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TEMPERATURE EFFECTS ON CATION AFFINITIES OF THE (Na^+, K^+)-ATPase OF MAMMALIAN BRAIN

ALAN C. SWANN [a,*] and R. WAYNE ALBERS [b]

[a] *Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06510 and* [b] *Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD 20857 (U.S.A.)*

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Effects of temperature on the Na^+-dependent ADP-ATP exchange and the *p*-nitrophenylphosphatase reactions catalysed by (Na^+, K^+)-ATPase were examined. Apparent Mg^{2+} affinity decreased with decreasing temperature. Arrhenius plots of *p*-nitrophenylphosphatase in the presence of Na^+ and ATP had discontinuities similar to those previously reported for ($Na^+ + K^+$)-ATPase, while those of *p*-nitrophenylphosphatase measured without Na^+ or ATP did not. The apparent activation energy for *p*-nitrophenylphosphatase was a function of the physical characteristics of the cation acting at the K^+ site.

The coupled transport of Na^+ and K^+ is mediated by conformational transitions in the ($Na^+ + K^+$)-ATPase that are associated with phosphorylation of the enzyme by ATP and subsequent dephosphorylation [1–4]. Some of the so-called 'partial reactions' of the ATPase appear to be expressions of particular conformational states. For example, ATP-ADP transphosphorylation is characteristic of an enzyme state, E_1, that is stabilized by nucleotide and Na^+ binding and by relatively low concentrations of Mg^{2+} [6,7]. This reaction is inhibited by higher concentrations of Mg^{2+} that favor the formation of a 'K^+-sensitive' conformation, E_2 [5,8]. A form, R, similar but perhaps not identical to E_2, catalyses the hydrolysis of phosphate esters, including *p*-nitrophenylphosphate [9–11]. The R form is stabilized by binding of K^+ or Na^+ to their respective moderate affinity 'regulatory' sites, and in addition requires K^+ binding at high affinity 'catalytic' sites [10,11]. Li^+ can activate at the catalytic sites but is ineffective at the regulatory sites [12].

In the case of bovine brain ($Na^+ + K^+$)-ATPase, we have shown that while little of the R form is present at 25°C in the absence of stabilizing ligands, the fraction of enzyme in the R form increases with decreasing temperature [12]. The present study examines the effect of temperature on the Na^+-dependent ATP-ADP transphosphorylation catalysed by the bovine brain ATPase and extends our earlier observations [12] concerning the temperature dependence of the *p*-nitrophenylphosphatase reaction.

Materials and Methods

Rabbit muscle pyruvate kinase and the Tris salts of ATP, ADP, and *p*-nitrophenylphosphate were obtained from Sigma Biochemical Corp., [8-^{14}C]ADP from Schwartz-Mann Biochemicals, and fresh beef brains from Frederick County Products, Frederick, MD. Tris ADP and Tris ATP were of the Sigma 'vanadium-free' grade.

NaI-treated deoxycholate microsomes were prepared as described by Nakao et al. [13] and modified by us [11]. They were stored in liquid nitrogen until use.

Na^+-dependent ADP-ATP transphosphorylation was determined essentially as previously described [8]. The reaction mixture contained 10 μg protein, 1.25 mM [^{14}C]ADP, 5 mM Tris ATP, 0.08 M imidazole, pH 7.2, and various concentrations of Mg^{2+}. The Na^+-dependent activity was taken as the increment in [^{14}C]ATP formed in the presence of 125 mM Na^+.

* Present address: Department of Psychiatry and Behavioral Sciences, University of Texas School of Medicine, P.O. Box 20708, Houston, TX 77025, U.S.A.

The ^{14}C-labeled nucleotides were separated by thin-layer chromatography and counted by liquid scintillation counting. The exchange rate was calculated using the equation of McKay [14].

The assay for *p*-nitrophenylphosphatase is based on the spectrophotometric determination of *p*-nitrophenol [11]. The reaction mixture contained 0.08 M imidazole, pH 7.2, 10 mM *p*-nitrophenylphosphate as the Tris salt, and various concentrations of Mg^{2+} and other cations as described in the text. For experiments using Na^+ and ATP, an ATP regenerating system consisting of 5 mM phospho*enol*pyruvate and pyruvate kinase was used as described previously [11]. For the temperature curves, preliminary experiments were carried out in order to determine optimal cation concentrations for the range of temperatures used [15]. Arrhenius plots were based on measurements of enzyme activity at at least ten temperatures.

Parameters of equations used to fit experimental data were determined by least-squares curve fitting as previously described [10,11,16].

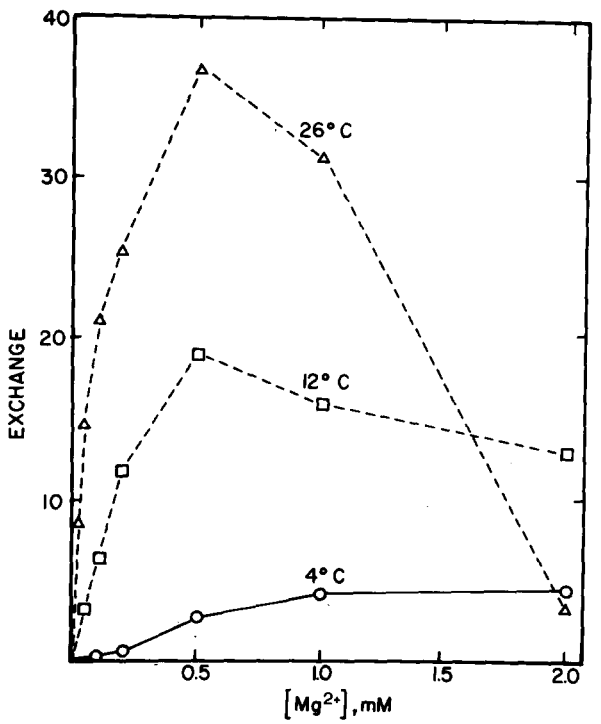

Fig. 1. Na^+-dependent ADP-ATP exchange as a function of magnesium and temperature. The ordinate shows Na^+-dependent exchange in nmol/mg protein per min; the abscissa shows $[Mg^{2+}]$ in mM.

Results

Effects of temperature on apparent magnesium affinities

Na^+-dependent ADP-ATP exchange, at 26°C, is a biphasic function of magnesium concentration (Fig. 1) [8,17] with very little activity at the higher Mg^{2+} concentration favorable for K^+-phosphatase and $(Na^+ + K^+)$-ATPase reactions [8]. As temperature decreases, however, apparent affinity of the activation site for Mg^{2+} decreases and Mg^{2+} inhibition virtually disappears.

The apparent Mg^{2+} affinity of the activation site for K^+-*p*-nitrophenylphosphatase also decreases with temperature (Fig. 2).

Monovalent cation activation of p-nitrophenylphosphatase as a function of temperature

The *p*-nitrophenylphosphatase can be activated by K^+ or its congeners through moderate affinity 'regulatory' sites or, if Na^+ and ATP are present, via high affinity 'catalytic' sites [10,11]. The temperature dependence of *p*-nitrophenylphosphatase is different under these two types of condition: with Na^+ and ATP present, there is an obvious discontinuity in the Arrhenius plot (Fig. 3). Without ATP and Na^+, the

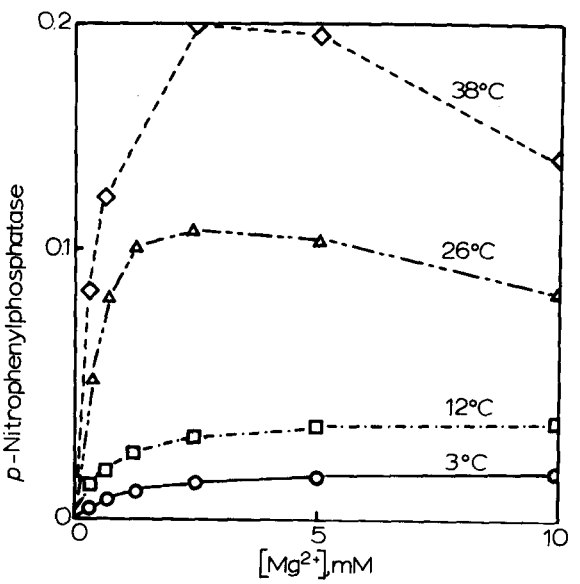

Fig. 2. K^+-dependent *p*-nitrophenylphosphatase as a function of magnesium and temperature. The ordinate shows the *p*-nitrophenylphosphatase activity in μmol/mg protein per min; the abscissa shows $[Mg^{2+}]$ in mM.

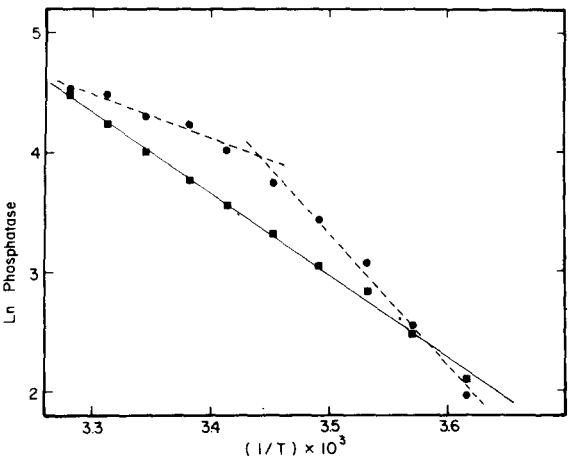

Fig. 3. Arrhenius plots for K^+-dependent *p*-nitrophenylphosphatase. The lines represent least squares linear regression fits giving the constants shown in Table I. – – –, with 25 mM NaCl, 0.1 mM ATP and 0.5 mM KCl. ▪——▪, 10 mM KCl; ATP and NaCl absent.

Arrhenius plot is essentially linear, being fit best by a straight line despite the slight curvature apparent at very low temperatures.

Table I summarizes the apparent activation energies and transition temperatures for *p*-nitrophenylphosphatase activation. As is shown for K^+ in Fig. 3, activation energy was essentially independent of temperature in the absence of ATP + Na^+, (with the exception of Li^+ [12]), while in the presence of these ligands there was a transition at about 18°C. Dimethyl sulfoxide, which increases phosphatase activity and K^+ affinity [9,11], did not appreciably change the apparent activation energy.

Discussion

Temperature effects on Mg^{2+} interactions with (Na^+, K^+)-ATPase

We have proposed that low temperatures favor conversion of (Na^+, K^+)-ATPase to the R form, with phosphatase activity, high K^+ affinity, and low ATP affinity [12], and that this form may have relatively low affinity for Mg^{2+} [8]. Both high-affinity MgATP binding, which activates ATP-ADP exchange [18], and the moderate-affinity binding, which stimulates *p*-nitrophenylphosphatase [9,18] and inhibits exchange [6,19,20], appear to characterize the 'E_1' form of enzyme, favored by higher temperatures [12].

Consistent with this formulation, Table II shows that the apparent affinity for both high and moderate affinity Mg^{2+} binding increase as temperature increases.

Temperature effects of cation activation of p-nitrophenylphosphatase

Both apparent affinity and maximal activation for *p*-nitrophenylphosphatase follow the sequence $Rb^+ > K^+ > Li^+$ [12]. The apparent activation energy in the absence of Na^+ and ATP increases with this sequence (Table I). Under these conditions, the cations are activating *p*-nitrophenylphosphatase via the moderate-affinity 'regulatory' site [11]. Dimethyl sulfoxide increases the apparent affinity and maximal activation for K^+ to levels comparable to Rb^+ [9–11], but does not appreciably affect the apparent activation energy.

TABLE I

APPARENT ACTIVATION ENERGIES FOR *p*-NITROPHENYLPHOSPHATASE

E_{act} figures are presented as kcal · mol^{-1}. DMSO, dimethyl sulfoxide.

	Without (Na + ATP) *				With (Na^+ + ATP) **		
	Rb^+	K^+	K^+/DMSO	Li^+	Rb^+	K^+	Li^+
E_{act} above T_c	−11.89	−13.56	−13.01	−27.15	−8.21	−7.33	−4.65
T_c (°C)	–	–	–	–	17.4	17.7	12
E_{act} below T_c	–	–	–	–	−20.39	−21.98	−22.2

* With 20 mM KCl or LiCl or 15 mM RbCl.

** 25 mM NaCl, 0.1 mM ATP. With 0.5 mM KCl or RbCl or 5 mM LiCl.

TABLE II

KINETIC PARAMETERS FOR Mg^{2+}-ACTIVATION OF (Na^+, K^+)-ATPase PARTIAL REACTIONS

Constants are obtained from least squares curve fitting to $v = V[(K_a/[Mg^{2+}]) + 1 + ([Mg^{2+}]/K_i)]^{-1}$ [8,11].

ADP-ATP exchange

T (°C)	V *	K_a **	K_i **
5	5.62	0.48	5.10
12	23.48	0.16	2.38
26	42.75	0.08	1.30

K^+-*p*-nitrophenylphosphatase: without Na + ATP

T (°C)	V ***	K_a	K_i
4	0.018	0.949	
12	0.040	0.640	
26	0.121	0.357	17.043
38	0.235	0.346	11.364

* In nmol/mg protein per min.
** In mM Mg^{2+}.
*** In μmol/mg protein per min.

In contrast to the case with the regulatory sites, cation activation via the catalytic sites in the presence of Na^+ and ATP yields different apparent activation energies at high and low temperatures. This implies that the discontinuity previously reported in Arrhenius plots of (Na^+, K^+)-ATPase [21–28] is associated with formation of phosphorylenzyme [11]. Table III compares energies of *p*-nitrophenylphosphatase activation, physical properties, and kinetic parameters of *p*-nitrophenylphosphatase activation, for Li^+, K^+, and Rb^+. It is evident that apparent K_m, the ionic radius, and the apparent activation energy above T_c are strongly correlated; in fact, linear or exponential correlations of these properties have $r = 0.9995$. Activation energy increases with increasing apparent affinity for the catalytic site and with increasing ionic radius. Below T_c, as in the absence of Na^+ and ATP, the opposite relationship holds.

Goldman and Albers have reported that, in hamster brain, E_{act} for *p*-nitrophenylphosphatase in the absence of Na^+ and ATP is similar at high and low temperatures, implying a linear Arrhenius plot [29]. (Na^+, K^+)-ATPase and Na^+-dependent ADP-ATP transphosphorylation had markedly different E_{act} at high and low temperatures. The discontinuity in the Arrhenius plot for the exchange reaction implies that the step in (Na^+, K^+)-ATPase or in the activation of *p*-nitrophenylphosphatase by Na^+ and ATP that has a discontinuous Arrhenius plot is the initial stage of phosphorylation [6,7]. Barnett and Palazzotto, however, reported linear Arrhenius plots for K^+-*p*-nitrophenylphosphatase whether or not Na^+ and ATP were present [30]. Under the conditions of their experiments, with relatively low Na^+ and high K^+, much of the enzyme activation may have been via K^+ rather than Na^+ and ATP [11], especially at low temperatures [12]; relative affinities for Na^+ and K^+ as activa-

TABLE III

ACTIVATION PARAMETERS OF *p*-NITROPHENYLPHOSPHATASE COMPARED TO CHARACTERISTICS OF ACTIVATING CATIONS

The energy of hydration figures (from Ref. 32) are presented as kcal · mol^{-1}, in the presence of 25 mM NaCl and 0.1 mM ATP. E_{act} figures are presented as kcal · mol^{-1}, in the presence of 25 mM NaCl and 0.1 mM ATP (from Table I). ΔE_{act}, change in E_{act} at T_c.

	Radius (Å) [25]		Energy of	E_{act}		ΔE_{act}	V (rel.) *	K (mM) *, **
	Ion.	Hyd.		Above T_c	Below T_c			
Li^+	0.68	3.4	124.4	−4.65	−22.2	17.53	0.2	0.150
K^+	1.33	2.32	77	−7.33	−21.9	14.6	1.0	0.095
Rb^+	1.47	2.28	71.9	−8.21	−20.4	12.2	1.6	0.080

* From Ref. 12, $K^+ = 1.0$, $T = 25$°C.
** Extrapolated to zero Mg^{2+} (see Ref. 12).

tors and competitive inhibitors are also known to vary widely among enzyme sources [11,31].

The contrasting order of activation energies below and above the transition temperature could indicate that activation by cations is influenced by phase transitions in membrane lipids [17,21] or that the rate-limiting step for *p*-nitrophenylphosphatase is different, at least regarding the role of cation binding, below the transition temperature. This difference would be expected to involve phosphorylation. Combined with the previously reported effects of Rb^+, K^+ and Li^+ on apparent ATP affinity for activation of *p*-nitrophenylphosphatase [12], these data suggest that the ionic radius (or hydration energy) is proportional to apparent affinity and maximal activation of *p*-nitrophenylphosphatase. A possible explanation is that, while at lower temperatures the equilibrium $T \rightleftharpoons R$ is shifted toward R, at higher temperatures the R form exists only when stabilized by cation binding [11,12], so the critical step in regeneration of E_1 would be dissociation of the cation bound to the high-affinity catalytic site for K^+ [5,11], permitting binding of ATP and subsequent phosphorylation [5].

General conclusions

These data support the existence of an equilibrium between forms of (Na^+, K^+)-ATPase that is influenced by temperature. Low temperature favors a form with high potassium and low magnesium affinity which hydrolyzes *p*-nitrophenylphosphate. A discontinuity in Arrhenius plots, similar to that previously described for ($Na^+ + K^+$)-ATPase [21,28], characterizes the *p*-nitrophenylphosphate reaction in the presence of Na^+ and ATP. This discontinuity is absent from plots of *p*-nitrophenylphosphatase when Na^+ and ATP are not present. Above the transition temperature, activation energy increases with cation affinity and correlates closely with physical properties of the activating cation.

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