

Selective and Reversible Inhibition of Heart Sarcolemmal (Na⁺ + K⁺)-ATPase by p-Bromophenyl Isothiocyanate. Evidence for a Sulfhydryl Group in the ATP-Binding Site of the Enzyme

A. ZIEGELHÖFFER, A. BREIER, A. DŽURBA and N. VRBJAR

Department of Biochemistry, Institute of Experimental Surgery, Centre of Physiological Sciences, Slovak Academy of Sciences, Dúbravská cesta 9, 842 33 Bratislava, Czechoslovakia

Abstract. Isothiocyanates are potent modifiers of thiol groups, and they have been successfully applied in studying the active site structure of renal (Na⁺ + K⁺)-ATPase. However, very little has been known on interactions of isothiocyanates with myocardial sarcolemmal ATPases. In the present study the mode of interaction and inhibitory effect of p-bromophenyl isothiocyanate (BPITC) on isolated rat heart sarcolemmal preparation ATPase activities not exhibiting (Mg-Ca)-ATPase activity was investigated. BPITC in concentrations of 10⁻⁷—10⁻⁴ mol.l⁻¹ inhibited selectively and non-competitively the (Na⁺ + K⁺)-ATPase activity in the sarcolemma with an ID₅₀ around 2.10⁻⁷ mol.l⁻¹. The non-specific interaction of BPITC with bivalent cations, namely with Mg²⁺ and Ca²⁺, in the reaction system was eliminated by preincubation of membranes with BPITC keeping the ratio of inhibitor to membrane protein concentration constant. Under these conditions no considerable inhibitory effects were observed on Mg²⁺-ATPase or the low-affinity Ca²⁺-ATPase of sarcolemma. Preincubation of membranes with 2 mmol.l⁻¹ ATP protected (Na⁺ + K⁺)-ATPase activity against inhibition by BPITC. The interaction of BPITC with the sarcolemma proved to be reversible in the presence of beta-mercaptoethanol or dithiothreitol.

Key words: p-Bromophenyl isothiocyanate — Heart sarcolemma — Sulfhydryl groups — (Na⁺ + K⁺)-ATPase active site

Introduction

Isothiocyanates are well known as potent modifiers of nucleophilic groups. Their specificity toward thiols may be secured by selection of appropriate reaction pH (Drobica and Gemeiner 1976). Owing to their high reactivity as well as to the availability of various isothiocyanates with different degree of lipophilicity these

compounds have been successfully used for modification and/or labelling of amphiphatic membrane proteins such as Ca^{2+} -ATPase in the sarcoplasmic reticulum (Sigrist and Zahler 1978, 1980). To investigate the active structure of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ in the plasma membrane, however, only the slightly lipophilic fluorescein isothiocyanate has been used (Carilli et al. 1982). Moreover, the latter study was based on interaction of the isothiocyanate with the COOH terminal on the 77,000 dalton peptide fragment obtained by chymotryptic cleavage of alpha-subunit of the purified enzyme.

The aim of our present study was to investigate the interaction of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ in isolated purified cardiac sarcolemmal fraction with p-bromophenyl isothiocyanate (BPITC), and: i.) to utilize in the investigation the higher lipophilicity of BPITC which should enable the latter compound to reach membrane proteins from the apolar membrane phase; ii.) to restrict as far as possible interactions of BPITC to sulfhydryl groups by choosing appropriate reaction conditions, iii.) to avoid or minimize interactions of BPITC with other sarcolemmal ATPases, such as Mg^{2+} -ATPase or Ca^{2+} -ATPase with low affinity to calcium (Ziegelh offer and Dhalla 1979).

Material and Methods

Membrane preparation. Isolated rat heart membrane preparation enriched in sarcolemma was obtained by the method of hypotonic shock combined with NaI treatment (Kostka et al. 1981). The resulting fraction consisted of predominantly right side out oriented membrane vesicles. As determined by the activities of 5'-nucleotidase, succinic dehydrogenase, oligomycin-sensitive Mg^{2+} -ATPase and Mg^{2+} -dependent, Ca^{2+} -stimulated ATPase present in the sarcolemmal preparation the latter has been contaminated to less than 3 per cent by other subcellular membrane particles (Kostka et al. 1981). Further details concerning the preparation procedure were described in our earlier paper (Ziegelh offer et al. 1979).

Estimation of ATPase activities. Specific activities of the sarcolemmal $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, Mg^{2+} -ATPase and Ca^{2+} -ATPase were investigated by determining the amount of phosphate liberated from ATP-splitting during 10 min reaction at 37 °C in 1 ml of medium containing 20 mmol.l^{-1} histidine-HCl buffer pH = 7.0, usually 35–60 μg of membrane protein and optimal stimulatory concentrations of Na^+ , K^+ , Mg^{2+} or Ca^{2+} ions (see Table 1). The reaction was started by adding ATP (final concentration 2 mmol.l^{-1}) and it was terminated by adding 1 ml of ice-cold trichloroacetic acid (0.73 mmol.l^{-1}). $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity was established as the difference in the enzyme activity in the presence of optimal stimulatory concentrations of Na^+ , K^+ and Mg^{2+} and in the absence of the former monovalent cations. Around 70 per cent of the enzyme activity proved to be ouabain-sensitive in the presence of 1 mmol.l^{-1} ouabain. Further details concerning the estimation of phosphate, proteins, calculations of the enzyme activities etc., have been described elsewhere (Fedelešova et al. 1976).

Investigation of the inhibitory effect of BPITC. Various concentrations (between 10^{-8} – 10^{-4} mol.l^{-1}) of BPITC prepared by the thiophosgene procedure according to Gemeiner and Drobnica (1979), were dissolved in dioxane and added to the incubation medium prior to starting the enzyme reaction. Final concentrations of dioxane in the reaction system varied with the amount of the inhibitor added but they

Table 1. Specific activities of ATPases in the isolated sarcolemmal fraction and the ionic composition of incubation medium. Means from 15 experiments \pm S. E. M.

ATPase	Specific activity ($\mu\text{mol P}_i \cdot \text{mg}^{-1} \text{ membrane protein} \cdot \text{h}^{-1}$)	Ionic composition
Mg ²⁺ -ATPase	22.16 \pm 1.38	2 mmol.l ⁻¹ MgCl ₂
(Na ⁺ + K ⁺)-ATPase	12.05 \pm 1.02	20 mmol.l ⁻¹ KCl 100 mmol.l ⁻¹ NaCl
Ca ²⁺ -ATPase	22.42 \pm 1.27	2 mmol.l ⁻¹ MgCl ₂
(Mg ²⁺ - Ca ²⁺)-ATPase	0.35 \pm 0.20	2 mmol.l ⁻¹ MgCl ₂ 0.1 mmol.l ⁻¹ CaCl ₂

never exceeded 55 mmol.l⁻¹. From the point of view of experimental conditions, the experiments with BPITC were essentially divided into three main groups consistent with those in Table 2:

Group A — Inhibitory action of BPITC in the presence of bivalent cations (protection of (Na⁺ + K⁺)-ATPase by Mg²⁺ ions). Membranes (35–60 μg protein) were preincubated in the presence of BPITC (10 $\mu\text{mol.l}^{-1}$) at 37 °C for a period of 10 min at pH=6.8 in 1 ml of the reaction medium for ATPase (see above) containing optimal concentrations of the respective cations. The enzyme reaction was started immediately after preincubation by adding ATP.

Group B — Inhibitory effect of preincubation with BPITC. Membranes (200 μg protein.ml⁻¹) were preincubated in the presence of BPITC (100 $\mu\text{mol.l}^{-1}$) for a period of 10 min at pH=6.8 in TRIS-HCl buffer solution (10 mmol.l⁻¹). The preincubation medium containing excess of the free inhibitor was then removed by centrifugation for 10 min at 1500 g. The pellet was repeatedly washed

Table 2. Protective effect of magnesium ions and ATP on inhibition of the sarcolemmal (Na⁺ + K⁺)-ATPase and Mg²⁺-ATPase activities by BPITC

Reaction conditions	Inhibition of (Na ⁺ + K ⁺)-ATPase activity [%]	Inhibition of Mg ²⁺ -ATPase activity [%]
A	56.94 \pm 1.10 ^a	5.24 \pm 3.21 ^b
B	99.64 \pm 0.36 ^a	10.12 \pm 2.05 ^a
C	25.00 \pm 3.61 ^a	1.45 \pm 0.70 ^b

A — Preincubation with BPITC in the presence of optimal concentrations of Na⁺, K⁺ and Mg²⁺ (see Table 1), for other experimental conditions see Methods.

B — Preincubation with BPITC in absence of Na⁺, K⁺ and Mg²⁺. For other experimental conditions see Methods.

C — Preincubation in the presence of ATP, Mg²⁺ and Na²⁺. Reaction started by adding K⁺ immediately after the addition of BPITC. For other experimental conditions see Methods.

Means of 6 experiments \pm double standard deviation. Significance: a — p<0.01, b — p>0.05

out by its resuspension in the same buffer and by centrifugation. The resulting pellet was, again, resuspended in TRIS-HCl medium and adjusted to a final concentration of 400 $\mu\text{g protein}\cdot\text{ml}^{-1}$. For estimation of ATPase activities 40 $\mu\text{g protein}$ aliquots of the inhibitor-treated membrane fraction were used.

Group C — Inhibitory action of BPITC in the presence of both bivalent cations and ATP (protection of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by Mg^{2+} ions and ATP, respectively). Membranes (35–60 $\mu\text{g protein}$) were preincubated in the presence of ATP, Mg^{2+} and Na^+ ions (2; 2; and 100 $\text{mmol}\cdot\text{l}^{-1}$, respectively) in 1 ml of 20 $\text{mmol}\cdot\text{l}^{-1}$ histidine-HCl reaction medium, $\text{pH} = 6.8$ at 37 °C for 10 min. After preincubation had been completed, BPITC (10 $\mu\text{mol}\cdot\text{l}^{-1}$) was added to the reaction system. The enzyme reaction for $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity measurements was started by adding of potassium ions (final concentration 20 $\text{mmol}\cdot\text{l}^{-1}$), and it was kept running for 10 min. The activity of $\text{Mg}^{2+}\text{-ATPase}$ was established in parallel experiments, both at the end of the preincubation period and after 10 min of enzyme reaction in the presence of BPITC.

All the other results shown have been obtained under one of the above 3 experimental conditions, the peculiarities being indicated in the legends to the respective tables and figures. All chemicals were purchased from Sigma and Lachema and they were of analytical grade.

Results

The typical specific activities of ATPases in the sarcolemmal fraction are given in Table 1 together with the corresponding ionic composition of the reaction medium. The data in the table show that the above preparation exhibits a very low $(\text{Mg-Ca})\text{-ATPase}$ activity.

This in fact proves that no considerable contamination of the fraction by membranes of sarcoplasmic reticulum or by myofibrils occurred.

The administration of BPITC in a concentration range of 10^{-6} to 10^{-4} $\text{mol}\cdot\text{l}^{-1}$ to isolated sarcolemmal membranes in the presence of optimal stimulatory concentrations of Na^+ , K^+ and Mg^{2+} ions led to an approximately similar degree of inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$ activity by 50–60%, and 5 per cent respectively. The inhibition obtained with 10^{-5} $\text{mol}\cdot\text{l}^{-1}$ of BPITC amounting to 56.94 and 5.24 per cent is illustrated in Table 2, group A. When investigating the influence of the same concentrations of BPITC on the sarcolemmal $\text{Ca}^{2+}\text{-ATPase}$ an approximately 10 per cent inhibition of the enzyme activity could only be observed in the presence of 2 $\text{mmol}\cdot\text{l}^{-1}$ CaCl_2 (not shown).

Preincubation of membranes with BPITC at 4 °C in the absence of Mg^{2+} ions, however, resulted in a considerable increase in the inhibitory effect of isothiocyanate on $(\text{Na}^+, \text{K}^+)\text{-ATPase}$ activity (see Table 2, group B). Nevertheless, in contrast to the findings of Sigrist and Zahler (1981) on sarcoplasmic Ca^{2+} -stimulated ATPase, no considerable inhibition of the sarcolemmal $\text{Ca}^{2+}\text{-ATPase}$ activity by BPITC was observed even under these conditions.

The protective effect of magnesium against BPITC-induced inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ illustrated in the Table 2, group A, was even more pro-

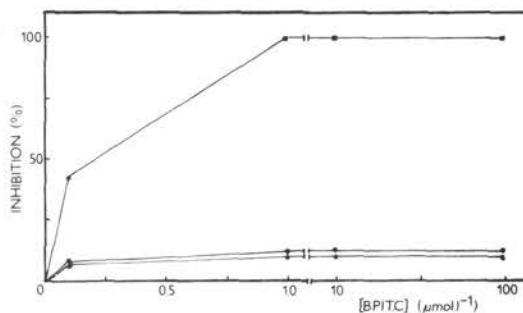


Fig. 1. Inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, $\text{Mg}^{2+}\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$ activities in isolated rat heart sarcolemma by increasing concentrations of p-bromophenyl isothiocyanate. Experimental conditions are similar to those described in Table 2 (B). Results are means from 6 experiments and represent inhibition-induced loss in enzyme activities (in per cent.).

squares — $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$; triangles — $\text{Mg}^{2+}\text{-ATPase}$; circles — $\text{Ca}^{2+}\text{-ATPase}$.

nounced if the membranes were preincubated besides Mg^{2+} ions also with equimolar concentrations of ATP in addition to Mg^{2+} ions (Table 2, group C).

Further investigation of the inhibitory effect of BPITC on $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, $\text{Mg}^{2+}\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$ activities under experimental conditions indicated in Table 2 (B) revealed a selective inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ over the whole concentration range of the inhibitor action, i. e. from 10^{-7} up to 10^{-4} mol.l $^{-1}$ with an ID_{50} value of approximately $2 \cdot 10^{-7}$ mol.l $^{-1}$ (Fig. 1).

Kinetic parameters of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ were investigated primarily with the aid of the Lineweaver — Burk plot. The results revealed a V_{max} value of $20.83 \mu\text{mol P}_i \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ in the absence of BPITC. In the presence of various concentrations of the inhibitor within the range of $5 \cdot 10^{-8}$ to $5 \cdot 10^{-7}$ mol.l $^{-1}$, decreased V_{max} values from 16.81 to $2.50 \mu\text{mol P}_i \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$ and an unchanged K_m value of 0.833 mol.l $^{-1}$ could be established (Fig. 2, panel A). This indicated a non-competitive type of inhibition. Further investigation of inhibition kinetics in the classical Dixon plot revealed concave upward curves pointing to the presence of a tight-binding inhibitor (Fig. 2, panel B). This exactly reflects the actual situation, since, in contrary to ATP which is bound to the SH-group in the active site of the enzyme with a hydrogen bond (Patzelt—Wenzler and Schoner 1981), BPITC is bound to this thiol group covalently. It has to be taken in consideration that, due to unavoidable experimental conditions (see Material and Methods and Table 2, both sub B) the concentration of the total inhibitor added does probably not equal to the concentration of the free inhibitor. In such a case, for a definitive decision about the type of inhibition it is advantageous to apply the graphical method of Dixon (1972). The values of K obtained by this method (Fig. 3, panel A) proved to be independent on the concentration of ATP (Fig. 3, panel B) and they also indicate

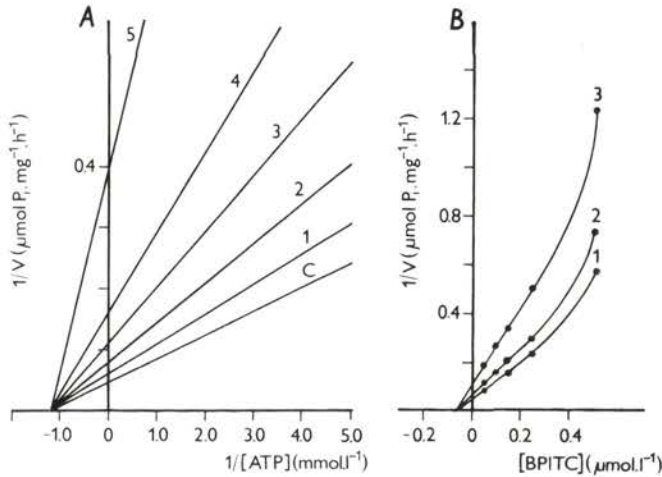


Fig. 2. Investigation of inhibition-kinetics of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by BPITC using the Lineweaver — Burk (A) and Dixon (B) plots. Straight lines were obtained by linear regression from results of 5 experiments. Correlation coefficients (0.988—0.998) show significance at $p < 0.01$. For experimental conditions not indicated in the plots, see Material and Methods, (group B). Enzyme activities were measured in the presence of excess of ATP-regeneration system (phosphoenol pyruvate-pyruvate kinase). A: Concentrations of BPITC in the Lineweaver — Burk plot; C — controls with no inhibitor present, 1 — 0.05; 2 — 0.1; 3 — 0.15; 4 — 0.25; 5 — 0.5 $\mu\text{mol} \cdot \text{l}^{-1}$. B: ATP concentration (in $\text{mmol} \cdot \text{l}^{-1}$): 1 — 2; 2 — 1; 3 — 0.4.

a non-competitive type of inhibition where $K = K_i$ and the latter amounts to $0.067 \pm 0.005 \mu\text{mol} \cdot \text{l}^{-1}$.

The binding of BPITC to $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ could be reversed by dithiothreitol or beta-mercaptoethanol as illustrated in Fig. 4. The BPITC-induced inhibition of sarcolemmal $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity (for the conditions see Material and Methods and Table 2, both sub B) exceeding 99 % could be reduced by beta-mercaptoethanol and dithiothreitol to 35.5 and 15.25 %, respectively.

Discussion

The basic enzyme characteristics of the sarcolemmal preparation used in the present experiments (Table 1) correspond to those reported earlier for similar preparations (Ziegelhöffner et al. 1979; Kostka et al. 1981). It should be considered as an advantage that the preparation represents sarcolemmal membranes contaminated only minutely by other subcellular particles, and which have not been particularly purified in respect to any of the present ATPase. This may keep the

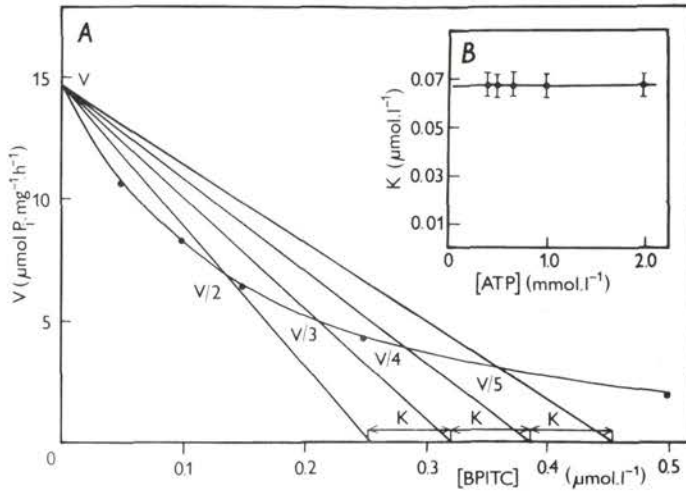


Fig. 3. Type of inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by BPITC estimated with the aid of the graphical method for tight-bound inhibitors. The calculations as well as the plotting were made according to Dixon (1972). A — Estimation of K -values for an ATP concentration of $2 \text{ mmol} \cdot \text{l}^{-1}$. Similar plots have been made for ATP concentrations of 0.4; 0.5; 0.67; and $1 \text{ mmol} \cdot \text{l}^{-1}$ (not shown). Single points have been determined from the corresponding lines in Lineweaver-Burk plots obtained by linear regression (Fig. 2, A). Experimental conditions were similar to those described in Fig. 2. B — Comparison of K -values determined graphically from the Dixon plots (Fig. 2, A) in the presence of different concentrations of ATP. Single points are means \pm S. E. M. from 3 K — values obtained for each concentration of ATP. The absence of any dependence of mean K -values on the concentration of ATP indicates a non-competitive type of inhibition.

mutual ratio of sarcolemmal ATPase activities as well as their specific properties more close to those in the native state.

Experimental data mainly from Table 2 show that the presence of ATP and/or bivalent cations such as Mg^{2+} and Ca^{2+} , both proved to decrease the inhibitory effect of BPITC. The relatively weak inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by BPITC observed in the presence of magnesium ions is in a good agreement with the findings of Mårdh (1982) and points to a stabilizing effect of Mg^{2+} ions on the enzyme. This stabilizing effect manifests itself as an antiinhibitory effect against BPITC.

Adenosine triphosphate was shown to protect the ATP hydrolysis site of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ against fluorescein isothiocyanate-induced inactivation (Carilli et al. 1982). This inactivation was described to occur at a binding-stoichiometry of approximately one isothiocyanate binding site per one ATP binding site. Because of the pH value of 7.5 chosen by the above investigators in their experiments, both thiol and amino groups may be involved in the observed binding of isothiocyanate. On the other hand, in our experiments with ATP-protectable

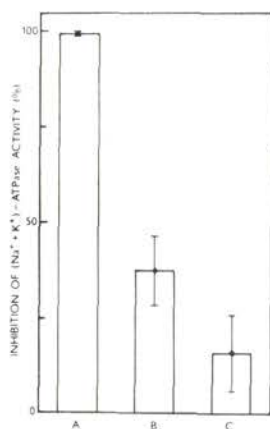


Fig. 4. Reversal of *p*-bromophenyl isothiocyanate-induced inhibition of the sarcolemmal (Na⁺ + K⁺)-ATPase by beta-mercaptoethanol and dithiothreitol. Experimental conditions were essentially similar to those described in Table 2 *B*. Thiols were added to BPITC-pretreated membranes (see Material and Methods, *B*). Results are means \pm double SD from 6 experiments and they represent inhibition-induced loss of enzyme activity expressed in per cent. *A*—Inhibition of (Na⁺ + K⁺)-ATPase activity in the presence of BPITC and absence of thiols; *B* — Influence of beta-mercaptoethanol (64 mmol.l⁻¹) on (Na⁺ + K⁺)-ATPase activity in BPITC-pretreated membranes; *C* — Influence of dithiothreitol (1 mmol.l⁻¹) on (Na⁺ + K⁺)-ATPase activity in BPITC pretreated membranes.

inactivation of (Na⁺ + K⁺)-ATPase (Table 2) the binding of BPITC was highly restricted to sulfhydryl groups by the reaction pH of 6.8 (Drobnica and Gemeiner 1976). Thus, the observed high inhibition and protective effects (Table 2) support the view that the inhibitor binding locus at the active site of the enzyme is a thiol group. This is also in agreement with data reported by Patzelt—Wenczler and Schoner (1981) and Schoner et al. (1982) indicating that one of the 36 sulfhydryl groups of (Na⁺ + K⁺)-ATPase is involved in the recognition of ATP — presumably by forming a hydrogen bond with the 6-amino group of the purine moiety of ATP. The observed additive effects of Mg²⁺ and ATP in protection of (Na⁺ + K⁺)-ATPase against the BPITC-induced inactivation support the view of M ardh (1982) that Mg²⁺ ions which play a role in dephosphorylation of the phosphoenzyme may induce a conformational change at the ATP-binding site of the ATPase.

An interpretation of the finding that BPITC is a non-competitive inhibitor of (Na⁺ + K⁺)-ATPase (Fig. 2 and 3) is difficult from following reasons:

- the *K_d* value of ATP which has been found to amount to 2.95 and 77 μ mol.l⁻¹ for the high affinity and low affinity ATP binding sites, respectively (Patzelt-Wenczler and Schoner 1981), exceeds at least by two orders the *K_i* value of BPITC established in Fig. 3, panel *B*,
- the probably different distribution between the aqueous and lipid environments of the high hydrophilic ATP and the high lipophilic BPITC with a partition

coefficient of 10 800 (octanol — water; unpublished data) indicates that the latter compound will be accumulated more in the lipid layer of the membranes, c) the K_i value of BPITC will be in fact lower than it was estimated in the kinetic studies since a portion of the inhibitor which had not been bound to the membranes was washed out before starting the measurement of the enzyme activity (see Material and Methods, group B).

The ID_{50} value of 2.10^{-7} mol.l⁻¹ estimated from Fig. 1 for BPITC points to a high sensitivity of the sarcolemmal (Na⁺ + K⁺)-ATPase toward this arylisothiocyanate, since the ID_{50} value of the same compound against the highly purified, commercially available D-glyceraldehyde-3-phosphate dehydrogenase was found to be higher by three orders (Gemeiner and Drobica 1979).

It has to be noted that the conditions for reversal of BPITC-induced (Na⁺ + K⁺)-ATPase-inhibition by beta-mercaptoethanol and dithiothreitol have been chosen arbitrarily and could be far from the optimal conditions. Thus the finding that the two above high reactive thiol compounds proved to be capable of reversing BPITC-induced inactivation of the enzyme is another argument in support of the presence of an isothiocyanate-accessible sulfhydryl group in the ATP-splitting site of heart sarcolemmal (Na⁺ + K⁺)-ATPase.

The aim of the present study was first of all to find and to describe conditions for differential inhibition of (Na⁺ + K⁺)-ATPase in a sarcolemmal fraction containing also Ca²⁺-ATPase. Hence, the absence of inhibition by BPITC of the heart sarcolemmal Ca²⁺-ATPase in the presence of calcium ions or after pretreatment of membranes with isothiocyanate at 4 °C is only in a seeming contradiction to findings obtained with rabbit skeletal muscle sarcoplasmic reticulum Ca²⁺-ATPase (Pick 1981a, b; Pick and Bassilian 1981; Sigrist and Zahler 1981). Namely, after preincubation of sarcolemmal membranes with BPITC in the absence of Ca²⁺ ions at 37 °C, Ca²⁺-ATPase was also found to be considerably inhibited. This inhibition is subject of our further investigations.

References

- Carilli C. T., Farley R. A., Perlman D. M., Cantley L. C. (1982): The active site structure of Na⁺- and K⁺-stimulated ATPase. Location of a specific fluorescein isothiocyanate reactive site. *J. Biol. Chem.* **257**, 5601—5605
- Dixon M. (1972): The graphical determination of K_m and K_i . *Biochem. J.* **129**, 197—202
- Drobica L., Gemeiner P. (1976): Use of isothiocyanate as „reporter“ groups in modification of enzymes. In: *Protein Structure and Evolution* (Eds. J. L. Fox, Z. Deyl, A. Blažej), pp. 105—115, Marcel Dekker Inc., New York and Basel
- Fedelesová M., Džurba A., Ziegelhöffer A. (1976): Studies on specific properties of dog heart (Na⁺ + K⁺)-ATPase. In: *Recent Advances in Studies on Cardiac Structure and Metabolism*. Vol. 9. *The Sarcolemma* (Eds. P. E. Roy, N. S. Dhalla), pp. 303—309. University Park Press, Baltimore
- Gemeiner P., Drobica L. (1979): Selective and reversible modification of essential thiol groups of D-glyceraldehyde-3-phosphate dehydrogenase by isothiocyanates. *Experientia* **35**, 857—858

- Kostka P., Ziegelhoffer A., Džurba A., Vrbjar N. (1981): A comparative study of the enzyme characteristics of different sarcolemmal preparations from the rat heart. *Physiol. Bohemoslov.* **30**, 173
- Mårdh S. (1982): Stabilizing effects by Mg^{2+} on Na, K-ATPase. *Acta Chem. Scand.* **B36**, 269—271
- Patzelt—Wenzler R., Schoner W. (1981): Evidence for two different reactive sulfhydryl groups in the ATP-binding sites of $(Na^+ + K^+)$ -ATPase. *Eur. J. Biochem.* **114**, 79—87
- Pick U. (1981 a): Dynamic interconversion of phosphorylated and non-phosphorylated intermediates of the Ca-ATPase from sarcoplasmic reticulum followed in a fluorescein-labelled enzyme. *FEBS Letters* **123**, 131—136
- Pick U. (1981 b): Interaction of fluorescein isothiocyanate with nucleotide-binding sites of the Ca-ATPase from sarcoplasmic reticulum. *Eur. J. Biochem.* **121**, 187—195
- Pick U., Bassilian S. (1981): Modification of the ATP binding site of the Ca^{2+} -ATPase from sarcoplasmic reticulum by fluorescein isothiocyanate. *FEBS Letters* **123**, 127—130
- Schoner W., Serpersu E. H., Pauls H., Patzelt—Wenzler R., Kreickmann H., Rempeters G. (1982): Comparative studies on the ATP-binding sites in Ca^{2+} -ATPase and $(Na^+ + K^+)$ -ATPase by the use of ATP-analogues. *Z. Naturforsch.* **37c**, 692—705
- Sigrist H., Zahler P. (1978): Characterization of phenylisothiocyanate as a hydrophobic membrane label. *FEBS Letters* **95**, 116—124
- Sigrist H., Zahler P. (1981): Arylisothiocyanate modification of sarcoplasmic Ca^{2+} -stimulated ATPase. *J. Bioenerg. Biomembrane* **13**, 89—101
- Ziegelhoffer A., Dhalla N. S. (1979): Regulation of sarcolemmal Mg^{2+} -ATPase and Ca^{2+} -ATPase activities by electrical stimulation and membrane phosphorylation. *J. Mol. Cell. Cardiol.* **11**, Suppl. 2, 69
- Ziegelhoffer A., Anand-Srivastava M. B., Khandelwal R. L., Dhalla N. S. (1979): Activation of heart sarcolemmal Ca^{2+}/Mg^{2+} -ATPase by cyclic AMP-dependent protein kinase. *Biochem. Biophys. Res. Commun.* **89**, 1073—1081

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