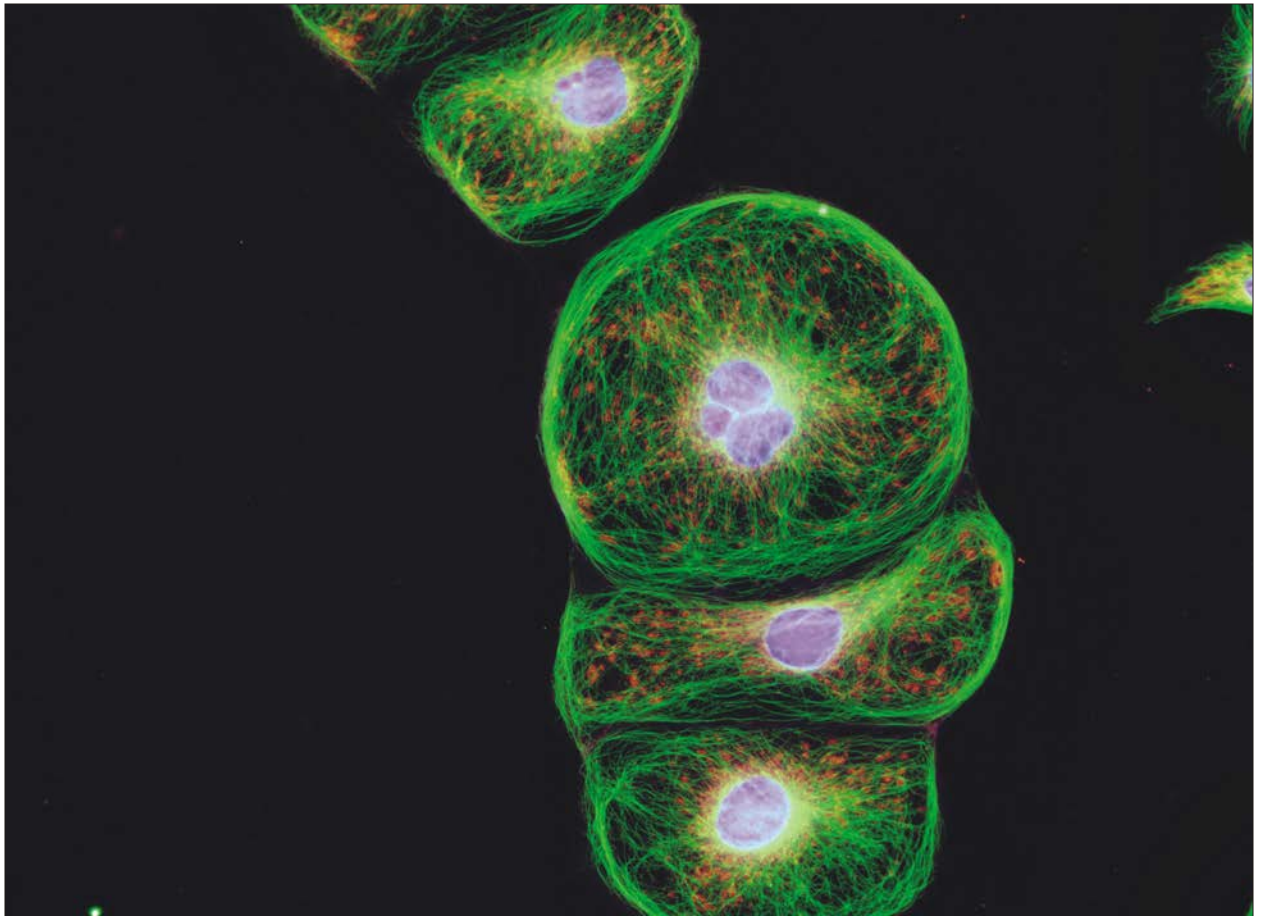




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**Comparative of Effect of Inhibitors on the ATPases from the
Excretory System of the Usherhopper, *Poekilocerus bufonius*
and Desert Locust, *Schistocerca gregaria***

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Abstract: The bafilomycin A₁ and *N*-ethylmaleimide (NEM)-sensitive (V-type) ATPase was partially purified from the apical membranerich fractions of excretory system (Malpighian tubules and hind gut) of *Poekilocerus bufonius* and *Schistocerca gregaria*. Enzymatic activity was inhibited by bafilomycin A₁ (IC₅₀ = 0.48 and 1.3 μM for *S. gregaria* and *P. bufonius*, respectively) and NEM (IC₅₀ = 10.1 and 2.8 μM of *P. bufonius* and *S. gregaria*, respectively). The V-type ATPase activity is confined to the apical membrane fraction, while the activity of Na⁺/K⁺ -ATPase forms the major part of the basal membrane fraction of both insects. The chloride salts also caused an increase in activity in the following ascending order: RbCl, LiCl, choline Cl, NaCl, KCl and *tris*-HCl of both insects. The present results show that the properties of V-type ATPase of *P. bufonius* and *S. gregaria* are similar to those reported for other insect tissues.

Key words: *Poekilocerus bufonius*, *Schistocerca gregaria*, excretory system, V-type ATPase, Na⁺/K⁺ -ATPase, bafilomycin A₁, *N*-ethylmaleimide

Introduction

It has been proposed that in *Locusta* Malpighian tubules an electrogenic (Na⁺/K⁺)- ATPase energizes the basolateral membrane by the active efflux of Na⁺ into the haemolymph, enabling the cotransport of Na⁺ and K⁺ into the cell by a loop diuretic-sensitive Na⁺/K⁺/2Cl⁻ cotransporter (Baldrick *et al.*, 1988). Such a cotransporter has been identified on the basolateral membrane Malpighian tubules of *Manduca sexta* (Klein *et al.*, 1991) and indirect evidence exists for its presence in *Locusta* (Fogg *et al.*, 1991; Hopkin *et al.*, 1999; 2001). Further, a basolateral conductance for K⁺ may be present on the basal membrane, since the permeability of this cation is considerably greater than that of Na⁺ (Morgan and Mordue, 1983).

One of the characteristic features of the (Na⁺/K⁺)- ATPase is its inhibition by ouabain and other cardiac glycosides (Keynes, 1973; Anstee and Bowler, 1984; Al-Robai *et al.*, 1990; 1993b). However, it has been reported that some insect species are able to ingest and store cardiac glycosides, to act as a deterrent to predators (Duffy and Scudder, 1972; Martel and Malcolm, 2004). Indeed, the Malpighian tubules of *Oncopeltus fasciatus* are able to concentrate ouabain (Maddrell, 1977). The usherhopper, *Poekilocerus bufonius*, is known to feed on the usher milkweed, *Calotropis procera*, which is rich in cardiac glycosides (Euw *et al.*, 1967). The (Na⁺/K⁺)- ATPase of the excretory system of *P. bufonius* is considerably less sensitive to ouabain than that of *Schistocerca gregaria*, a non-cardenolide ingesting species (Al-Robai *et al.*, 1990).

Harvey *et al.* (1983) initially suggested that a K⁺-activated ATPase might transport K⁺ across the apical membrane and subsequent investigations suggest that secondary active transport of K⁺ into the tubule lumen occurs via a K⁺/nH⁺ antiporter driven by a V-type ATPase-generated proton gradient

(Bertram *et al.*, 1991; Wieczorek *et al.*, 2000; for additional references, see Al-Fifi *et al.*, 1998a). A similar, dedicated Na^+/H^+ exchanger may be present in *Locusta* tubule cells (Pivovarova *et al.*, 1994; Hopkin, 2001) though a common cation antiporter has not been ruled out (Al-Fifi *et al.*, 1998b). In view of the physiological importance of the Malpighian tubule apical membrane V-type ATPase and the unusual insensitivity of the usherhopper basolateral (Na^+/K^+)-ATPase to ouabain, the aim of the following study was to investigate the biochemical properties of a putative V-type ATPase in the apical membrane fraction of the excretory system of *P. bufonius*.

A number of recent studies have reported the presence of V-type H^+ -ATPase activity at the apical surface of the membrane of epithelial cells (Klein *et al.*, 1991; Garayoa *et al.*, 1995; Al-Fifi *et al.*, 1998a; 2002; O'Donnell and Spring, 2000). The properties of the enzyme, in the all species studied to date, support that the enzyme create a proton motive force across the apical membrane and acts in parallel with a K^+/H^+ antiporter to affect the active transport of K^+ across the apical membrane which in turn is the driving force for fluid secretion (Al-Fifi *et al.*, 1998a, b; Hopkin *et al.*, 1999).

The activity of V-type H^+ -ATPase has been measured at the apical membrane of the insect epithelial cells from different species (Harvey, 1992; Klein *et al.*, 1991; Garayoa *et al.*, 1995; Al-Fifi *et al.*, 1998a, 2002; Wieczorek *et al.*, 1999b; O'Donnell and Springk, 2000). It has been stated that the V-type H^+ -ATPase creates a Proton Motive Force (PMF) across the apical membrane (Schweikl *et al.*, 1989; Wieczorek *et al.*, 1989; 1991; 1999a; 2000; Wieczorek, 1992; Zhang *et al.*, 1994; Harvey *et al.*, 1998). The PMF acts in parallel with a K^+/H^+ antiporter to affect the active transport of K^+ across the apical membrane, which in turn is the driving force for fluid secretion (Harvey *et al.*, 1983; O'Donnell *et al.*, 1996; Hopkin *et al.*, 1999). The activity of the V-type H^+ -ATPase and hence of cation transport, is controlled hormonally (Davies *et al.*, 1995; Hopkin *et al.*, 1999; Al-Fifi *et al.*, 1998b; O'Donnell and Spring 2000; Coast *et al.*, 2001). The insect physiologist sees the enzyme as an energizer of plasma membranes; the transmembrane voltage it generates drives nutrient uptake and fluid secretion, in some cases alkalizing the gut lumen (Harvey *et al.*, 1998; Wieczorek *et al.*, 1999b; Grüber *et al.*, 2001).

It has been reported that insects feeding on plants high in K^+ were shown to be insensitive to ouabain (Jungreis and Vaughan, 1977; Moore and Scudder, 1986). The usherhopper, *Poecilocus bufonius*, in nature probably feeds exclusively on toxic plants, one of which is *Calotropis procera* (Euw *et al.*, 1967; Duffey, 1980; Al-Robai *et al.*, 1998). This plant contains cardiac glycosides (Al-Robai *et al.*, 1998) and a minute amount of its latex is toxic to both vertebrates and invertebrates (Detweiler, 1967; Mahmoud *et al.*, 1979a, b; Al-Robai *et al.*, 1993a, b; Al-Robai, 1997).

It has been previously investigated that the activity of Na^+/K^+ -ATPase in the microsomal preparations of the excretory system (Malpighian tubules and hindgut) and mid gut of usherhopper and other species was resistant to inhibition by ouabain (Al-Robai *et al.*, 1990; Al-Robai, 1993; Mebs *et al.*, 2000, 2005). Al-Fifi *et al.* (2002) recently, reported that the properties of V-type H^+ ATPase of the excretory system of usherhopper, *Poecilocus bufonius*, are similar to those reported for other insect tissues.

Materials and Methods

Mature adult usherhoppers *Poecilocus bufonius* (Klug) were collected from Gizan area. They were kept in cages in the department of biological sciences at $28 \pm 1^\circ\text{C}$, with very access to branches of fresh *Calotropis procera*. Mature adult locusts, *Schistocerca gregaria*, were used and these were taken from a population maintained under crowded conditions at $28 \pm 1^\circ\text{C}$ and 60% relative humidity. The photoperiod was 12 h light: 12 h dark.

Preparation of Membrane Microsomes

Animals were killed by decapitation and the excretory system, comprising of the Malpighian tubules and the hindgut, was removed and the contents discarded. Tissue from approximately 30 animals was added to 10 mL of ice-cold homogenization medium (250 mM sucrose and 5 mM Tris-

HCl buffer, pH 7.5). All subsequent steps were carried at 4°C. Homogenization was performed in a glass homogenizer with a Teflon pestle (clearance 0.1-0.15 mm) with 20 passes of the plunger at 1000 revs/min. Membrane microsomes were then isolated by differential centrifugation according to the protocol of Al-Fifi *et al.* (1998a).

Assay of ATPases Activity

The ATPase activity of the isolated membrane fractions was measured according to the method of Al-Fifi *et al.* (1998a). Briefly, each incubation medium, containing 250 μ L of an appropriate ionic medium (see below) and 125 μ L of membrane preparation, was equilibrated in a waterbath for 15 min at 35°C. The assay was initiated by the addition of 125 μ L of 12 mM ATP (Tris salt) and run for 30 min at 35°C. ATP (Tris salt) concentrations were varied between 0 and 4 mM in assays employed to elucidate the effect of ATP concentration on V-type ATPase activity. One thousand microliter of a 1:1 mixture of 1% Lubrol and 1% ammonium molybdate in 0.9 M sulphuric acid was used to halt the reaction (Atkinson *et al.*, 1973), after which the tubes were left for 10 min at room temperature to allow colour development to occur. Following centrifugation at 10000 rpm for 10 min, absorbancy was measured at 390 nm. The intensity of the yellow colour developed was proportional to the amount of inorganic phosphate in the assay. Thus, enzyme activity was measured by determining the amount of inorganic phosphate released. The following ionic media were used to assay different ATPase activities (final concentrations):

Assay of Na⁺/K⁺-ATPase Activity

(1) 4 mM MgCl₂; (2) 4 mM MgCl₂, 100 mM NaCl and 20 mM KCl; (3) 4 mM MgCl₂, 100 mM NaCl, 20 mM KCl plus 1 mM ouabain. Each medium contained 20 mM imidazole/HCl (pH 7.2). Na⁺/K⁺-ATPase activity was determined as the difference in the amount of inorganic phosphate liberated in the presence of ionic medium 2 and 3.

Assay of V-Type ATPase Activity

This was performed as described by Schweikel *et al.* (1989), by assaying the azide- and orthovanadate-insensitive ATPase activity of the membrane fraction (Al-fifi *et al.*, 1998a, 2002). The ionic media used were; (1) 1 mM MgCl₂, 20 mM KCl, 50 mM Tris-MOPS, 0.1 mM EGTA, 1 mM 2-mercaptoethanol, 0.5 mM NaN₃, 0.1 mM sodium orthovanadate, 0.3 mg BSA/mL and 0.05% Triton X-100 (pH = 7.5); (2) Medium (1) plus *N*-ethylmalimide (NEM) (0.0-1.0 mM); (3) Medium (1) plus bafilomycin A₁ (0.0-1.0 mM).

Additionally, the effect of various salts on V-type ATPase activity was determined in the presence of 1 mM MgCl₂, 5 mM Tris-HCl (pH 7.5), 0.1 mM EGTA, 0.5 mM NaN₃ and 0.1 mM Na₃VO₄. The salts used included monovalent chlorides (K⁺, Na⁺, Rb⁺ and Li⁺) and choline chloride and anion salts of K⁺ (SO₄²⁻, F⁻, Br⁻, gluconate and NO₃⁻). All the above salts were used at a concentration of 30 mM.

V-type ATPase activity was also examined over a pH range of 6.0 to 10.0. The reaction medium used contained 50 mM Tris-MOPS, 20 mM KCl, 1 mM EGTA, 0.5 mM NaN₃, 0.1 mM Na₃VO₄, 0.05% Triton X-100 and 0.3 mg mL⁻¹ BSA (pH 7.5), allied to a 30 mM Bis-Tris Propane buffer system.

Appropriate controls were used to determine the extent of non-enzymatic hydrolysis of ATP. All ATPase activities are expressed in nmoles Pi liberated mg protein⁻¹ min⁻¹.

Estimation of Protein Concentration

Protein concentrations were determined according to the method of Lowry *et al.* (1951), with Bovine Serum Albumin (BSA) fraction V as the standard.

Reagents

All solutions were prepared in glass-distilled deionised water. All inorganic salts were AnalaR grade or the purest commercially available. Bafilomycin A₁ was dissolved in DMSO before adding it to the appropriate solution and the final concentration of DMSO in the experimental solution was <0.1%. The same concentration of solvent was included in the controls. All reagents were purchased from Sigma, St. Louis, MO.

Results

ATPases Distribution of the Apical and Basal Membranes

The present extensive studies on the distribution of ATPases (Total ATPase, Mg²⁺-dependent ATPase, Na⁺/K⁺-ATPase and V-type ATPase) activity of the basal membrane fraction (P₄), in Fig. 1 and apical membrane fraction (P₅), in Fig. 2, of *S. gregaria* and *P. bufonius* excretory system. The results indicate that the four types of enzyme activities are found in both basal and apical membrane fractions. However, the activity of V-type ATPase is confined to the apical membrane fraction, while that of Na⁺/K⁺-ATPase and NEM sensitive (V-type) ATPase activities in the basal and apical membrane fractions of *P. bufonius* and *S. gregaria* are summarized in Table 1. V-type ATPase activity was found to be greatest in the apical membrane fractions and was 1897.2±137.6 nmoles Pi liberated

The Na⁺/K⁺-ATPase and NEM sensitive (V-type) ATPase activities in the basal and apical membrane fractions of *P. bufonius* and *S. gregaria* are summarized in Table 1. V-type ATPase activity was found to be greatest in the apical membrane fractions and was 1897.2±137.6 nmoles Pi liberated

Table 1: Distribution of ATPases activity in the Apical and Basal Membrane fractions obtained from the excretory system of *S. gregaria* and *P. bufonius*

Insect	Membrane fractions	ATPases activity *(nmoles Pi liberated mg Protein ⁻¹ min ⁻¹)	
		Na ⁺ /K ⁺ -ATPase	V type -ATPase
<i>S. gregaria</i>	Basal membrane fraction (P ₄)	562.6±112.8	301.3±89.4
	Apical membrane fraction (P ₅)	58.1±13.4	2087.9±155.6
<i>P. bufonius</i>	Basal membrane fraction (P ₄)	539.8±119.3	277.1±89.4
	Apical membrane fraction (P ₅)	39.7± 9.4	1897.2±137.6

*Each point represents the mean±SEM (n = 3)

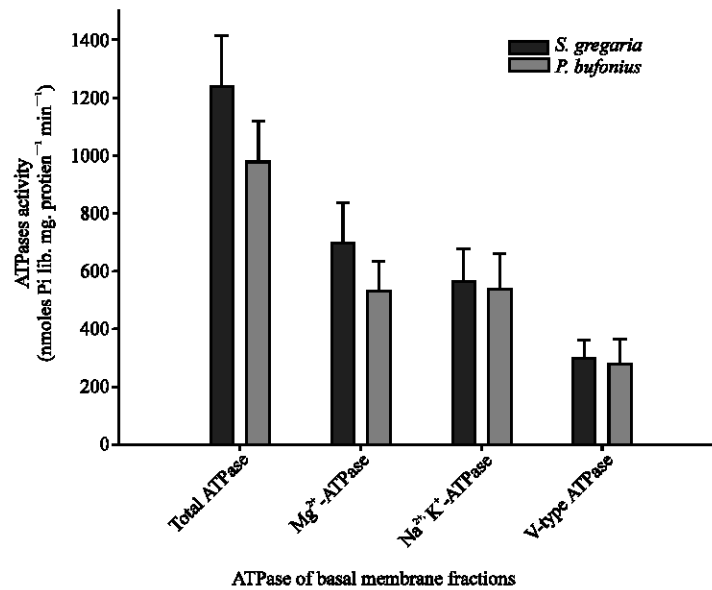


Fig. 1: Distribution of ATPases activity from basal membrane fraction of *S. gregaria* and *P. bufonius*. (Each point represents the mean ±SEM n = 3)

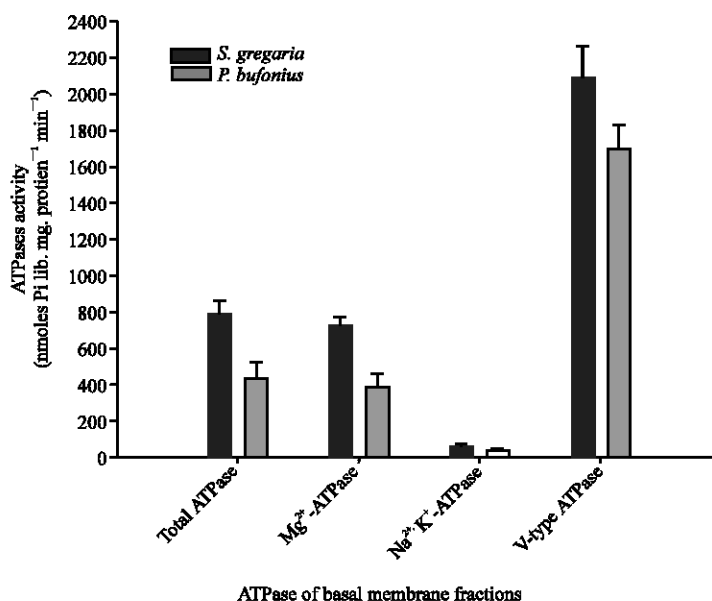


Fig. 2: Distribution of ATPases activity from apical membrane fraction of *S. gregaria* and *P. bufonius*. (Each point represents the mean $\pm$ SEM n = 3)

mg protein⁻¹ min⁻¹ of *P. bufonius* and 2087.9 $\pm$ 155.6 nmol Pi liberated mg protein⁻¹ min⁻¹ of *S. gregaria*. In contrast, Na⁺/K⁺-ATPase activity was highest in the basal membrane fraction, the maximum activity being 562.6 $\pm$ 112.8 and 539.8 $\pm$ 119.3 nmol Pi liberated mg protein⁻¹ min⁻¹ of *S. gregaria* and *P. bufonius*, respectively. The low activities of Na⁺/K⁺-ATPase and V-type ATPase activities in the apical and basal fractions respectively may be due to contamination.

Effect of NEM concentration on V-type ATPase

Figure. 3 and 4 show the effect of different concentrations of NEM (0.0-1 mM) on the V-type ATPase of *P. bufonius* and *S. gregaria*, respectively. The sensitivity of V-type ATPase to NEM in which residual activity plotted as a percentage of the inhibited rate against the -log₁₀ NEM concentrations. The concentration resulting in 50% inactivation of V-type ATPase activity (IC₅₀) was 10.1 and 2.8 μ M of *P. bufonius* and *S. gregaria*, respectively. It is clear that V-type ATPase activity in the apical fraction of both insects are highly sensitive to NEM.

Effect of Bafilomycin A₁ Concentration on V-type ATPase

The V-type ATPase Activity was measured in incubation medium (1 mM MgCl₂, 20 mM KCl, 50 mM Tris-MOPS, 0.1 mM EGTA, 5 mM NaN₃, 0.1 mM Na₃VO₄ and 0.3 mg mL⁻¹ BSA, pH 7.5) containing different concentrations of Bafilomycin A₁ (0.0-1000 nM). The enzyme activity of the excretory system of *P. bufonius* (Fig. 5) and *S. gregaria* (Fig. 6) was extremely sensitive to Bafilomycin A₁. However, the enzyme of *P. bufonius* was more tolerant to Bafilomycin A₁. The enzyme activity was reduced by approximately 19 and 7% of that of the controls of *P. bufonius* and *S. gregaria*, respectively, when 10⁻⁸ M Bafilomycin A₁ included in incubation medium. Almost total inhibition of V-type ATPase activity was obtained 10⁻⁷ M Bafilomycin A₁. The calculated IC₅₀ of V-type ATPase activity was 0.48 and 1.3 μ M for *S. gregaria* and *P. bufonius*, respectively.

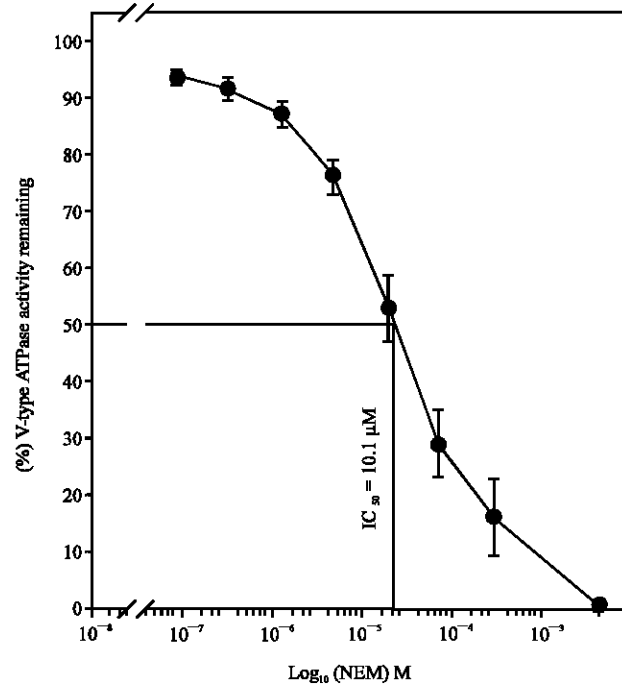


Fig. 3: Effect of different concentrations of NEM on V-type ATPase activity of *P. bufonius* excretory system. Enzyme activity expressed as n moles Pi liberated mg protein⁻¹ min⁻¹. (Each point represents mean $\pm$ SEM n = 3)

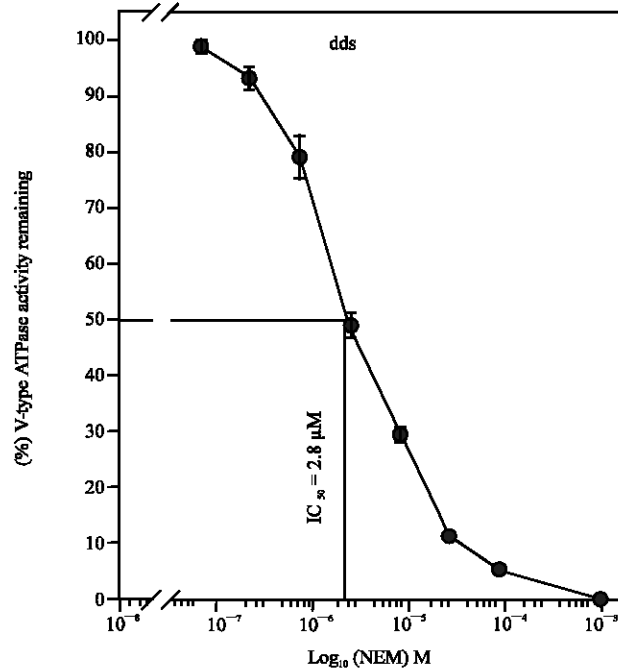


Fig. 4: Effect of different concentrations of NEM on V-type ATPase activity of *S. gregaria* excretory system. Enzyme activity expressed as n moles Pi liberated mg protein⁻¹ min⁻¹. (Each point represents mean $\pm$ SEM n = 3)

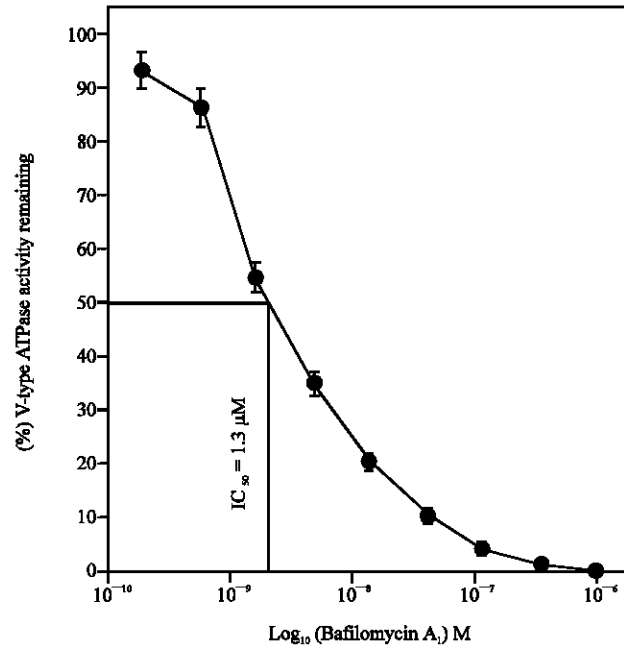


Fig. 5: Effect of different concentrations of bafilomycin A₁ on V-type ATPase activity of *P. bufonius* excretory system. Enzyme activity expressed as n moles Pi liberated mg protein⁻¹ min⁻¹. (Each point represents mean±SEM n = 3)

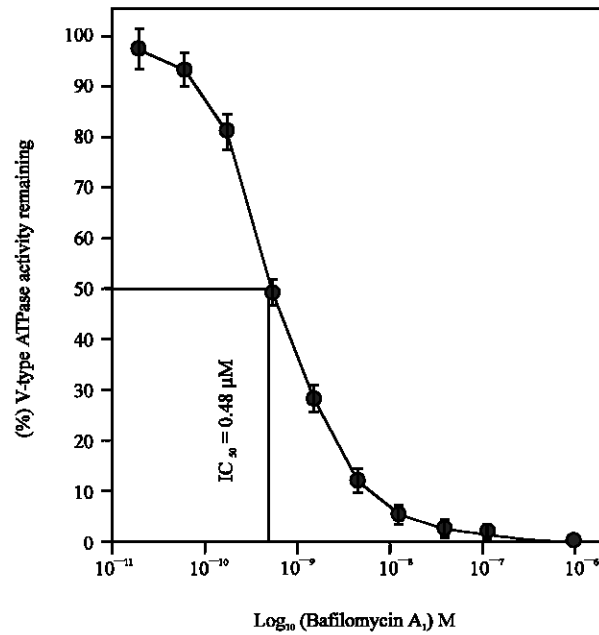


Fig. 6: Effect of different concentrations of bafilomycin A₁ on V-type ATPase activity of *S. gregaria* excretory system. Enzyme activity expressed as n moles Pi liberated mg protein⁻¹ min⁻¹. (Each point represents mean ±SEM n = 3)

Table 2: Comparative influence of various salts on V-type ATPase activity of excretory system of *S. gregaria* and *P. bufonius*

Salt (30 mM)	Relative specific activity of V-type ATPase (Mean $\pm$ SEM)			
	<i>S. gregaria</i>	P	<i>P. bufonius</i>	P
No salt*	100%		100%	
KCl	224.3 $\pm$ 20.1	<0.001	183.9 $\pm$ 13.1	<0.002
NaCl	180.7 $\pm$ 19.5	<0.002	174.9 $\pm$ 22.0	<0.01
Choline Cl	161.3 $\pm$ 20.3	<0.01	154.1 $\pm$ 18.3	<0.02
RbCl	134.7 $\pm$ 11.5	<0.05	129.7 $\pm$ 13.5	<0.05
LiCl	167.1 $\pm$ 19.8	<0.05	138.4 $\pm$ 17.6	<0.05
KHCO ₃	278.4 $\pm$ 28.1	<0.0001	261.3 $\pm$ 31.2	<0.0001
NaHCO ₃	262.4 $\pm$ 8.4	<0.0001	241.9 $\pm$ 29.1	<0.0001
Tris-HCl	241.5 $\pm$ 20.1	<0.0001	212.5 $\pm$ 26.7	<0.001
KBr	115.9 $\pm$ 11.5	ns	109.1 $\pm$ 19.4	ns
K Gluconate	140.4 $\pm$ 14.0	<0.05	156.3 $\pm$ 21.0	<0.05
KSO ₄	138.3 $\pm$ 8.2	<0.05	132.8 $\pm$ 27.8	<0.05
KF	130.3 $\pm$ 11.6	<0.05	138.2 $\pm$ 16.7	<0.05
KNO ₃	29.2 $\pm$ 2.1	<0.0001	33.6 $\pm$ 5.7	<0.0001

*(No salt) Unstimulated ATPase activity obtained in assay medium: 1 mM MgCl₂, 5 mM Tris-HCl (pH 7.5), 0.1 mM EGTA, 0.5 mM NaN₃ and 0.1 mM Na₃VO₄. The specific activity recorded in this assay medium depended on each salt experiment. Mean values $\pm$ SEM. p-values were obtained by comparing the activity in the presence and absence of salt using Student's t-test, (n = 3). ns = non significant

Effect of Various Salts on the Activity of V-type ATPase

The results presented in Table 2 summarizes the response of the V-type ATPase of *P. bufonius* and *S. gregaria* to a number of different salts, in comparison to the activity of the enzyme in the standard reaction medium (1 mM MgCl₂, 5 mM Tris-HCl (pH 7.5), 0.1 mM EGTA, 0.5 mM NaN₃ and 0.1 mM Na₃VO₄). With the exception of KBr, all the salts tested had a significant effect on the specific activity of V-type ATPase.

Firstly, the effect of changing the concentrations of a range of monovalent cations (K⁺, Na⁺, Rb⁺, Li⁺, Choline and Tris) indicates that maximal activation occurred with Tris and was approximately 213 and 241% of V-type ATPase of *P. bufonius* and *S. gregaria*, respectively. The cations were stimulatory in the following sequence; Tris > K⁺ $\approx$ Na⁺ > choline > Li⁺ $\approx$ Rb⁺. K⁺ had a stimulatory effect of approximately 175%, whilst Rb⁺ effect was the smallest, at approximately 130%.

The effect of the anion salts of (HCO₃⁻, SO₄²⁻, F⁻, Br⁻, gluconate and NO₃⁻) is also illustrated. KHCO₃ produced the greatest stimulation of enzyme activity, with a value of approximately 261% of *P. bufonius* enzyme activity and 278% of *S. gregaria* enzyme activity; in contrast, NO₃⁻ was inhibitory to V-type ATPase activity, which was reduced to approximately 34 and 29% of the control value of *P. bufonius* and *S. gregaria*, respectively. The anions were stimulatory in the following sequence; HCO₃⁻ > gluconate $\approx$ F⁻ $\approx$ SO₄²⁻ > Br⁻ > NO₃⁻. Br⁻ elicited no effect on ATPase activity, though the sodium salt of HCO₃⁻ caused a similar level of stimulation to its potassium salt counterpart of both insects.

Discussion

The present results indicate that four components of ATPase activity has been demonstrated in microsomal fractions of the apical and basal membranes of excretory system of *S. gregaria* and *P. bufonius*. They are total ATPase, Mg²⁺-dependent ATPase, Na⁺/K⁺-transporting ATPase and V-type ATPase. The activity of V-type ATPase is confined to the apical membrane fraction, while that of Na⁺/K⁺-ATPase form part of basal membrane fraction of both insects. Similar enzyme components have been reported in microsomes from a variety of tissues of various animals' sources (Forgac, 1989; Gluck *et al.*, 1995; Al-Fifi *et al.*, 1998b, 2002). It appears that the V-type ATPase sensitive to NEM is an important component of the apical membrane, whereas the Na⁺/K⁺-transporting ATPase is

confined to the basal membrane of epithelial cells of the excretory system of both insects. The present results substantiates the study of Wieczorek *et al.* (1989) and Al-Fifi *et al.* (1998a and 2002), which carried out on Malpighian tubules of *Locusta migratoria* and *P. bufonius* and proved that including 1 mM NEM to the incubation medium abolished the enzyme activity (Al-Fifi *et al.*, 1998a). It has been reported that NEM is an alkylating agent and is relatively selective for sulphhydryl groups (Forgac, 1989) and may have toxic effect on V-type protein (Weltens *et al.*, 1992). All the evidence reported yet support the role played by V-type ATPase in ion and fluid transport across the excretory system of animals (Bertram *et al.*, 1991; Klein 1992; Klein *et al.*, 1991; Dow, 1994; Dijkstra *et al.*, 1994).

Although the present study is still in progress, it shows that the properties, studied yet, of V-type ATPase as well as Na⁺/K⁺-ATPase of excretory system of *S. gregaria* and *P. bufonius* (influence the concentration of ATP, Na⁺, K⁺, Mg²⁺ and NEM) are similar. Other studies on various tissues (Forgac, 1989; Bertram *et al.*, 1991; Al-Fifi *et al.*, 1998a, 2002) coincide with results reported in the present study.

The question that ought to be answered is whether the V-type ATPase of both insects, used in the present study, differ in its properties or no. Pervious study showed that locust refrained from feeding on usher leaves (Al-Robai, 1997), which contains high concentration of toxic cardiac glycosides in all parts (Al-Robai *et al.*, 1998). On the other hand, usherhopper consumed, ingest, sequesters and concentrates toxic cardiac glycosides in bilobed poison gland and various parts of its body (Al-Robai *et al.*, 1998). It is already established that the Na⁺/K⁺ - transporting ATPase of the excretory system the usherhopper is tolerant to toxic cardiac glycosides while that of locust is very sensitive to such compounds (Al-Robai *et al.*, 1990; Al-Robai, 1993). The present study proved that the properties of V-type ATPase of both insects are similar and insensitive to food type. It appears that Na⁺/K⁺-transporting ATPase is liable to be influenced by food type consumed.

The molecular studies on the cardenolide binding site of the Na⁺, K⁺-ATPase confirmed that African *D. chrysippus aegyptius* specimens have an identical amino acid sequence in this particular region of the enzyme like specimens from Turkey and Papua New Guinea (Mebs *et al.*, 2000; 2005). The same applies to *A. ochlea ochlea*, another member of the Danaidae family. Since a mutation at position 122 (Asn to His) renders the enzyme of *D. plexippus* insensitive to cardenolides (Holzinger and Wink, 1996; Holzinger *et al.*, 1996; Mebs *et al.*, 2000; 2005), the other members of this butterfly family must have developed different modifications at the Na⁺, K⁺-ATPase preventing cardenolide binding or other strategies to tolerate the high toxicity of these compounds. Whether the replacement of glutamine by lysine (position 111) in *A. ochlea ochlea* is affecting cardenolide binding is an open question.

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