Published in final edited form as:

Mol Biochem Parasitol. 2008 January ; 157(1): 107–111.

A Single S1034C Mutation Confers Altered Drug Sensitivity to PfMDR1 ATPase Activity That is Characteristic of the 7G8 Isoform

Jacqueline K. Lekostaj, Linda E. Amoah, and Paul D. Roepe

Department of Chemistry, Department of Biochemistry, Cellular & Molecular Biology, Tumor Biology Program, Lombardi Cancer Center, and Center for Infectious Disease, Georgetown University, 37th and O Streets, Washington, DC 20057

PfMDR1 is a member of the ATP-Binding Cassette (ABC) superfamily, many members of which (e.g. human P-glycoprotein; HuMDR1) have been implicated in drug resistance phenomena. PfMDR1 is composed of two homologous cassettes, each containing 6 putative transmembrane (TM) helices and 1 nucleotide binding domain (NBD). Similar to HuMDR1, PfMDR1 has been proposed to confer multidrug resistance. A number of drug resistance-conferring ABC proteins have been proposed to function as drug transporters, using the energy from ATP hydrolysis to catalyze drug efflux from a cell or cellular compartment containing the relevant drug target, thereby promoting drug resistance. ATP-dependent small molecule or ion pumps in general do not hydrolyze ATP well in the absence of transported substrate. Thus, although basal ATPase activity in the absence of drugs remains to be understood, support for ABC drug transport models includes stimulation of ABC ATPase activity by the drugs to which the ABC transporter confers resistance. For example, stimulation of HuMDR1 ATPase is 3–10 fold for a number of drugs to which it confers resistance [1,2], and also up to 10 fold for compounds that reverse the resistance phenotype (e.g. verapamil [VPL] see [1]). Calculated drug effects on K_m span two orders of magnitude, and different patterns of significant K_m vs. V_{max} alterations have been noted [1]. Although strict correlations between the magnitude of ATPase stimulation vs. ability of the ABC transporter to confer resistance are typically not found, in general, a higher degree of ATPase stimulation by drugs to which the ABC transporter confers the highest degree of resistance is a frequent observation (e.g. [1,2] and references within).

Many questions surround the role of PfMDR1 in antimalarial drug resistance phenomena. The presumed importance of PfMDR1 in drug resistance has been significantly diminished by genetic studies that culminated in the recent discovery that *Pfcr1* mutations are the main molecular determinant of chloroquine resistance (CQR) for *P. falciparum* [4–6]. It has therefore subsequently been suggested that PfMDR1 modulates the level of CQR and resistance to other quinoline-based antimalarials in a PfCRT-dependent manner. Another hypothesis is that PfMDR1 mutations could represent fitness adaptations in CQR *P. falciparum* harboring mutated PfCRT. In terms of mechanism, the protein has been localized to the membrane of the parasite digestive vacuole (DV; [3]), with NBDs disposed towards the cytoplasm. Therefore, if the above drug pumping model is applicable, PfMDR1 would paradoxically act to translocate drugs *into* the DV, which is believed to be the principle site of action for most quinoline antimalarials. How concentrating drugs *at* the site of action would promote resistance is a puzzle. However, for non quinoline drugs (such as artemisinin) with multiple unknown targets,

Correspondence to PDR at: roepeg@georgetown.edu; 202 687- 7300; 202 687- 7186 (fax).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

some of which may reside in the cytosol, sequestration in the DV could act to promote resistance. If PfMDR1 recognizes and alters the diffusion of a wide variety of substrates (some of which have targets within the DV and others without), it may help to explain why increased resistance to one compound can be accompanied by hypersensitivity to another.

Transfection studies suggest that PfMDR1 mutations may make small contributions to sensitivity vs. quinine (QN), mefloquine (MQ), halofantrine (HF), and artemisinin (ART), but in a strain dependent fashion [7,8]. However, field data link over expression (not necessarily mutation) of the gene with clinical drug treatment failure [9]. Thus the relative importance of PfMDR1 mutation vs. overexpression in adding to resistance profiles is currently debated. Laboratory experiments with field isolates commonly report mutations at five codons: N86Y and Y184F in the N-terminal cassette, and S1034C, N1042D and D1246Y in the C-terminal cassette. Using allelic exchange, the Cowman group [7] reported that introduction of the three C-terminal mutations into a chloroquine sensitive (CQS) strain conferred resistance to QN and mildly increased susceptibility to MQ, HF and ART; the MQ and HF effects were even more apparent in a strain bearing only the 1246Y mutation. However, effects of the individual mutations on drug stimulated PfMDR1 ATPase activity are not known. Reversion of the same three mutations back to wildtype in a strain 7G8 background resulted in the opposite phenotype (MQ, HF, and ART resistance and QN sensitivity). These results were later confirmed in additional transfection studies by Sidhu *et al.* [8], which also suggested the 1042D mutation was particularly influential.

Taking all of the above together, it is commonly expected that if these PfMDR1 mutations do indeed promote additional degrees of resistance to particular drugs (e.g. QN, MQ), then effects of these drugs on the ATPase activity of the PfMDR1 mutants should be significant, and/or differ vs. effects for wild type PfMDR1. If this is the case, defining the specific contributions of individual mutations would assist identification of drug interaction sites. However, all previous work with PfMDR1 has until now been done with parasite isolates or transfectants, systems greatly complicated by the presence of other proteins involved in drug resistance (e.g. PfCRT) and/or other changes due to antimalarial drug selection pressure. Moreover, isolating parasite fractions within which PfMDR1 ATPase activity is easily separable from other ATPases is extremely difficult, if not impossible.

Thus, we have recently reported the successful heterologous overexpression of several PfMDR1 isoforms found in either CQS or CQR parasites [10], which now greatly facilitates molecular dissection of PfMDR1, including quantification of drug effects on ATPase activity, which as mentioned is a hallmark of drug resistance-conferring ABC transporters. While all overexpressed isoforms were active as measured by their ability to hydrolyze ATP [10], there were significant differences among them. In particular, the PfMDR1 isoform expressed in CQR strain Dd2 exhibited higher ATPase activity relative to that expressed in CQS 3D7, while activity of the CQR strain 7G8 isoform was by far the lowest of the three. Also, in contrast to Dd2 and 3D7 isoforms, ATPase activity of 7G8 PfMDR1 was largely unaffected by quinoline antimalarial drugs, except at very high [CQ]. Since Dd2 PfMDR1 possesses a single amino acid change relative to wildtype (N86Y) some changes in activity relative to wild type can be assigned to this substitution. However, 7G8 PfMDR1 contains four additional substitutions relative to wild type: Y184F, S1034C, N1042D, D1246Y. In order to investigate which of these mutations is responsible for unusual 7G8 isoform ATPase activity (e.g. low V_{max} , high K_m , conspicuous loss of QN and MQ stimulation), we have created yeast strains expressing PfMDR1 harboring various combinations of these mutations. This is the first step in identifying regions of the PfMDR1 molecule that may interact with specific drugs.

Initial data indicated that a mutant containing the three C-terminal substitutions (called “triple mutant” or TM by Reed *et al* [7]) behaved similarly relative to 7G8 PfMDR1 (see below and

also [7]). We therefore focused attention on the effects of 1034C, 1042D, and 1246Y substitutions, both alone and in various possible combinations. Using recently published methods [10] we constructed 3 yeast strains expressing PfMDR1 with each single mutation as well as 3 strains expressing PfMDR1 with each possible double mutation (Table I). We then purified plasma membrane fractions from each strain (Fig. 1) and quantified PfMDR1 ATPase activity as described [10]. All mutants tested had an optimum pH near 7.0 or 7.5 and all but one showed optimal activity near 5.0 or 7.5 mM ATP (Table 1). In terms of basal activity, the S1034C mutant closely approximates 7G8, whereas N1042D is more similar to Dd2, and D1246Y exhibits the highest ATPase activity of any mutant yet tested (with a $V_{\max}$ identical to that of Dd2). However, interestingly, K_m for S1034C is conspicuously higher relative to 7G8. That is, no single C terminal substitution recapitulates both $V_{\max}$ and K_m seen for the 7G8 isoform. Two or more mutations must therefore act in concert to produce an enzyme with 7G8 kinetic characteristics.

Interestingly, the 1034/1042 and 1034/1046 double mutants both show elevated $V_{\max}$ and lower K_m relative to S1034C. The 1042/1246 double mutant is similar to the wildtype 3D7 isoform, and showed higher K_m relative to the single site 1042 and 1246 mutants. Thus the lower $V_{\max}$ and higher K_m relative to wild type that is seen for TM and 7G8 is not seen in any of the double mutants; all three C-terminal substitutions are required for this behavior (Table I). The remaining mutation that converts TM to 7G8 (Y184F) produces a small effect that then mildly raises $V_{\max}$ and lowers K_m but, importantly, does not alter drug response (see below), consistent with previous suggestions from analysis of transfectants [7].

We have previously reported [8] that the ATPase activity of wild type (3D7) PfMDR1 is mildly stimulated over a wide range of estimated cytoplasmic and digestive vacuolar concentrations of MQ (0–300 nM and 5–60 μ M, respectively). Amongst the C-terminal mutants now tested (Figure 2A), the N1042D and D1246Y single mutants as well as the 1034C/1042D double mutant (not shown) are similarly stimulated by MQ. In contrast, remarkably, the S1034C single mutant showed very little response upon addition of any concentration of MQ, very similar to the behavior of TM and 7G8. Other double mutants (1034/1246, 1042/1246) showed intermediate behavior (not shown, see caption).

A significant QN stimulatory effect was previously seen for wild type and Dd2 isoforms, but only at very high concentrations that correspond to the upper range of what we calculate to be expected within the DV [10]. None of our newly-created C-terminal mutants display this stimulation, and indeed some even seem to be inhibited by high amounts of QN (Figure 2B). However, D1246Y was stimulated to a similar extent by QN, but at much lower concentrations of QN.

Considering the fact that CQ is generally not believed to interact with PfMDR1 [7,8], it was surprising that we earlier found CQ had the greatest effect on ATPase activity: high DV-compatible concentrations of CQ severely inhibit both 3D7 and 7G8 isoforms. Under similar conditions, all C-terminal mutants exhibit some degree of inhibition, but only S1034C and TM are rendered almost completely inactive (Figure 2C). In sum, of all mutants tested, only S1034C exhibited a nearly identical “drug profile” relative to 7G8 PfMDR1. Also, interestingly, S1034C and 7G8 are the only two isoforms that were not affected at all by the chemosensitizer verapamil (VPL) (Table 1).

Some parallels can be drawn between these PfMDR1 mutation sites and corresponding positions in the human MDR1 sequence. Topologically, the N86Y mutation is found in the DV-disposed loop between the first and second predicted TM domains. Similar to our previous results with Dd2 [10], deletions in this region of HuMDR1 lead to altered verapamil sensitivity [11]. The 1034 and 1042 mutations lie in the middle of predicted TM helix 11, which has been

hypothesized to be part of a drug binding site in HuMDR1 [12–14]. TM11 has also been implicated in the release of drug during ATP hydrolysis [15]. Thus our finding that the S1034C substitution has the most significant effect on PfMDR1 ATPase drug stimulation is consistent with previous drug interaction domain analyses for other ABC proteins. The D1246Y mutation is situated within the C-terminal NBD. While there are no direct comparisons to HuMDR1 currently in the literature, it seems likely that any mutation within the NBD is liable to affect ATP binding or hydrolysis. How this domain then interacts with others predicted to interact with drug (e.g. TM 11) to further modify $V_{\max}$ and K_m remains to be determined, but ongoing crystallographic analyses of other ABC proteins (e.g. [16]) will be helpful in this regard.

Since mutations in PfCRT originated in at least four independent foci worldwide [17], PfMDR1 mutations may have arisen in similar fashion, against a backdrop of selective drug pressure and fitness adaptation in response to different PfCRT mutations. In evolutionary terms, a number of scenarios are possible since it is unclear which quinoline drugs (if any) selected for Dd2 and 7G8 PfMDR. But based on our inspection of $V_{\max}$, K_m , and drug stimulation of ATPase for all the mutants described herein, and assuming quinoline exposure was the penultimate driving force for PfMDR1 mutation, we speculate that the 7G8 strain first acquired the S1034C mutation in order to bias against drug effects on the enzyme, followed by the other substitutions, which merely act to “fine tune” $V_{\max}$ and K_m . Regardless the order, the final consequence is an enzyme that has reduced catalytic efficiency and that is insensitive to quinoline based antimalarial drugs, both of which would seem to be advantageous based on conventional drug transport models for MDR proteins and the DV localization of both PfMDR1 and the quinoline target (see above). That is, both features would presumably act synergistically to lower quinoline drug accumulation into the DV via an ABC transporter with cytosolically disposed NBD. However, importantly, if we compare the behavior of Dd2, 7G8 (both CQR strains) and 3D7 (CQS) PfMDR1 isoforms [10] we draw the immediate conclusion that the level of basal ATPase activity is not necessarily relevant for quinoline drug resistance. Dd2 has higher $V_{\max}$ relative to 3D7, whereas 7G8 has lower. The degree of QN and MQ stimulation could be related to resistance to these specific compounds, since although the differences between Dd2 and 3D7 isoforms are quite small 3D7 PfMDR1 showed the greatest proportional drug stimulation of ATPase activity (see also [10]). Interestingly, strain 7G8 exhibits VPL insensitive CQR, whereas the strain Dd2 CQR phenotype is VPL reversible. Perhaps correspondingly, Dd2 PfMDR1 ATPase is inhibited by VPL, but the 7G8 isoform is not. Interestingly, the S1034C mutation is responsible for this loss of VPL sensitivity along with reduced catalytic efficiency (Table 1). However, most importantly, these results indicate no simple pattern among quinoline drug effects on ATPase activity for PfMDR1 isoforms found in these strains vs. the level or pattern of quinoline drug resistance exhibited by the strain. Also, PfMDR1 function is clearly quite different from that of its close homologue HuMDR1 (P-glycoprotein), since VPL has the strongest effects (up to 10-fold stimulation) of any compound on HuMDR1 ATPase [1], whereas effects are minor to nonexistent for PfMDR1 isoforms associated with resistance. We suggest these data indicate that the relative contribution of PfMDR1 to drug resistance is likely different for various CQR strains (e.g. Dd2 vs. 7G8).

The PfMDR1 isoforms that are currently found in CQR isolates may illustrate symbiotic relationships between mutant PfCRTs and mutant PfMDR1 that confer either preferred resistance patterns, fitness adaptations, or perhaps both. Perhaps the different $V_{\max}$ and K_m for Dd2 vs 7G8 PfMDR1 isoforms, along with over expression levels (gene copy number), reflect fitness adaptations relevant to distinct Dd2 and 7G8 PfCRT mutations, whereas decreased quinoline drug stimulation of both Dd2 and 7G8 PfMDR1 ATPase activity reflects selection to further subtly modify quinoline drug resistance conferred by PfCRT.

These experiments with purified membranes allow more precise and accurate quantification of PfMDR1 ATPase activity and other molecular characteristics (e.g. perhaps binding of some

drugs). However, obviously only transfection with PfMDR1 alleles into various parasite strains can provide precise quantification of their minor role in modulating drug resistance profiles. Put together, the two approaches are synergistic; transfection of some of the mutants constructed in the present work may offer one convenient way in which to test some concepts. Also, we note that although specific PfMDR1 codons are often sequenced for CQR isolates, and that some parallels between those data and the results in this paper can be drawn, more complete sequencing of PfMDR1 alleles as well as additional quantification of the frequency of 7G8 vs. Dd2 allele over expression in CQS vs. CQR isolates [9] will eventually be required to distinguish between current models generated by our *in vitro* work.

Acknowledgements

This work was supported by NIAID/NIH (RO1 AI056312).

References

1. Kimura Y, Kioka N, Kato H, Matsuo M, Ueda K. Modulation of drug – stimulated ATPase activity of human MDR1/P-glycoprotein by cholesterol. *Biochemical Journal* 2007;401:597–605. [PubMed: 17029589]
2. Sarkadi B, Price EM, Boucher RC, Germann UA, Scarborough GA. Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug – stimulated membrane ATPase. *Journal of Biological Chemistry* 1992;267:4854–4858. [PubMed: 1347044]
3. Cowman AF, Karcz S, Galatis D, Culvenor JG. A p-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole. *Journal of Cell Biology* 1991;113(5):1033–42. [PubMed: 1674943]
4. Wellems TE, Panton LJ, Gluzman IY, do Rosario VE, Gwadz RW, Walker-Jonah A, Krogstad DJ. Chloroquine resistance not linked to *mdr*-like genes in a *Plasmodium falciparum* cross. *Nature* 1990;345:253–5. [PubMed: 1970614]
5. Wellems TE, Walker-Jonah A, Panton LJ. Genetic mapping of the chloroquine-resistance locus on *Plasmodium falciparum* chromosome 7. *Proceedings of the National Academy of Sciences* 1991;88(8):3382–6.
6. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LM, Sidhu AB, Naude B, Deitsch KW, Su XZ, Wootton JC, Roepe PD, Wellems TE. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol Cell* 2000;6(4):861–71. [PubMed: 11090624]
7. Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF. Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. *Nature* 2000;403(6772):906–9. [PubMed: 10706290]
8. Sidhu AB, Valderramos SG, Fidock DA. *pfmdr1* mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in *Plasmodium falciparum*. *Molecular Microbiology* 2005;57(4):913–26. [PubMed: 16091034]
9. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, Patel R, Laing K, Looareesuwan S, White NJ, Nosten F, Krishna S. Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. *Lancet* 2004;364:438–47. [PubMed: 15288742]
10. Amoah LE, Lekostaj JK, Roepe PD. Heterologous expression and ATPase activity of wildtype vs mutant PfMDR1 protein. *Biochemistry* 2007;46(20):6060–73. [PubMed: 17469853]
11. Welker E, Szabo K, Hollo Z, Muller M, Sarkadi B, Varadi A. Drug-stimulated ATPase activity of a deletion mutant of the human multidrug-resistance protein (MDR1). *Biochemical and Biophysical Research Communications* 1995;216(2):602–9. [PubMed: 7488154]
12. Bruggemann EP, Germann UA, Gottesman MM, Pastan I. Two different regions of P-glycoprotein are photoaffinity labeled by azidopine. *Journal of Biologic Chemistry* 1989;264:15483–88.
13. Bruggemann EP, Currier SJ, Gottesman MM, Pastan I. Characterization of the azidopine and vinblastine binding site of P-glycoprotein. *Journal of Biologic Chemistry* 1992;267:21020–26.

14. Morris DI, Greenberger LM, Bruggemann EP, Cardarelli CO, Gottesman MM, Pastan I, Seamon KB. Localization of the forskolin labeling sites to both halves of P-glycoprotein: similarity of the sites labeled by forskolin and prazosin. *Molecular Pharmacology* 1994;46:329–37. [PubMed: 7915819]
15. Frelet A, Klein M. Insight in eukaryotic ABC transporter function by mutation analysis. *FEBS Letters* 2006;580:1064–84. [PubMed: 16442101]
16. Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature* 2006;443 (7108): 180–5. [PubMed: 16943773]
17. Wootton JC, Feng X, Ferdig MT, Cooper RA, Mu J, Baruch DI, Magill AJ, Su XZ. Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. *Nature* 2002;418:320–23. [PubMed: 12124623]

Figure 1.

Representative polyHis blot showing approximately equal inducible expression of new PfMDR1 mutants in the constructed yeast strains. Expression levels are similar to those of 3D7, Dd2 and 7G8 isoforms reported previously [8]. “ZC” indicates negative control carrying empty zeocin selectable vector. Yeast were grown under standard conditions, induced with methanol for ~20 hours and plasma membranes purified by acid precipitation as described [8]. Protein was quantified by amido black and an equivalent amount of membrane protein was loaded into wells of a 7.5% polyacrylamide gel, which was run at 110V for 100 min and subsequently transferred to a PVDF membrane overnight at 40 mA. The blot was developed using the PentaHis detection kit from Qiagen according to manufacturer’s instructions. After averaging densitometry from two such plots, we calculate that for each mutant, PfMDR1 constitutes 3.63, 2.45, 2.71, 1.94, 2.85, 2.05, and 2.46 % of total membrane protein, respectively.

Figure 2.

ATPase activity of C-terminal mutants in the presence of varying amounts of MQ (**A**), QN (**B**), and CQ (**C**). Activity for 3D7, Dd2 and 7G8 is also reproduced in ref [10]. The ATPase activity of purified PM fractions was measured using the colorimetric determination of orthophosphate released from ATP as described in detail previously [8]. Briefly, plates were set up on ice: assay buffer pH 7.5 (180 mM NH_4Cl /100 mM Mes-Tris/10 mM MgCl_2 /.01% NaN_3) was added to each well followed by relevant drug solutions, and finally membrane samples, to a total volume of 100 μL . The plate was shaken, warmed to 37°C, and ATP was then added. After shaking at 37°C for an additional 15 minutes, the reaction was stopped, stabilized 10 minutes later, and absorbance at 720 nm read after 30 minutes. Results shown

are the average ($\pm$ s.d.) for at least 2 independent membrane preparations, each done at least in triplicate, and values are normalized to PfMDR1 content via densitometry as described in detail elsewhere [8]. Single mutants, solid lines, closed symbols: S1034C, closed squares; N1042D, closed triangles; D1246Y, closed circles. Strain isoforms, dashed lines, open symbols: 3D7, open squares; 7G8, open circles. Double mutant data are omitted for clarity but are available from the authors upon request: 1034/1042 ATPase activity is very similar to that of D1246Y when plotted vs. these concentrations of MQ, QN or CQ, whereas 1034/1246 and 1042/1246 show behavior that lies between that of D1246Y vs. 7G8 and S1034C. Note behavior for D1246Y is reminiscent of that seen for isoform Dd2 [8] indicating that single site mutations at two widely separate positions (1246 and 86 respectively) are capable of approximately doubling $V_{\max}$.

Table 1

Summary of PfMDR1 Mutant Kinetic Parameters

Kinetic parameters and inhibitor sensitivities of PfMDR1 isoforms. Basal ATPase activity $\pm$ VPL (rows 1,2) are measured under optimum conditions (temp, [ATP], pH) in the absence of antimalarial drugs, as described previously [8] and numbers shown are avg of 6 measurements (see Methods) $\pm$ S.D. For row 3, activity was tested at [ATP] = 2.5, 5.0, 7.5 and 10.0 mM; for row 4 activity was tested at pH = 6.0 to 8.0 in 0.5 unit increments, and the [ATP] and pH at which highest activity was measured are then listed. For other rows, values were computed after curve fitting the relevant plotted variables as described in detail elsewhere [8]. VPL is verapamil, N/A denotes not analysed, as the measured activity in the presence of vanadate was too low to yield a statistically reliable calculation.

	3D7	Dd2	1034C	1042D	1246Y	1034C/ 1042D	1034C/ 1246Y	1042D/ 1246Y	TM (triple)	7G8
basal ATPase (μ mol Pi/mg/min)	44.04 $\pm$ 4.72	78.73 $\pm$ 8.88	27.17 $\pm$ 3.33	70.08 $\pm$ 5.49	92.69 $\pm$ 7.87	69.59 $\pm$ 8.67	56.94 $\pm$ 5.69	45.34 $\pm$ 5.24	14.07 $\pm$ 1.32	25.38 $\pm$ 4.23
ATPase with 2 μ M VPL	41.06 $\pm$ 4.46	52.69 $\pm$ 9.78	27.09 $\pm$ 0.86	61.56 $\pm$ 3.52	98.73 $\pm$ 9.86	78.05 $\pm$ 10.71	63.19 $\pm$ 7.20	34.79 $\pm$ 1.43	11.80 $\pm$ 1.54	25.38 $\pm$ 3.29
[ATP] optimum (mM)	5.0	5.0	5.0	5.0	7.5	7.5	2.5	7.5	7.5	5.0
pH optimum	7.0	7.5	7.0	7.5	7.0	7.0	7.0	7.0	7.0	7.0
V _{max} (μ mol Pi/mg/ min)	62.9	109.9	46.9	70.9	109.9	93.5	59.5	73.0	49.3	42.7
K _m (mM)	2.14	2.00	5.53	0.369	0.879	1.82	0.226	2.78	8.15	3.42
Vanadate IC ₅₀ (μ M)	2.25	4.00	4.36	4.55	4.36	7.00	3.65	4.29	N/A	1.25