Biochimica et Biophysica Acta 861 (1986) 259–266
Elsevier

BBA 73302

Nucleotide specificity of the $E_2K \rightarrow E_1K$ transition in $(Na^+ + K^+)$-ATPase as probed with tryptic inactivation and fragmentation

F.M.A.H. Schuurmans Stekhoven, H.G.P. Swarts, R.S. Zhao *
and J.J.H.H.M. de Pont

Department of Biochemistry, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen (The Netherlands)

(Received 10 April 1986)
(Revised manuscript received 11 July 1986)

Key words: $(Na^+ + K^+)$-ATPase; Nucleotide specificity; Tryptic inactivation; Conformational transition; (Rabbit kidney)

The nucleotide specificity for the $E_2K \rightarrow E_1K$ conformational transition in $(Na^+ + K^+)$-ATPase as the key step for overall hydrolytic activity and coupled cation transport has been investigated. Use has been made of tryptic inactivation, which is biexponential in time for the enzyme in the presence of Na^+ with or without nucleotides (E_1 conformation) and monoexponential in the presence of K^+ (E_2 conformation). ATP, AdoPP[NH]P and CTP in order of decreasing effectivity induce the biphasic tryptic inactivation pattern in the presence of K^+. Their order of effectivity is inversely related to the rate constant of the second (slow) phase of inactivation. In the presence of K^+ and either ITP or GTP tryptic inactivation remains monoexponential, indicating that these nucleotides cannot drive the $E_2K \rightarrow E_1K$ transition. Tryptic inactivation has been compared with tryptic fragmentation of the α-subunit (apparent mol. wt. 94 kDa) of $(Na^+ + K^+)$-ATPase. In the E_1 conformation (Na^+ present) a 71 kDa fragment is formed during the second phase of inactivation. In the E_2 conformation (K^+ present) the α-subunit is split to fragments of 41 and 52 kDa. In the presence of K^+ and ATP, ADP, AdoPP[NH]P or CTP the 71 kDa fragment is formed in amounts which decrease in the order ATP ≈ ADP > AdoPP[NH]P > CTP. In the presence of K^+ and AMP, ITP or GTP the 71 kDa fragment is absent and only the E_2 fragments are formed. From these and literature data we arrive at a specificity order for the $E_2K \rightarrow E_1K$ transition of ATP > ADP > AdoPP[NH]P > CTP > ITP = GTP = AMP. The same order holds for K^+ transport in the K^+–K^+ exchange and for overall hydrolytic activity ($Na^+ + K^+$ present) with the natural nucleoside triphosphates as substrates. This marks the $E_2K \rightarrow E_1K$ transition as the step in the reaction mechanism that determines nucleotide specificity for $(Na^+ + K^+)$-activated hydrolysis and coupled cation transport.

Introduction

In a previous article from our laboratory [1] we have shown that the locus of nucleotide specificity in the reaction mechanism of $(Na^+ + K^+)$-ATPase, as determined with ATP and GTP as substrates, is the conformational change known as the $E_2K \rightarrow$

* Present address: Department of Biochemistry, Institute of Experimental Medicine, Hebei Academy of Medical Sciences, Shijiazhuang, Hebei Province, The People's Republic of China.

Abbreviation: 5-IAF, 5-iodoacetamidofluorescein.

Correspondence: Dr. F.M.A.H. Schuurmans Stekhoven, Department of Biochemistry, University of Nijmegen, P.O.B. 9101, 6500 HB Nijmegen, The Netherlands.

E_1K transition. ATP can enhance the speed of this transition, leading to a conformation (E_1) that can be phosphorylated by the substrate in the presence of $Na^+ + Mg^{2+}$, but GTP cannot. Other partial reactions, such as phosphorylation, the subsequent conformational change to a K^+-sensitive phosphoenzyme, and its K^+-activated dephosphorylation do not appear to differ much for the two substrates.

In the light of the above findings it is of interest to acquire a broader overview of the nucleotide specificity for the $E_2K \rightarrow E_1K$ transition and compare it with the nucleotide specificity for the overall ATPase reaction. Only few methods allow determination of nucleotide specificity for this transition in a straightforward fashion without interference with nucleotide binding. From the fluorimetric methods intrinsic tryptophan fluorescence [2] should be mentioned, but the signal (about 3%) is small. An extrinsic fluorescent probe that binds outside the ATP binding centre is 5-iodoacetamidofluorescein (5-IAF, Ref. 3), allowing a much higher signal (8–9%) to be recorded, and it has been used to demonstrate that GTP, unlike ATP, is unable to drive the $E_2K \rightarrow E_1K$ transition [1]. However, the response of the 5-IAF fluorescence to ligands, such as Na^+ or nucleoside triphosphates, and K^+ showed some unpredictable variability, possibly due to variability in 5-IAF incorporation or inactivation during labeling with the fluorescent probe.

Hence, we applied tryptic inactivation and fragmentation as originally presented by Jørgensen and Petersen [4] as a fluorescence independent assay for following the $E_2K \rightarrow E_1K$ transition. This is an elegant and reproducible method to recognize conformational states and transitions in the enzyme by means of which we have extended our study of nucleotide specificity for the $E_2K \rightarrow E_1K$ transition to nucleotides other than ATP and GTP. The results confirm those obtained for ATP and GTP with the 5-IAF fluorescence method, showing that these nucleotides represent the two extremes on the specificity scale.

Materials and Methods

Preparation of the enzyme

Isolation of $(Na^+ + K^+)$-ATPase from rabbit kidney outer medulla [5], removal of contaminating ATP, present as a stabilizer during preparation, washing and storage of the resulting preparation [1], determination of its activity [6] and of protein [5] follow the indicated references.

Tryptic inactivation

Inactivation of $(Na^+ + K^+)$-ATPase (0.2 mg protein/ml) by trypsin (2.5 μg/ml) is carried out at 37°C in a medium containing 50 mM imidazole-HCl, pH 7.0, 1 mM EDTA (imidazole salt), pH 7.0, 5 mM NaCl or KCl and 95 mM choline chloride as ionic strength control. The inactivation is started by addition of an aqueous solution of trypsin (25 μg/ml) to the incubation medium in a 1 : 9 volume ratio and is stopped after 5, 10, 20, 30, 50 and 60 min by mixing 50-μl aliquots of the resulting medium with a solution of trypsin inhibitor (1 mg/ml) in 50 mM imidazole-HCl, pH 7.0. The volume of inhibitor added is reduced from 400 μl on 5 min incubation to 100 μl at 50 and 60 min to diminish dilution 3-fold and thus obtain a sufficient number of units in the 20-μl aliquot taken in the later stages for assay of residual $(Na^+ + K^+)$-ATPase activity (see below). Inhibitor to trypsin is in large excess throughout: 800–3200-fold on a weight basis. The zero-time control is prepared by mixing 500 μl of inhibitor solution with 45 μl of the enzyme incubation medium before addition of trypsin (5 μl).

The effect of nucleotides (imidazole salts, 5 mM each) on tryptic inactivation is tested according to the same procedure, but the choline chloride and imidazole-HCl concentrations in the incubation medium are reduced to 50 and 20 mM, respectively, to compensate for ionic strength and imidazole introduced by the nucleotide.

After stopping tryptic inactivation by trypsin inhibitor 20-μl aliquots, containing 0.36–1.33 μg of $(Na^+ + K^+)$-ATPase protein, are assayed for 30 min at 37°C for ATPase activity according to the non-radioactive procedure of Schoot et al. [6]. Residual specific activity as percent of the zero-time control is plotted semilogarithmically versus time. Kinetic constants for the inactivation are graphically determined from the slopes. Biphasic exponential decay of activity is represented by two straight rather than curvilinear lines, indicating that the initial rapid phase is followed by rather

than parallel to the subsequent slow phase, i.e. is not a sum of two monoexponentials. Hence the size of the rapid phase is simply determined from the point of intersection with the slow phase.

Tryptic fragmentation and gel electrophoresis of the tryptic fragments

Tryptic fragmentation of $(Na^+ + K^+)$-ATPase is determined by gel electrophoretic separation of the resulting polypeptides, followed by staining of the polypeptide bands. $(Na^+ + K^+)$-ATPase (0.67 mg protein/ml) is incubated for 30 min at 37°C and pH 7.0 in a medium, containing 50 mM imidazole-HCl, 0.7 mM EDTA (imidazole salt), 10 mM Na^+ or K^+ and 1 μg trypsin/ml. Choline chloride is omitted to enhance the rate of tryptic proteolysis. The effect of nucleotides (imidazole salts, 5 mM each) is assayed in the same medium, but containing 1 mM K^+, sufficient to induce the E_2K conformation, but making it easier for the less potent nucleotides to induce or retain the E_1K conformation. The imidazole-HCl concentration is reduced to 20 mM to compensate for the imidazole contributed to the medium by the nucleotide.

Proteolysis is stopped by adding 5 μl of an aqueous solution of trypsin inhibitor (0.1 mg/ml) to 50 μl of the medium used for tryptic fragmentation, thus yielding a 10-fold excess by weight of the inhibitor over trypsin.

Samples obtained after stopping proteolysis with inhibitor are mixed with an equal volume of sample buffer (100 mM Tris-HCl, pH 6.8, containing 5% (w/v) dithioerythritol, 4% (w/v) sodium dodecyl sulfate, 20% (v/v) glycerol and 0.012% (w/v) bromophenol blue as tracking dye) and heated for 30 min at 60°C to solubilize the membrane-bound proteins. Aliquots of 50 μl (15 μg of $(Na^+ + K^+)$-ATPase protein) are loaded onto a slab gel (10% acrylamide, 0.268% bisacrylamide) and the polypeptide components are separated by sodium dodecyl sulfate gel electrophoresis according to Laemmli [7] at pH 8.8 (stacking gel pH 6.8). A mixture of six marker proteins, 5 μg of each dissolved in 50 μl of twice diluted sample buffer, runs in parallel. Their molecular weights are: phosphorylase *b* (94000), serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20100) and α-lactalbumin (14400).

Gels are stained with 0.17% (w/v) Coomassie brilliant blue in 50% (v/v) methanol/10% (v/v) acetic acid and destained by diffusion in 5% (v/v) methanol/7.5% (v/v) acetic acid according to Weber and Osborn [8]. The stained protein bands are scanned with a laser densitometer at 633 nm and their percent of total absorbance recorded. Their apparent molecular weights are read from a semilogarithmic calibration graph relating the molecular weights of the marker proteins to their mobility relative to that of the tracking dye. The position of the tracking dye is marked with Chinese ink prior to staining the gel.

Biochemicals and enzymes

Bovine pancrease trypsin, treated with the chymotrypsin-specific inhibitor *N*-tosyl-L-phenylalanyl chloromethane, is purchased from Merck (Darmstad, F.R.G.). Its specific activity is 3.5 μmol/min per mg protein at pH 8 and 25°C with *N*α-benzoyl-L-arginine-4-nitroanilide as substrate.

Soybean trypsin inhibitor is from Sigma Chem. Co. (St. Louis, MO). Marker proteins are from Pharmacia (Uppsala, Sweden). Nucleotides are from Boehringer Mannheim GmbH (F.R.G.). They are converted into their imidazole salts by passage over a Dowex 50W-X4 exchange resin in the corresponding cation form [9] and neutralized to pH 7.0 with imidazole. Their residual Na^+ and K^+ concentrations are determined by flame photometry, contributing about 15 μM Na^+ and 5 μM K^+ per 5 mM nucleotide to the assay media. Maximal contribution by other components in the assay media (imidazole-HCl, EDTA, choline chloride and the enzyme preparation) is 8 μM Na^+ and 6 μM K^+, of which the enzyme preparation is almost completely (99%) responsible for the K^+ contribution.

Results

Inactivation of $(Na^+ + K^+)$-ATPase by trypsin

In agreement with earlier data of Jørgensen and Petersen [4], the enzyme is inactivated by trypsin treatment in the presence of Na^+ (E_1Na conformation) in a biexponential fashion, and in the presence of K^+ (E_2K conformation) in a monoex-

ponential fashion (Fig. 1A).

The concentration of Na^+ or K^+ (5 mM) applied must have been saturating in view of the $K_{0.5}$ for Na^+ in 50 mM buffer without added K^+ (0.24 mM, Ref. 10) and a $K_{0.5}$ for K^+ of 0.01 mM under similar conditions without Na^+, increasing to 0.05 mM upon addition of 90 mM choline chloride [1,11]. Rapid and slow phase of inactivation are both linear and individually monoexponential, indicating that the slow phase is following upon the rapid phase. This conclusion is supported by the finding that the second proteolytic split in the catalytic subunit of $(Na^+ + K^+)$-ATPase, yielding a 78 kDa fragment, only occurs in the second phase of inactivation [12].

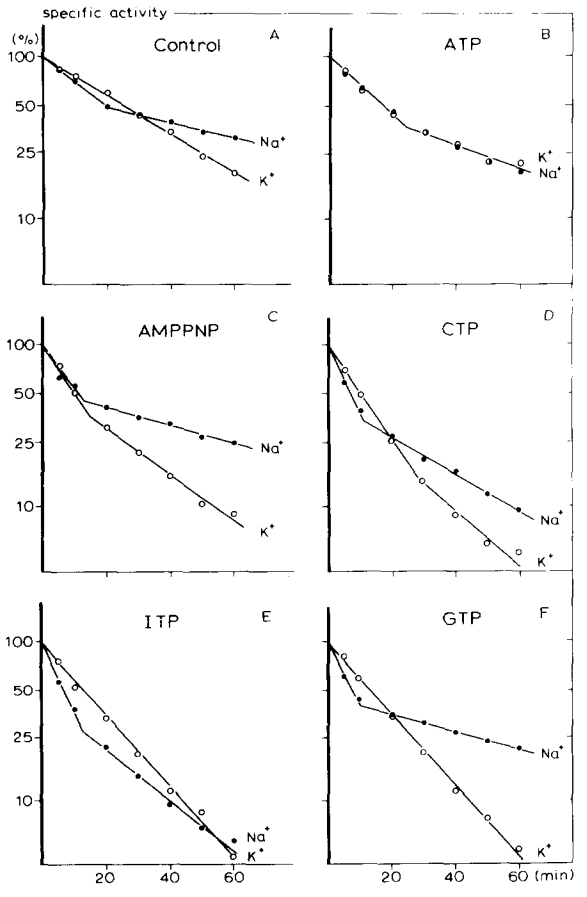

Fig. 1. Tryptic inactivation of $(Na^+ + K^+)$-ATPase. Tryptic inactivation occurs at pH 7 and 37 °C in the presence of 5 mM Na^+ or K^+, in the absence (control) or presence of the indicated nucleotides (5 mM each; AMPPNP = AdoPP[NH]P).

The dual monoexponential course of inactivation also occurs in the presence of nucleotides (ATP, AdoPP[NH]P, CTP, ITP and GTP) and Na^+ (Fig. 1B–F), demonstrating that the E_1 conformation is retained. Yet among them differences in the kinetics and size of the sequential phases of inactivation are observed (Table I), indicating subconformational differences in the E_1 family. In the table the nucleotides are arranged according to their binding affinity to a kidney preparation in the E_1 conformation [9,13]. It can be seen from the table that all low-affinity nucleotides below ATP display a 2–3-fold increase in rate constants of inactivation in the rapid phase. The kinetics of the slow phase show more variability, with AdoPP[NH]P and GTP yielding k values, which are as low as in the absence of nucleotides. From these data it can be inferred that the low-affinity nucleotides open up the structure for the more easily trypsin-accessible sites.

In the presence of 5 mM K^+ only ATP gives the same inactivation pattern as in the presence of Na^+ (Fig. 1B), indicating that a similar E_1 conformation is obtained. From the other nucleotides only AdoPP[NH]P and CTP give a biphasic inactivation pattern (Fig. 1C and D). ITP and GTP are not able to bring the enzyme into the E_1 conformation and thus give the monophasic inactivation pattern indicative for E_2K (Fig. 1E and F).

From the kinetics of inactivation in the presence of K^+ it appears that among the nucleotides which give a biexponential inactivation, the same increase in rapid-phase kinetics is observed upon decreasing nucleotide affinity as in the presence of Na^+ (Table I), indicating that low-affinity nucleotides increase the sensitivity to trypsinolysis. This is accompanied by a steady increase in size of the rapid phase.

The rate constant of the slow phase also increases with decreasing nucleotide affinity and capability to drive the $E_2K \rightarrow E_1K$ transition from 0.018 min^{-1} in the presence of ATP (full capability to drive the transition) to 0.048 min^{-1} in the presence of ITP or GTP (no capability to drive the transition). When we take the fraction of E_1 (f_1) in the presence of ATP as 1 (rate constant for the slow phase is k_1) and the fraction of E_2 (f_2) in

TABLE I

KINETICS OF TRYPTIC INACTIVATION

The data are averages (±S.E.) from three experiments (including that from Fig. 1), each with a different enzyme preparation. The nucleotides (5 mM) are arranged according to their order of decreasing binding affinity to the enzyme in the E_1 conformation [9,13]. Size refers to the size of the initial rapid phase as percent of total inactivation, determined from the intersection of the rapid and slow phase (see Materials and Methods section).

Nucleotide added	$+Na^+$ (5 mM)			$+K^+$ (5 mM)		
	rapid phase k (min^{-1})	size (%)	slow phase k (min^{-1})	rapid phase k (min^{-1})	size (%)	slow phase k (min^{-1})
none	0.030 ± 0.004	51 ± 2	0.009 ± 0.001	–	–	0.026 ± 0.003 [a]
ATP	0.035 ± 0.002	61 ± 3	0.017 ± 0.001	0.039 ± 0.004	48 ± 1	0.018 ± 0.003
AdoPP[NH]P	0.086 ± 0.009	53 ± 2	0.011 ± 0.001	0.061 ± 0.002	59 ± 6	0.034 ± 0.004
CTP	0.071 ± 0.013	60 ± 3	0.023 ± 0.002	0.065 ± 0.007	84 ± 1	0.038 ± 0.002
ITP	0.063 ± 0.007	69 ± 2	0.034 ± 0.002	–	–	0.048 ± 0.004 [a]
GTP	0.077 ± 0.011	61 ± 1	0.010 ± 0.001	–	–	0.048 ± 0.006 [a]

[a] Kinetics of the monoexponential inactivation.

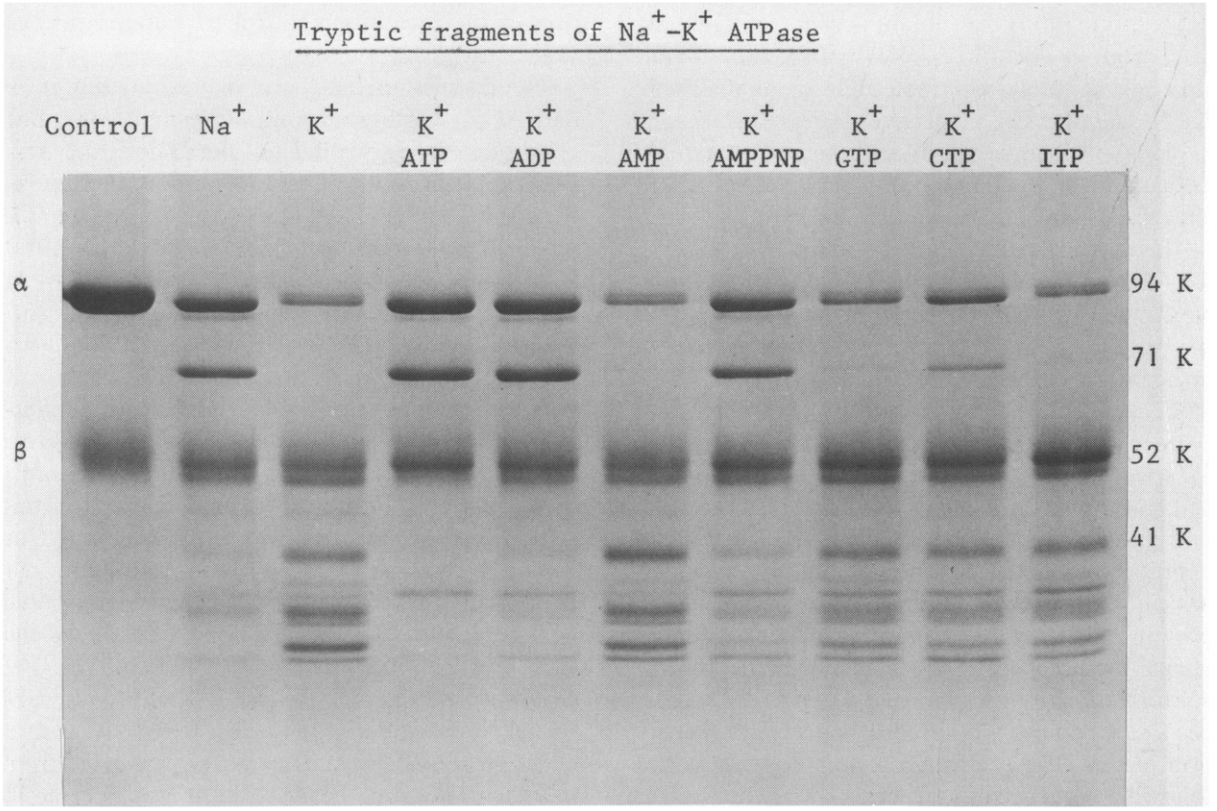

Fig. 2. Tryptic fragmentation of $(Na^+ + K^+)$-ATPase. Tryptic fragmentation occurs at pH 7 and 37°C in the presence of 10 mM Na^+ or K^+, or 1 mM K^+ plus the indicated nucleotides (5 mM each; AMPPNP = AdoPP[NP]P). Shown are Coomassie blue-stained polypeptide bands following polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Apparent molecular weights as determined from the mobility of marker proteins of known molecular weights are indicated in the right hand margin (K is kilodalton). Control is the α- and β-subunit pattern of an unfragmented sample of $(Na^+ + K^+)$-ATPase.

the presence of ITP or GTP and K^+ as 1 (rate constant of inactivation is k_2) we then may calculate f_1 for the other nucleotides in the presence of K^+, assuming that k_{app} (slow phase) $= k_1 f_1 + k_2 f_2$, where $f_1 + f_2 = 1$. Using the data from Table I ($+K^+$) we arrive at values for f_1 in the presence of AdoPP[NH]P and CTP of 0.47 and 0.33, respectively. From these data we conclude that the order of nucleotide specificity for the $E_2K \rightarrow E_1K$ transition is ATP > AdoPP[NH]P > CTP > ITP = GTP.

Tryptic fragmentation of ($Na^+ + K^+$)-ATPase

Another criterion for the conformation of the enzyme is tryptic cleavage of the α-subunit (apparent molecular weight 94 kDa, Fig. 2: control). In the slow phase of tryptic inactivation in the presence of Na^+ (E_1 conformation) a fragment of 78 kDa is formed, whereas in the presence of K^+ (E_2 conformation) the subunit is cleft to fragments of 58 and 48 kDa [4,12]. We confirm these data, although the apparent molecular weights differ: 71, 52 and 41 kDa, respectively (Fig. 2, Na^+ and K^+ lane), probably due to the different gel system, using a higher percentage of acrylamide, cross-linker and a higher pH.

In the presence of K^+ only ATP, ADP, AdoPP[NH]P and CTP give rise to the 71 kDa band, indicative of the E_1 conformation (Fig. 2). Its percentage of total Coomassie blue-stained protein absorbance is 13.3 ± 5.1, 14.6 ± 5.6, 10.4 ± 3.2 and 2.8 ± 1.1 (average ± S.E. for three enzyme preparations) for this nucleotide sequence. This yields percentages for the E_1 conformation of 100, 100, 78 and 21, respectively. AMP, GTP and ITP give only rise to the 52 and 41 kDa protein bands, plus a number of smaller polypeptides indicative of the E_2 conformation. The 52 kDa protein coincides on the gel with the β-subunit. Hence the latter nucleotides cannot retain the E_1 conformation in the presence of K^+. From the percent Coomassie blue absorbance in the position of the 71 kDa protein band we arrive at the following sequence of nucleotide specificity for the $E_2K \rightarrow E_1K$ transition: ATP ≈ ADP > AdoPP[NH]P > CTP > ITP = GTP = AMP. This is the same sequence as obtained for the nucleotides used in experiments in which the inactivation kinetics have been studied (Fig. 1).

Discussion

We have demonstrated in this paper by tryptic inactivation and fragmentation of ($Na^+ + K^+$)-ATPase that the order of nucleotide specificity for the $E_2K \rightarrow E_1K$ transition is qualitatively equal to that for binding to the high-affinity phosphorylating site with the enzyme in the E_1 conformation [9,13]. Data from tryptic inactivation and fragmentation confirm each other logically, since both were related to the slow phase of inactivation in the presence of K^+. We have taken the ability to obtain the E_1 conformation in the presence of K^+ as a measure of the ability to stimulate the $E_2K \rightarrow E_1K$ transition. This appears to be justified in view of the fact that this transition is reversible, and studies on K^+–K^+ exchange and overall ($Na^+ + K^+$)-ATPase activity, to be discussed below, indicate that not only the equilibrium position of the transition is involved but the rate as well.

No distinction has been made between ATP and ADP, which we found equipotent at 5 mM concentration, as we did not determine $K_{0.5}$ values. From the work of Jørgensen and Petersen [4] it appears that ATP ($K_{0.5} = 0.2$ mM) is 3–4-times more effective than ADP. For the electroneutral K^+–K^+ exchange in resealed red cell ghosts, in which the $E_2K \rightarrow E_1K$ transition is involved, $K_{0.5}$ values for ATP and ADP of 0.1 mM [14] and 0.8 mM [15], respectively, have been reported. Apparently in the tryptic fragmentation experiments at 1 mM K^+ both ATP and ADP were saturating at 5 mM concentration for obtaining the E_1 conformation. On the other hand, the concentration of 5 mM may not have been saturating for AdoPP[NH]P and CTP, or still saturating but sustaining a lower V_{max} for the transition than ATP or ADP. They retain the E_1 conformation with a relative effectivity of 47–78% and 21–33%, respectively, depending on the conditions used during tryptic inactivation and fragmentation of ($Na^+ + K^+$)-ATPase. This is the same order of effectivity as displayed by these nucleotides in stimulation of the K^+–K^+ exchange, in which AdoPP[NH]P is 55% and CTP 26.5% as effective as ATP [14,16]. ITP and GTP do not support the K^+–K^+ exchange [14] and also do not lead to the E_1 conformation in the presence of K^+, hence

cannot drive the $E_2K \rightarrow E_1K$ transition. An identical conclusion has been drawn for GTP from a different study in our laboratory [1].

It is then interesting to find a relationship with specific activities for the overall $(Na^+ + K^+)$-stimulated nucleoside triphosphatase reaction in the presence of various nucleotides that can be hydrolyzed by the enzyme. For nucleotides that are modified in the terminal phosphate moiety the phosphorylation step and the $E_1P \rightarrow E_2P$ conformational change, rather than the $E_2K \rightarrow E_1K$ transition, are rate-limiting [17,18]. On the other hand, for nucleotides modified in the purine nucleus we have drawn the conclusion [1] that the $E_2K \rightarrow E_1K$ transition is rate-limiting. This is in agreement with the order of nucleotide specificity ATP > CTP > ITP > GTP known for hydrolytic activity in the presence of $Na^+ + K^+$ [19] and the corresponding order of nucleotide specificity for the $E_2K \rightarrow E_1K$ transition established in this paper.

Although K^+ via low-affinity sites ($K_{0.5}$ of the order of 10 mM, Refs. 20 and 21) stimulates dissociation of E_2K and transition to the E_1 conformation when Na^+ or nucleotides are present, their occupation during the assay of hydrolytic activity at 5–25 mM K^+ [1,13,22,23] apparently does not change the order of nucleotide affinity determined in the present paper (at 1–5 mM K^+). Also quantitatively the relationship holds. CTP is hydrolyzed with 1/4th the V_{max} for ATP [24] and stimulates the transition with a relative effectivity of 21–33% depending on the presence or absence of choline, which exerts an Na^+-like action on this transition [11,25] and the K^+ concentration. ITP and GTP are hydrolyzed with relative activities of 2.4–13.5 and 0.6–8%, respectively [1,13,22,23], but apparently are unable to drive the transition. The residual hydrolytic activity can be considered as a K^+-inhibited Na^+-activated process [1]. A small stimulation of hydrolytic activity at submillimolar K^+ via dephosphorylation of E_2P is brought about by stimulation of the subsequent $E_2K \rightarrow E_1Na$ transition by Na^+. At higher K^+ concentrations Na^+ becomes unable to compensate for the inhibition exerted by E_2K and the stimulation turns over into an inhibition.

The order of nucleotide specificity for the $E_2K \rightarrow E_1K$ transition differs from the kinetically determined order of affinity for binding to the low-affinity nucleotide binding site [26]: GTP > CTP > AdoPP[NH]P > ATP > ADP > AMP > ITP. The discrepancy, as mentioned before [26], may be caused by the difference in nucleotide specificity for binding to E_2K and for driving the subsequent conformational change to E_1K.

In conclusion, the nucleotide specificity for high-affinity nucleotide binding, the $K^+ \rightarrow K^+$ exchange, overall hydrolytic activity for purine base modified nucleoside triphosphates and the $E_2K \rightarrow E_1K$ transition is the same, for the last three processes also in a quantitative respect, i.e. for the effectivity as a percentage of that for ATP. This marks the $E_2K \rightarrow E_1K$ transition as the step in the reaction mechanism determining nucleotide specificity for $(Na^+ + K^+)$-activated hydrolysis and linked cation transport.

References

1 Fu, Y.F., Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., De Pont, J.J.H.H.M. and Bonting, S.L. (1985) Biochim. Biophys. Acta 817, 7–16
2 Karlish, S.J.D. and Yates, D.W. (1978) Biochim. Biophys. Acta 527, 115–130
3 Kapakos, J.C. and Steinberg, M. (1982) Biochim. Biophys. Acta 693, 493–496
4 Jørgensen, P.L. and Petersen, J. (1975) Biochim. Biophys. Acta 401, 399–415
5 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36–52
6 Schoot, B.M., Schoots, A.F.M., De Pont, J.J.H.H.M., Schuurmans Stekhoven, F.M.A.H. and Bonting, S.L. (1977) Biochim. Biophys. Acta 483, 181–192
7 Laemmli, U.K. (1970) Nature 227, 680–685
8 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
9 Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., De Pont, J.J.H.H.M. and Bonting, S.L. (1981) Biochim. Biophys. Acta 649, 533–540
10 Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., De Pont, J.J.H.H.M. and Bonting, S.L. (1985) Biochim. Biophys. Acta 815, 16–24
11 Hastings, D. and Skou, J.C. (1980) Biochim. Biophys. Acta 601, 380–385
12 Jørgensen, P.L. and Petersen, J. (1977) Biochim. Biophys. Acta 466, 97–108
13 Hegyvary, C. and Post, R.L. (1971) J. Biol. Chem. 246, 5234–5240
14 Simons, T.J.B. (1974) J. Physiol. 237, 123–155
15 Kaplan, J.H. and Kenney, L.J. (1982) Ann. N.Y. Acad. Sci. 402, 292–295
16 Simons, T.J.B. (1975) J. Physiol. 244, 731–739
17 Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., De Pont,

J.J.H.H.M. and Bonting, S.L. (1983) Biochim. Biophys. Acta 736, 73–78
18 Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., Fu, Y.F., Kuijpers, G.A.J., De Pont, J.J.H.H.M. and Bonting, S.L. (1984) Biochim. Biophys. Acta 774, 277–287
19 Schuurmans Stekhoven, F. and Bonting, S.L. (1981) Phys. Rev. 61, 1–76
20 Skou, J.C. and Esmann, M. (1983) Biochim. Biophys. Acta 746, 101–113
21 Forbusch III, B. (1985) in the Sodium Pump, 4th Int. Conf. Na,K-ATPase (Glynn, I.M. and Ellory, J.C., eds.), pp. 599–611, The Company of Biologists Ltd., Cambridge
22 Matsui, H. and Schwartz, A. (1966) Biochim. Biophys. Acta 128, 380–390
23 Schoner, W., Von Ilberg, C., Kramer, R. and Seubert, W. (1967) Eur. J. Biochem. 1, 334–343
24 Robinson, J.D. (1982) Arch. Biochem. Biophys. 213, 650–657
25 Skou, J.C. and Esmann, M. (1980) Biochim. Biophys. Acta 601, 386–402
26 Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., De Pont, J.J.H.H.M. and Bonting, S.L. (1983) Biochim. Biophys. Acta 732, 607–619