanadate interacts with ATPase in the same fashion for all the conformations that occurs during the ATP-hydrolysis coupled calcium translocation. These recent findings emphasise the crucial need to properly understand and compare the ion pump inhibition processes of several types of inhibitors, since they may well turn out to be significantly different.

Given the specific activity of these structures towards the ion pumps, polyoxometalates are promising as drugs for anti-cancer activity, protection against viral, bacterial and protozoa infections, and antidiabetic activity.^{49–51,56}

5. Conclusions

Although some 18 classes of ion pump inhibitors are known, the biochemical mechanisms are only known for a handful of compounds. The majority of these compounds – such as

ouabain, omeprazole, thapsigargin, CPA and BHQ – seem to interact selectively with the E2 or E2P conformations of the target enzyme, which can be understood in light of the binding site being located in the transmembranal region, at or close to the calcium binding site. The binding is normally reversible, but, in the case of omeprazole, nearby cysteine units are also directly affected, which leads to the irreversible inhibition of the pump.

Thapsigargin is one of the most potent inhibitors of the Ca^{2+} -ATPases, and of all the E1–E2 ion pump inhibitors discussed in the present Perspective, with an IC_{50} in the nM range of concentration. While vanadium and tungsten mono- and polyoxometalates have IC_{50} values which are three orders of magnitude ($IC_{50} = 2–15 \mu M$) higher than TG, they still have activities comparable with other relevant ion pump inhibitors.

The mode of action of decavanadate and the other oxometalates implies that the main focus of the interaction is on the cytoplasmic domain of the protein, more specifically at the nucleotide or phosphorylation domains or at a pocket involving several cytoplasmic domains. Although decavanadate interacts most strongly with the E2 conformation of the Ca^{2+} -ATPase, as do some of the other drugs described above, decavanadate also interacts with other conformations.^{6,8} Therefore, decavanadate exhibits a particular feature that may be useful for the understanding of the mode of action of ion pump inhibitors in general. Besides, the specific behaviour of decavanadate towards ion pumps may guide the development of new drugs that would have these pumps as therapeutic targets. Finally, it is believed that the present observations will contribute to the understanding of oxometalate interactions with the

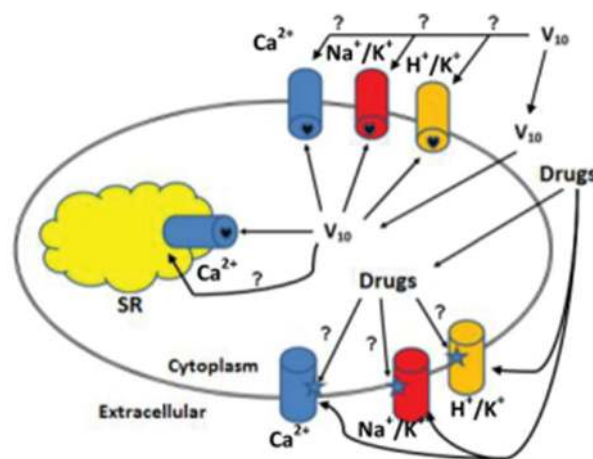


Fig. 2 E1–E2 ATPases, a family of cation pump enzymes, are known targets for specific drugs including decavanadate (V_{10}). The majority of the drugs bind within the bilayer near the entrance of the cation exit channel whereas decavanadate binds to a pocket formed by protein domains at the cytoplasmic side. Decavanadate and other polyoxometalates need to cross the bilayer before targeting the ion pumps, something which probably occurs through transport membrane systems such as channels, or specific transporters and/or pumps. In contrast, established organic drugs do not apparently need to cross the membrane, and probably for V_{10} this mechanism of action cannot be completely excluded. Na^+/K^+ , H^+/K^+ , and Ca^{2+} stand for Na^+ , K^+ -ATPase, H^+ , K^+ -ATPase, and Ca^{2+} -ATPase, respectively. SR denotes sarcoplasmic reticulum.

calcium pump and the role of decavanadate in biology, particularly the contribution of decavanadate to calcium homeostasis.

In summary, E1–E2 ATPases are targets for several classes of compounds, including decavanadate, and present several potential binding sites. The majority of the established drugs bind near the entrance of the cation binding sites within the bilayer, whereas decavanadate binds to a pocket formed by protein domains at the cytoplasmic side. Decavanadate and other polyoxometalates need to cross the bilayer before targeting the ion pumps, something which probably occurs through transport membrane systems such as channels, or specific transporters and/or pumps. In contrast, a majority of established organic drugs do not appear to need to cross the membrane. However, the former mechanism of action cannot be completely excluded for decavanadate (Fig. 2).

Abbreviations

BHQ	Benzohydroquinone
CPA	Cyclopiazonic acid
CTS	Cardiotonic steroids
E1	Protein conformation E1
E1P	Protein conformation E1 phosphorylated
E2	Protein conformation E2
E2P	Protein conformation E2 phosphorylated
PCAB	Potassium competitive acid blockers
PPIs	Proton pump inhibitors
SR	Sarcoplasmic reticulum
SERCA	Sarco(end) reticulum calcium ATPase
TG	Thapsigargin
TM	Transmembranal helices
V ₁₀	Decavanadate, a vanadate oligomer containing 10 vanadate units
W ₁₂	Polyoxotungstate containing 12 tungstate units

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References

- L. C. Cantley Jr., L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, *J. Biol. Chem.*, 1977, **252**, 7421–7423.
- L. Yatime, M. J. Buch-Pedersen, M. Musgaard, J. P. Morth, L. Anne-Marie, B. Winther, P. Pedersen, C. Olesen, J. P. Andersen, B. Vilsen, B. Schiøtt, M. G. Palmgre, J. V. Møller, P. Nissen and N. Fedosova, *Biochim. Biophys. Acta, Bioenerg.*, 2009, **1787**, 207–220.
- L. de Meis and A. L. Vianna, *Annu. Rev. Biochem.*, 1979, **48**, 275–292.
- C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa, *Nature*, 2004, **405**, 647–655.
- M. Aureliano, *World J. Biol. Chem.*, 2011, **2**, 215–238.
- M. Aureliano and V. M. C. Madeira, *Biochim. Biophys. Acta, Mol. Cell Res.*, 1994, **1221**, 259–271.
- P. L. Pedersen and E. Carafoli, *Trends Biochem. Sci.*, 1987, **12**, 146–150.
- G. Fraqueza, C. A. Ohlin, W. H. Casey and M. Aureliano, *J. Inorg. Biochem.*, 2012, **107**, 82–89.
- G. Fraqueza, L. A. E. Batista de Carvalho, M. Paula, M. Marques, L. Maia, C. André Ohlin, W. H. Casey and M. Aureliano, *Dalton Trans.*, 2012, **41**, 12749–12758.
- M. Juhaszova and M. P. Blaustein, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 1800–1805.
- K. Ihenetu, H. M. Qazzaz, F. Crespo, R. Fernandez-Botran and R. Valdes Jr., *Clin. Chem.*, 2007, **53**, 1315–1322.
- H. Bottinger and E. Habermann, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1984, **325**, 85–87.
- D. N. Almotrefi, *Gen. Pharmacol.*, 1991, **22**, 403–406.
- A. N. Wansapura, V. Lasko, Z. Xie, O. V. Fedorova, A. Y. Bagrov, J. B. Lingrel and J. N. Lorenz, *Am. J. Physiol.: Heart Circ. Physiol.*, 2009, **296**, 1833–1839.
- J. Joep, H. H. M. De Pont, H. G. P. Swarts, A. Karawajczyk, G. Schaftenaar, P. H. G. M. Willems and J. B. Koenderink, *Pfluegers Arch.*, 2009, **457**, 623–634.
- C. Scheiner-Bobis and H. Schneider, *Eur. J. Biochem.*, 1997, **248**, 717–723.
- Z. Zhang, Z. Li, J. Tian, W. Jiang, Y. Wang, X. Zhang, Z. Li, Q. You, J. I. Shapiro, S. Si and Z. Xie, *Mol. Pharmacol.*, 2010, **77**, 961–967.
- D. Bhattacharyya and P. C. Sen, *Mol. Cell. Biochem.*, 1999, **198**, 179–185.
- P. R. S. Kodavanti, W. R. Mundy, H. A. Tilson and G. Jean Harry, *Fundam. Appl. Toxicol.*, 1993, **21**, 308–316.
- H. Kutchai and L. M. Geddis, *Pharmacol. Res.*, 2001, **43**, 399–403.
- R. Coca, F. Soler and F. Fernández-Belda, *Arch. Biochem. Biophys.*, 2008, **478**, 36–42.
- Y. Ishida, K. Takagi, M. Takahashi, N. Satake and S. Shibata, *J. Biol. Chem.*, 1983, **258**, 7900–7902.
- H. Chau Wu, *Toxicol.*, 2009, **54**, 1183–1189.
- Y. Takada, K. Matsuo and T. Kataoka, *Mol. Cell. Biochem.*, 2008, **319**, 99–103.
- M. B. Colović, D. V. Bajuk-Bogdanovic, N. S. Avramovic, I. D. Holclajtner-Antunovic, N. S. Bošnjaković-Pavlovic, V. M. Vasić and D. Z. Krstić, *Bioorg. Med. Chem.*, 2011, **19**, 7063–7069.
- H. Matsuya, M. Okamoto, T. Ochi, A. Nishikawa, S. Shimizu, T. Kataoka, K. Nagai, H. H. Wasserman and S. Ohkuma, *Biochem. Pharmacol.*, 2000, **60**, 1855–1863.
- D. Seto-Young, B. Monk, A. B. Mason and D. S. Perlin, *Biochim. Biophys. Acta, Biomembr.*, 1997, **1326**, 249–256.

- 28 W. Beil, U. Staar and K. F. Sewing, *Eur. J. Pharmacol.*, 1992, **218**, 265–271.
- 29 D. J. Keeling, C. Fallowfield, K. J. Milliner, S. K. Tingley, R. J. Ife and A. H. Underwood, *Biochem. Pharmacol.*, 1985, **34**, 2967–2973.
- 30 R. A. Farley, S. Schreiber, S.-G. Wang and G. Scheiner-Bobis, *J. Biol. Chem.*, 2001, **276**, 2608–2615.
- 31 W. A. Simon, M. Herrmann, T. Klein, J. M. Shin, R. Huber, J. Senn-Bilfinger and S. Postius, *J. Pharmacol. Exp. Ther.*, 2007, **321**, 866–874.
- 32 S. Asano, M. Mizutani, T. Hayashi, N. Morita and N. Takeguchi, *J. Biol. Chem.*, 1990, **265**, 22167–22173.
- 33 T. Hayashi, K. Okamura, M. Kakemi, S. Asano, M. Mizutani, N. Takeguchi, M. Kawasaki, Y. Tezuka, T. Kikuchi and N. Morita, *Chem. Pharm. Bull.*, 1990, **38**, 2740–2745.
- 34 T. Kataoka, M. Muroi, S. Ohkuma, T. Waritani, J. Magae, A. Takatsuki, S. Kondo, M. Yamasaki and K. Nagai, *FEBS Lett.*, 1995, **359**, 53–59.
- 35 A. J. Johnson, A.-L. Hsu, H.-P. Lin, X. Song and C.-S. Chen, *Biochem. J.*, 2002, **366**, 831–837.
- 36 Y. Geng and M. Lotz, *J. Cell Biol.*, 1995, **129**, 1651–1657.
- 37 Y. Sagara and G. Inesi, *J. Biol. Chem.*, 1991, **266**, 13503–13506.
- 38 I. Moreno, L. Norambuena, D. Maturana, M. Toro, C. Vergara, A. Orellana, A. Zurita-Silva and V. R. Ordenes, *J. Biol. Chem.*, 2008, **283**, 9633–9641.
- 39 S. F. Plenge-Tellechea, I. Fortea and F. Fernandez-Belda, *Biochemistry*, 1998, **37**, 4266–4274.
- 40 N. J. Yard, M. Chiesi and H. A. Ball, *Br. J. Pharmacol.*, 1994, **113**, 1001–1007.
- 41 J. G. Bilmen, L. L. Wootton and F. Michelangeli, *Biochem. J.*, 2002, **366**, 255–263.
- 42 G. P. Ahern, P. R. Junankar, S. M. Pace, S. Curtis, J. A. Mould and A. F. Dulhunty, *J. Physiol.*, 1999, **514**, 313–326.
- 43 M. J. Logan-Smith, P. J. Lockyer, J. M. East and A. G. Lee, *J. Biol. Chem.*, 2001, **276**, 46905–46911.
- 44 J. G. Bilmen, S. Z. Khan, M. H. Javed and F. Michelangeli, *Eur. J. Biochem.*, 2001, **268**, 6318–6327.
- 45 M. J. Logan-Smith, P. J. Lockyer, J. M. East and A. G. Lee, *J. Biol. Chem.*, 2001, **276**, 46905–46911.
- 46 S. Grösch, T. J. Maier, S. Schiffmann and G. Geisslinger, *J. Natl. Cancer Inst.*, 2006, **98**, 736–747.
- 47 M. Aureliano, F. Henao, T. Tiago, R. O. Duarte, J. J. G. Moura, B. Baruah and D. C. Crans, *Inorg. Chem.*, 2008, **47**, 5677–5684.
- 48 M. Aureliano, T. Tiago, R. M. C. Gândara, A. Sousa, A. Moderno, M. Kaliva, A. Salifoglou, R. O. Duarte and J. J. G. Moura, *J. Inorg. Biochem.*, 2005, **99**, 2355–2361.
- 49 K. H. Thompson and C. Orvig, *Met. Ions Biol. Syst.*, 2004, **41**, 221–252.
- 50 M. J. Pereira, E. Carvalho, J. W. Eriksson, D. C. Crans and M. Aureliano, *J. Inorg. Biochem.*, 2009, **103**, 1687–1692.
- 51 F. Yraola, S. Garcia-Vicente, L. Marti, F. Albericio, A. Zorzano and M. Royo, *Chem. Biol. Drug Des.*, 2007, **69**, 423–428.
- 52 M. Aureliano and R. M. C. Gândara, *J. Inorg. Biochem.*, 2005, **99**, 979–985.
- 53 M. Aureliano and D. C. Crans, *J. Inorg. Biochem.*, 2009, **103**, 536–546.
- 54 M. Aureliano, *Dalton Trans.*, 2009, 9093.
- 55 R. M. C. Gândara, S. S. Soares, H. Martins, C. Gutiérrez-Merino and M. Aureliano, *J. Inorg. Biochem.*, 2005, **99**, 1238–1244.
- 56 T. L. Turner, V. H. Nguyen, C. C. McLauchlan, Z. Dymon, B. M. Dorsey, J. D. Hooker and M. A. Jones, *J. Inorg. Biochem.*, 2012, **108**, 96–104.
- 57 S. S. Soares, C. Gutiérrez-Merino and M. Aureliano, *J. Inorg. Biochem.*, 2007, **101**, 789–796.
- 58 S. S. Soares, F. Henao, M. Aureliano and C. Gutierrez-Merino, *Chem. Res. Toxicol.*, 2008, **21**, 607–618.
- 59 A. Maurer and S. Fleischer, *J. Bioenerg. Biomembr.*, 1984, **16**, 491–505.
- 60 B. Baruah, L. A. Swafford, D. C. Crans and N. E. Levinger, *J. Phys. Chem. B*, 2008, **112**, 10158–10164.
- 61 P. W. Winter, A. Al-Quati, A. L. Wolf-Ringwall, S. Schoeberl, P. B. Chatterjee, B. G. Barisas, D. A. Roess and D. C. Crans, *Dalton Trans.*, 2012, **41**, 6419–6430.
- 62 L. D. Faller, E. Rabon and G. Sachs, *Biochemistry*, 1983, **22**, 4676–4685.
- 63 K. L. Dürr, N. N. Tavraz and T. Friedrich, *PLoS One*, 2012, **7**, 33645.