Partial Purification and Properties of Adenosine Triphosphatase (ATPase) from Liver Fluke *Fasciola hepatica*

Karaciğer Kelebeği *Fasciola hepatica'* dan Adenozin trifosfataz (ATPaz)'ın Kısmi Saflaştırılması ve Özellikleri

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ABSTRACT

Objective: The adenosine triphosphatase (ATP phosphohydrolase, EC 3.6.1.3.;ATPase) is a membrane -bound enzyme which transport protons across the plasma membrane using ATP as an energy source.
Methods: The adenosine triphosphatase (ATPase ; EC: 3.6.1.3) was extracted from membrane preparations of adult *Fasciola hepatica* by chloroform treatment and purified by means of ammonium sulphate fractionation, gel filtration on sephadex G-200 and DEAE- Cellulose chromatography.
Results: The molecular weight was calculated to be 305.000 dalton by gel filtration. Kinetic experiments demonstrated a biphasic linear lineweaver - burk relationship (km=0.142 and 1.66 mM) thus revealing the existence of two substrate binding enzyme sites.
Conclusion: In our study revealed that partial inhibition of Mg^{2+} dependent purified enzyme by oligomycin suggest the absence of mitochondrial ATPase in *F. hepatica*. *(Turkiye Parazitol Derg 2014; 38: 26-31)*

Key Words: ATPase, *Fasciola hepatica*, gel filtration on sephadex G-200, DEAE- Cellulose chromatography
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ÖZET

Amaç: Adenozin trifosfataz (ATP fosfohidrolaz, EC 3.6.1.3, ATPaz) enerji kaynağı olarak ATP kullanarak plazma membranında proton taşıyan bir membran-bağlı enzimdir.
Yöntemler: Adenozin trifosfataz (ATPaz; EC: 3.6.1.3) yetişkin *Fasciola hepatica*'nın membran preparatlarından kloroform ile muamele edilerek ekstrakte edildi ve amonyum sülfat fraksiyonasyonu, sefadeks G-200 jel filtrasyonu ve DEAE-Selüloz kromatografisi vasıtasıyla saflaştırıldı.
Bulgular: Moleküler ağırlığı, jel filtrasyon ile 305000 dalton olarak hesaplandı. Kinetik deneyler, iki fazlı, doğrusal bir *Lineweaver-Burk* ilişkisini ortaya koydu (km=0.142 ve 1.66 mM), böylece subtrat bağlayan iki enzim bölgesinin varlığı gösterildi.
Sonuç: Çalışmamız ortaya koymuştur ki; oligomisin tarafından Mg^{2+} bağımlı saflaştırılmış enzimin kısmi inhibisyonu *F. hepatica*'da mitokondriyal ATPaz bulunmadığını düşündürmektedir. *(Turkiye Parazitol Derg 2014; 38: 26-31)*

Anahtar Sözcükler: ATPaz, *Fasciola hepatica*, Sefadeks G-200 jel filtrasyonu, DEAE-Selüloz kromatografisi
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INTRODUCTION

Fasciola hepatica is the species of parasitic flatworms that infects the liver of various mammals, including humans. The disease caused by the organism is called Fascioliasis or Fasciolosis. It is a parasitic flatworm of the class Trematoda, phylum Platyhelminthes that causes great economic losses in sheep and cattle.

The adenosine triphosphatase (ATP phosphohydrolase, EC 3.6.1.3.; ATPase) is a membrane -bound enzyme which transport protons across the plasma membrane using ATP as an energy source (1-4). It also plays a crucial role in oxidative phosphorylation upon the formation of ATP in biological membrane containing respiratory apparatus. Cell membrane ecto- ATPases are integral membrane glycoproteins that are millimolar divalent cation-dependent, low specificity enzymes that hydrolyze all nucleoside triphosphates. Their physiological role is still unknown. However, several hypotheses have been suggested such as; (i) protection from cytolytic effects of extracellular ATP, (ii) regulation of ectokinase substrate concentration, (iii) termination of purinergic signaling, (iv) involvement in signal transduction, and (v) involvement in cellular adhesion.

In contrasts to the large amount of information available about mitochondrial ATPase in mammals (5-8), bacteria (9, 10) and parasitic protozoa (11-15), little is known about this enzyme in parasitic helminthes .We have therefore report here the purification and properties of the ATPase from adult *Fasciola hepatica*.

METHODS

Organism: Adult *Fasciola hepatica* was collected from the bile duct of cattle freshly slaughtered at the local abattoir in Kirkuk and washed clean of host's tissue in normal saline.

Preparation of Crude Extract and Cellular Fractionation: All procedures were performed at 4°C. Crude homogenates of worms (1gm) were obtained as described previously after homogenized in a Potter-Elvehjem homogenizer (6) with 2 vols. of ice cold 50 mM Tris-HCl (pH7.2) containing 0.25M sucrose and 0.1 mM dithiothritol (TSD buffer). After centrifugation for 1 hr. at 105000g at 4°C, the material sediment was removed, resuspended in TDS buffer and used to investigate the subcellular localization of the enzyme.The adult worms were grounded in a chilled mortar with TSD buffer containing 0.2% Triton x-100 and subjected to differential centrifugation as previously described (16), yielding particle fraction P1 (2100g for 10 min.), P2 (15800g for 10min.) and P3 (240000 for 1 hr.). The four fractions produced were frozen and thawed three times to disrupt organelles, and were assayed for enzyme activity**.**

Partial purification of the ATPase:

1. Disruption of organisms: The worm was washed with 50 mM Tris-HCl (pH7.6) containing 0.25 M sucrose , 2.5 mM magnesium chloride and 2% bovine serum albumin (TSMA buffer) then suspended in the minimum volume of cold buffer , mixed with glass beads (75-150 µM dia.; 2g per g of worms wet wt.)and disrupted by grinding in a chilled mortar for 5 min. at 4°C as described by Frasch et al. (17), 1978.The homogenate was rapidly diluted with cold TSMA buffer and centrifuged at 800g at 4°C for 5 min. to remove the unbroken cells and glass beads .The homogenate was then centrifuged at 4500g at 4°C for 10 min. .The pellet was washed thrice and resuspended in TSMA buffer and used as membrane bound ATPase and as source for the solublilisation of the enzyme.

2. Chloroform extraction was performed as described by (18). The chloroform (2 vol.) was added to the particles suspended in TSMA buffer, thoroughly mixed and the precipitate protein eliminated by centrifugation for 2 min. at 500x g at room temperature. The supernatant was centrifuged at 114000xg for 60 min. at 10°C .The supernatant containing the solubilized ATPase was used for the purification of the enzyme.

3. Ammonium sulphat fractionation: The protein in the supernatant of the chloroform treatment was brought to 70% saturation by the slow addition of $(NH_4)_2$ SO_2 and after 10 min., the suspension was centrifuged at 27000 x g for 15 min. The precipitate was dissolved in 10 mM Tris-HCl (pH7.6) containing 1.0 mM EDTA (TE buffer).

4. Gel filtration on Sephadex G-200: The protein solution obtained above was applied to a column (1.6×34 cm) of sephadex G-200 previously equilibrated with TE buffer and the elution was accomplished with the same buffer at a flow rate of 8 ml / hr. Fractions of 4 ml were collected.

5. Column chromatography on DEAE- Cellulose: The active fractions from the sephadex G- 200 column were pooled and applied to a column (1.3×45cm)of DEAE- cellulose equilibrate with the same TE buffer and elution was preformed with TE buffer containing 10,20,25, mM KCl. The active fractions were pooled, concentrated by precipitation with ammonium sulphate at 70% saturation and used for the experiments described.

6. Molecular weight determination: Enzyme molecular weight was estimated by gel filtration on sephadex G-200 (column 1.6×34cm) equilibrated in TE buffer(19), using Ovalbumin (43000), bovine serum albumin (67000), lactate dehydrogenase (140000), catalase (248000), Ferritin (440000) as protein markers, under experimental conditions similar to those of the Sephadex step of the purification procedure. The void volume of the column was determined with Blue Dextran 2000.

Enzyme assay: ATPase activity was conducted at 37°C in a 1 mL reaction volume of 50 mM Tris-HCl buffer (pH7.2), 1.0 mM $MgCl_2$, 1.0 mM ATP, and sufficient enzyme to yield an appropriate reaction rate. Assays were terminated after 30 min. by the addition of 1 mL of trichloro acetic acid 10% (w/v) and the resulting mixture was centrifuged at 2000 g for 10 min. at 4°C. Liberated inorganic phosphate (Pi) was determined by the method of Fisk and Subbarow (20). One unit of ATPase activity is defined as the amount of enzyme which hydrolyses 1.0 µmol of ATPase per min. per mg protein.

Protein determination: Protein concentration were estimated by the method of Lowery et al. (21) with bovine serum albumin as standard.

Statistical Analysis: All data expressed as mean from triplicate experiments.

RESULTS

The activity of ATPase recovered in the various cell fractions of *F. hepatica* is shown in the Tables 1 and 2. ATPase was recovered in all fractions but the highest amount were in fractions P_1, P_2 and P_3. By the same procedure, succinate dehydrogenase which is known to be a particulate enzyme was recovered exclusively in the pellet fraction. It was concluded that the ATPase is particulate enzyme.

The purification of ATPase from *F. hepatica* is summarized in Table 3.

The yield of the chloroform extraction was consistently higher than 100%; this might be due to some latency of the ATPase activity in the particles. The solubilized enzyme by precipitation with ammonium sulphate was found to be efficient to eliminate the fat and contaminating proteins. Although precipitation with ammonium sulphate made it possible to concentrate the solubilized enzyme into a small volume with no effect on the enzyme specific activity. The enzyme was further purified with Sephadex G-200 and DEAE-Cellulose to eliminate the trace amount of contaminated protein. It has been shown that about 55% of the solubilized enzyme was recovered after gel filtration on Sephadex G-200 and about 84% of this fraction were eluted from DEAE-Cellulose column chromatography by KCl. The enzyme was purified by a factor of 112 with specific activity of 107 µ mol/min./mg protein. The molecular weight of the native enzyme determined by gel filtration on Sephadex G-200 was about 305 000 dalton (Figure 1).

Preliminary assay of ATPase was conducted to ensure that the reaction rate (liberation of inorganic phosphate) was a linear function of assay time and protein concentration in the assay mixture; under such conditions these reaction rates should provide a reasonable estimate of initial reaction velocities.

The pH optimum of ATPase activity was between (7.5- 9.0) and further ATPase assays were conducted at this pH. Under these assay conditions, the ATPase activity displayed a biphasic double reciprocal plot which allowed the calculation of two different Km values, namely 0.142 and 1.66 mM (Figure 2).

In the absence of divalent cations the purified ATPase displayed significant activity. The ATPase activity was inhibited nearly 100% when EDTA was added to the assay mixture (Table 4). The inhibition of ATPase activity by EDTA might be due to the presence of endogenous divalent cations in the crude homogenate or due to contamination of assay mixture with divalent cations from an unbroken source. It is evident (Table 4) that the ATPase activity was neither inhibited nor stimulated by the addition of Na^+ and K^+ (Na^+/K^+ ratio 5/1) to the assay mixture. Also, in the presence and absence of Na^+/K^+ the ATPase activity was not inhibited by 1Mm Ouabin (Table 4).

Table 1. Cellular fractionation of ATPase from *Fasciola hepatica*

Fractions	Total Volume (mL)	Specific activity µmol/min/mg protein	Total activity	Activity %
Crude homogenate	20	0.810	649	
Pellet (p)	17	0,567	386	60
Supernatant	15	0.435	261	40

Table 2. Distribution of ATPase in subcellular fractions of *Fasciola hepatica*

Fractions	Total volume (mL)	Activity*	Total activity	% Recovered activity
Crude homogenate	10	1.90	760	
Pellet (p1)	8	1.16	371.26	27
Pellet (p2)	7	1.27	355.6	26
Pellet (p3)	6	1.30	364	27
Supernatant	6	0.10	364	20

*The activities given are in µmol of inorganic phosphate liberated from hydrolysis of ATP

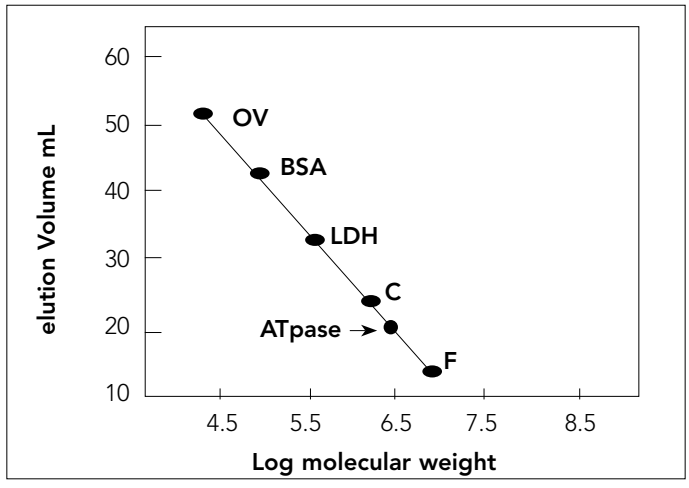

Figure 1. Estimation of the molecular weight of ATPase by gel filtration on Sephadex G-200. Standard proteins F=Ferritin; BSA=Bovine Serum albumi; OV=Ovalbumin; C=Catalase; LDH= Lactate dehydrogenase. The elution volume determined by Blu dextran 2000

Table 3. Purification of the ATPase from *Fasciola hepatica*

Step	Total protein (mg)	Total activity µmol /min	Specific activity µmol/min./mg protein	Purification fold	Yield %
800-4500x	23 700	22.6	0.953	1	100
Chloroform extraction	13 600	30.8	2260	2.30	136
Ammonium sulphate precipitation	10 400	28.6	2750	2.80	127
Sephadex G-200	0.203	15.7	77 300	81.10	69
DEAE-cellulose chromatography	0.123	13.1	106 500	111.3	58

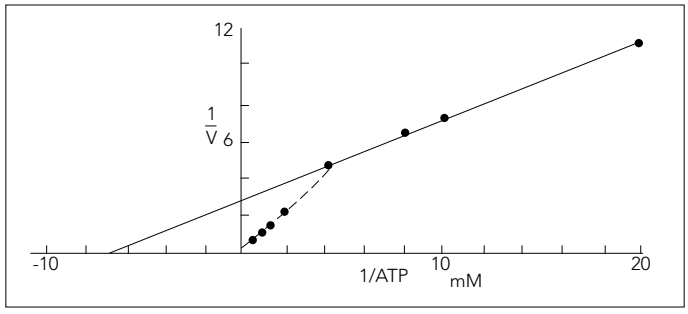

Figure 2. Lineweaver-Burke plot of the effect of substrate concentration (ATP) on purified ATPase. The reaction mixture contains Tris-HCl 50 mM, pH8.0, Mg Cl2 5 mM, ATP(0.05-20) mM

Table 4. The effects of various potential effectors on ATPase activity of *Fasciola hepatica*

	Assay mixture	ATPase Activity*
1.	Exract +1mMATP	48
2.	+ 2.5 m M EDTA	0.9
3.	+ Mg^{+2}	163.2
4.	+ Ca^{+2}	87.7
5.	+ Mn^{+2}	120.4
6.	+ Ca^{+2}, Mg^{+2}	163.6
7.	Mg^{+2}, Mn^{+2} +	109.5
8.	+ Ca^{+2}, Mn^{+2}	131.3
9.	+50 mM Na^{+} +10mM K^{+}	48.1
10.	+50 mM Na^{+} +10mM K^{+}	48
11.	+ 50mM Na^{+} +10mM K^{+} +1mM ouabain	48.2
12.	+ Mg^{+2} +10 mM fluorid	159.4
13.	+ Mg^{+2} +10 mM Molybdate	38.2

*The activities given are in µmol of inorganic phosphate liberated from hydrolysis of ATP, and values represent the mean of triplicate determinations using a single purified enzyme

Despite the significant ATPase activity in the absence of divalent cations, the addition of Mg^{2+}, Mn^{2+} or Ca^{2+} to the assay mixture activated the ATPase activity of *F. hepatica* (Figure 3). At low concentrations, all three divalent cations, increased ATPase activity with Mg^{2+} being the most effective activator.

On the other hand, at higher concentrations (>10mM) Mg^{2+} and Mn^{2+} were inhibitory while Ca^{2+} resulted in steady increasing ATPase activity (Figure 3). As shown in (Table 4), ammonium molybdate was found to be effective inhibitor of ATPase activity while sodium fluoride did not. Figure 4 shows the inhibition of ATPase activity by the antibiotic oligomycin. The maximum inhibition was about 53%.

DISCUSSION

The present investigation has shown that in several respects the ATPase of *F .hepatica* appear to be similar to those of parasitic protozoa (1, 2, 4, 11) and parasitic helminthes (15, 22, 23). The recovery of a significant proportion of ATPase activity in the particulate fractions suggest that this enzyme may be associated with

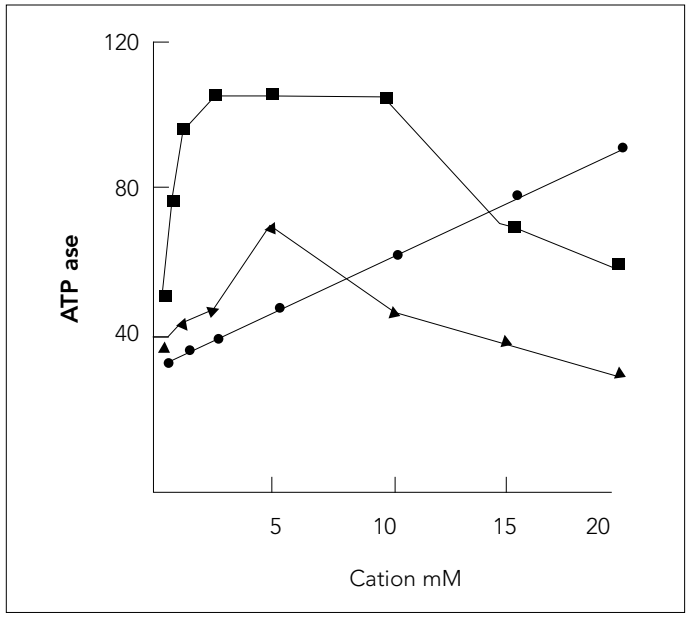

Figure 3. Effect of divalent cations on ATPase activity in *Fasciola hepatica*
Reaction mixture contains Tris-HCl 50 mM , pH8.0, ATP 1 mM, and divalent cations (0.1-20) mM Mg^{2+} (■), Ca^{2+}(●) , Mn^{2+} (▲)

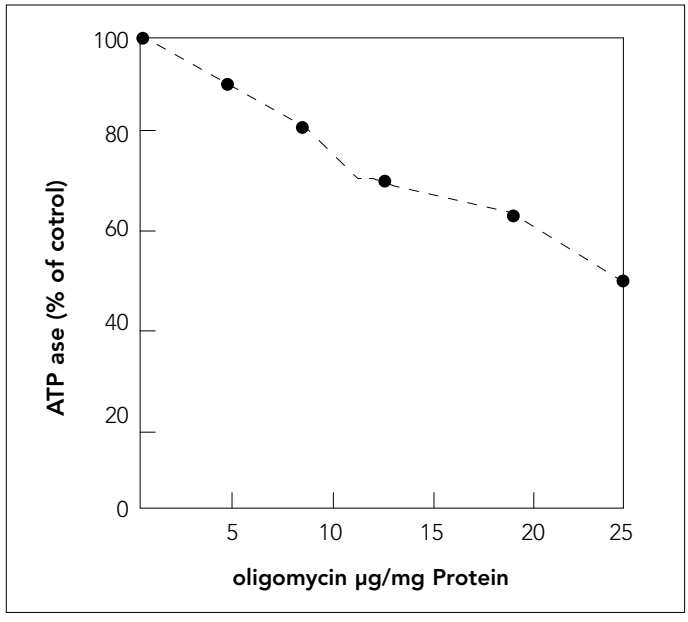

Figure 4. Inhibition of ATPase by Oligomycin in *Fasciola hepatica*

tegument as has been reported in the tapeworm *Hymenolepis dimiuta* (22). The ATPase of *F. hepatica* appear to function in the hydrolysis of nucleotide and the resultant nucleotide could be further catabolized by the surface located 5¢- nucleatidase (unpublished data) to yield nucleotides, which could be taken into the cells more easily and so it is possible that the ATPase may play a part in the interaction of the parasite with its host.

The specific activity of purified ATPase in this investigation was considerably higher than most ATPase attained values available from other parasite such as *Trypanosoma cruzi*; *Crithidia fasiculata* (2, 24). The molecular weight of ATPase from *F. hepatica* are

shown to be fairly consistent with those reported for other ATPase, among them the enzymes from *T. cruzi*[2] and *C. fasiculata* (24).The calculation of the different Km value for purified *F. hepatica* are very similar to those reported for the purified *T. cruzi* enzyme (25). The activity of *F. hepatica* ATPase is activated by cations and results showed that the effect of these cations(Ca^{2+}, Mn^{2+} , Mg^{2+}) are not additive, thus indicating the presence of single enzyme rather than there being multiple enzymes each specific for a single divalent cation.

Comparison of the ATPase activity reported in this study with that of other animal cell membranes indicates that the ATPase in the *F. hepatica* dose not resemble the more intensively studied ATPases. The ouabain insensitivity of the enzyme (in the absence or present of added Na^+ and K^+) in the *F. hepatica* suggest that it is not due to Na^+ - K^+ activated ATPase. In contrast, the existence of two (Na^+, K^+) ATPase isoforms has been reported in the related trematode *Scistosoma mansoni* (26). The absence of Ca^{2+} activation also indicate the absence of Ca^{2+} transport function usually associated with the Ca^{2+} activated, Mg^{2+}-dependent ATPase(23). Unlike mitochondria Mg^{2+} ATPase (24) the ATPase of *F. hepatica* was only partially sensitive to oligomycin at high inhibitor concentrations. The small inhibition by oligomycin may be due to contamination of membrane fraction with mitochondria and so it is possible to suggest that the origin of the major portion of ATPase activity in this was not mitochondrial. The result of this study firmly provided the first direct evidence for the existence of Mg^{2+} - dependent ATPase in *F. hepatica*, a fact which is of great interest from the phylogenetic point of view.

CONCLUSION

In our study revealed that partial inhibition of Mg^{2+} dependent purified enzyme by oligomycin suggest the absence of mitochondrial ATPase in *F. hepatica*.

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Informed Consent: N/A

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REFERENCES

1. Jesus JB, Lopes AHCS, Meyer-Fernandes JR. Characterization of an ecto-ATPase of Tritrichomonas foetus. Vet Parasitol 2002; 103: 29–42. [CrossRef]
2. Meyer-Fernandes JR, Saad-Nehme J, Peres-Sampaio CE, Belmont-Firpo R, Bisaggio DF, Do Couto LC, et al. A Mg-dependent ecto-ATPase is increased in the infective stages of Trypanosoma cruzi. Parasitol Res 2004; 93: 41-50. [CrossRef]
3. Cortes VF, Veiga-Lopes FE, Barrabin H, Alves-Ferreira M, Fontes CF. The gamma subunit of Na+, K+-ATPase: role on ATPase activity and regulatory phosphorylation by PKA. Int J Biochem Cell Biol 2006; 38: 1901-13. [CrossRef]
4. Borges FP, Gottardi B, Stuepp C, Larré AB, Tasca T, De Carli GA, et al. Characterization of an ecto-5'-nucleotidase (EC 3.1.3.5) activity in intact trophozoites of Trichomonas gallinae. Vet Parasitol 2007; 143: 106-11. [CrossRef]
5. Glynn I. The Na+, K+. transporting adenosine triphosphates. Martonosi, AN, editor. The enzymes of biological membranes. Volume 3: Membrane Transport. New York: Springer; 1989. p. 28-114.
6. Robinson JD. Modification of ligand to the Na+/K+ -activated ATPase. Biochem Biophs Acta 1989; 997: 41-8. [CrossRef]
7. Kurihara K, Hosoi K, Kodama A, Ueha T. A new elecrophoraretic variant of subunit of Na+/K+ -ATPase from the submandibular gland of rats. Biochem Biophs Acta 1990; 1039: 234-41. [CrossRef]
8. Ohkohchi N, Shibuya H, Seya K, Satomi S, Taguchi Y, Mori S. New evaluation method for viability of mitochondria in liver graft using fluorescent dye. Transplantation Proceedings. 1993; 25: 3210-2.
9. Cook GM, Keis S, Morgan HW, von Ballmoos C, Matthey U, Kaim G, et al. Purification and Biochemical Characterization of the F1Fo-ATP Synthase from Thermoalkaliphilic Bacillus sp. Strain TA2.A1. J Bacteriol 2003; 185: 4442-9. [CrossRef]
10. Sun Y, Fukamachi T, Saito H, Kobayashi H. ATP requirement for acidic resistance in escherichia coli. J Bacteriol 2011; 193: 3072-7. [CrossRef]
11. Meyer-Fernandes JR. Ecto-ATPases in protozoa parasites: looking for a function. Parasitol Inter. 2002; 51: 299-303. [CrossRef]
12. Meyer-Fernandes JR, Saad-Nehme J, Peres-Sampaio CE, Belmont-Firpo R, Bisaggio DF, Do Couto LC, et al. A Mg-dependent ecto-ATPase is increased in the infective stages of Trypanosoma cruzi. Parasitol Res 2004; 93: 41-50. [CrossRef]
13. Elandalloussi LM, Adams B, Smith PJ. ATPase activity of purified plasma membranes and digestive vacuoles from Plasmodium falciparum. Mol Biochem Parasitol 2005; 141: 49-56. [CrossRef]
14. de Sá Pinheiro AA, Cosentino-Gomes D, Lanfredi-Rangel A, Ferraro RB, De Souza W, Meyer-Fernandes JR. Giardia lamblia: Biochemical characterization of an ecto-ATPase activity. Exp Parasitol 2008; 119: 279–84. [CrossRef]
15. Moore CM, Hoey EM, Trudgett A, Timson DJ. A plasma membrane Ca2+ -ATPase (PMCA) from the liver fluke, Fasciola hepatica. Int J Parasitol 2012; 42: 851-8. [CrossRef]
16. Skou JC. The identification of the sodium-pump as the membrane-bound Na+/K+-ATPase: a commentary on 'The Influence of Some Cations on an Adenosine Triphosphatase from Peripheral Nerves'. Biochem Biophs Acta 1980; 1000: 435-8. [CrossRef]
17. Frasch AC, Cazzulo JJ, Stoppani AO. Solubilization and some properties of the Mg2+-activated adenosine triphosphatase from Trypanosoma cruzi. Comp Biochem Physiol 1978; 618: 207-12.

18. Stryer L. Biochemistry. San Francisco: W.H. Freeman and company; 1982.
19. Vieira L, Slotki I, Cabantchik ZI. Chloride Conductive Pathways Which Support Electrogenic H+ Pumping by Leishmania major Promastigotes . J Biol Chem 1995; 270: 5299-304. [CrossRef]
20. Fisk C, Sabbaraw Y. The colorimetric determination of phosphorous. J Biol Chem 1925; 66: 3254-400.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-75.
22. Wani HJ, Srivastava VML. Ca2+/Mg2+ -dependent ATPase activity in Hymenolepis diminuta mitochondria. Vet Parasitol 1995; 58: 17-26. [CrossRef]
23. Cunha VMN, Reis JMA, Noël F. Evidence for the presence of two (Ca2+-Mg2+) ATPases with different sensitivities to thapsigargin and cyclopiazonic acid in the human flatworm Schistosoma mansoni. Comp Biochem Physiol B 1996; 114: 199-205. [CrossRef]
24. Higa IA, Cazzulo JJ. Mg2+-Activated adenosine triphosphatase from Crithidia fasciculata: Purification and inhibition by suramin and efrapeptin. Mol and Bioch Parasitol 1981; 3: 357-67. [CrossRef]
25. Cataldi de Flombaum MA, Stoppani AO. High-affinity calcium-stimulated, magnesium-dependent adenosine triphosphatase in Trypanosoma cruzi. Comp Biochem Physiol B 1992; 103: 933-7. [CrossRef]
26. Noël F, Soares de Moura R. Schistosoma mansoni: Preparation, characterization of (Na+ + K+) ATPase from tegument and carcass. Experimental Parasitol 1986; 62: 298-307. [CrossRef]