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The temperature dependence of human erythrocyte transport of phosphate, phosphite and hypophosphite

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The temperature dependence of the erythrocyte anion transport protein (Band 3 or AE1) mediated influx of three nonspherical substrates, the divalent anions phosphate and phosphite, and the monovalent hypophosphite, were determined. Phase transitions were found in the temperature dependence of the influxes of all three anions. The 95% confidence limits for the transition temperatures were: 34.6–38.1°C, 7.4–9.1°C and 6.7–9.7°C for phosphate, phosphite and hypophosphite, respectively, while the critical influx rates at the transitions were 29–50, 64–102 and 26–58 ions/s per carrier, respectively. That the critical rates rather than the transition temperatures are of similar magnitude indicates that the transitions are related to transport mechanisms rather than to thermal protein conformational changes. These critical rates are two orders of magnitude lower than those reported for the self-exchange of Cl^- and Br^- (Brahm, J. (1977) J. Gen. Physiol. 70, 283–306). The critical rate of monovalent hypophosphite is similar to that of divalent phosphate and phosphite, but not to that of Cl^- indicating that this effect is mediated by the structure of the substrate rather than by its charge. The disparity in the rates r_c at which phase transitions occur in AE1-mediated transport of spherical and nonspherical anions indicates a difference in the interaction between the two classes of anions and the protein.

Introduction

The erythrocyte anion transport protein, AE1 *, is responsible for the rapid, tightly coupled exchange of Cl^- for bicarbonate in the respiratory cycle of the organism. The temperature dependence of AE1-mediated transport has previously been investigated for a variety of spherical, monovalent anions such as Cl^-, Br^- [1,2] and I^- [2] and for nonspherical, divalent anions such as sulfate [3] and phosphate [4]. Brahm has shown that the temperature dependence of the equilibrium self-exchanges of Cl^- and Br^- contain phase transitions at 15°C and 25°C, respectively [1]. The rates at which these phase transitions occurred were equal (4000 ions/s per carrier) and it was suggested that at this critical rate, r_c, a new step in the transport process becomes rate limiting. Glibowicka et al. [3] have determined that the equilibrium exchange of sulfate also contains a phase transition between 30 and 37°C. The r_c, however, is approx. 7 ions/s per carrier, much lower than that found for Cl^- and Br^-. That the r_c of sulfate transport is different than that of halide transport may result from any of a variety of factors such as charge, structure and relative transport rates. Sulfate is a nonspherical divalent anion whose transport rate is three orders of magnitude lower than that of Cl^- and Br^- [5].

The transport properties of the phosphate analogs have been reported previously by our laboratory [6,7]. The influx of phosphite, the phosphate analog that structurally most resembled bicarbonate in having three oxygen atoms, was found to be the most rapid. The influx of the monovalent hypophosphite was nearly as rapid as that of phosphite and much more rapid than that of phosphate. In the present study the temperature dependencies of the influx of phosphate and the phosphate analogs, phosphite and hypophosphite, were measured in order to determine which of the above factors

* The nomenclature for membrane anion transport proteins was proposed at the 1989 Fukuoka Conference on the Erythrocyte Anion Transporter. The proceedings have been published: (1989) Anion transport protein of the red blood cell membrane (Hamasaki, N. and Jennings, M., eds.), Elsevier, Amsterdam.

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are responsible for the disparity between the r_c of sulfate and that of the halides.

Materials and Methods

Reagents. Phosphite and hypophosphite, as free acids, and sodium gluconate were obtained from Alfa Products, Danvers, MA. Phosphoric acid was obtained from Fisher Scientific, Springfield, NJ. The phosphate assay reagents and 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS) were obtained from Sigma Chemical Company, St. Louis, MO.

Erythrocyte preparation. Erythrocytes were prepared from outdated blood obtained from the University of Illinois Hospital Blood Bank. For the phosphite and hypophosphite influx studies the red cells were washed four times in an isotonic 135 mM NaCl and 15 mM Hepes solution (pH 7.4). The cells were then resuspended in the Hepes-buffered saline solution and packed by centrifugation to a packed cell volume (PCV) of 95%. For the phosphate influx studies the red cells were incubated at 37°C in a buffer containing 135 mM NaCl and 15 mM Bistris (pH 6.0) for 3 h at a PCV of 15% to reduce the initial intracellular phosphate content. The cells were then incubated in a Hepes-buffered saline solution (pH 7.0) for 3 h at 25°C to restore the intracellular pH. Subsequent treatment was in the same manner as for the phosphite and hypophosphite influx studies.

Influx solutions. The anion influx solutions consisted of 120 mM phosphate, phosphite or hypophosphite. Phosphate and phosphite are titratable anions with a slight temperature dependence to their pK_a values. For example, the pK_a of phosphate ranges from 6.98 at 0°C to 6.84 at 60°C. Therefore, it is possible for changes in the ratio of monoionized/diionized species over the temperature ranges employed in this study to contribute to the temperature dependence of the influx rates for these compounds. To avoid this, the pH values of all influx solutions were fixed to pH 7.00 with NaOH at a constant temperature (25°C) prior to warming or cooling, and used without further pH adjustment, to hold the ratio of monoionized:diionized species invariant at all temperatures. The hypophosphite solution also contained 15 mM citrate for buffering. The osmolarity of each solution was adjusted to 295 ± 5 mosM by addition of sucrose when necessary. The pH of each influx solution was 7.00 ± 0.15 over the temperature range used.

Anion influx studies. For each anion studied, packed cells were rapidly mixed with the corresponding influx solution and after a specified time an excess of a 'stopping' solution was added to arrest further influx. The stopping solution contained 130 mM sodium gluconate, 15 mM Hepes and 0.75 mM SITS (pH 7.4) and was kept ice-cold. For the phosphate influx study the cells were kept at 25°C until mixed with the influx solution to minimize the time of exposure of the cells to high temperatures. The ratio of packed cells/influx buffer was 0.3 ml : 3.0 ml. For the phosphite and hypophosphite studies the cells and influx solutions were allowed to equilibrate to the specified temperature. The ratio of packed cells/influx buffer was 1.0 ml : 3.0 ml. Based upon the 95% PCV of the packed cells, these ratios led to an initial extracellular concentration of Cl^- in the incubation mixtures of about 0.7 mM for the phosphate studies and 2.2 mM for the phosphite and hypophosphite studies. The influx times chosen varied from 3 sec to 6 h depending on the anion and temperature. Two to four influx times were used to determine the rate at each temperature as described below. After the influx was stopped, the cells were washed once with the stop solution and packed by centrifugation. In control experiments 1 mM SITS was added to the influx solution prior to mixing with the cells. The packed cells were assayed for intracellular anion as described below.

Colorimetric phosphate assay. Perchloric acid extracts of the post incubation erythrocyte samples were made by adding 3.75 ml of ice cold 7% perchloric acid to the packed cells, followed by centrifugation. The supernatants were then assayed for phosphate colorimetrically by a modified method of Fiske and SubbaRow [8]. Pre-incubation red cell control samples were similarly assayed to determine the initial phosphate content.

^{31}P-NMR phosphite and hypophosphite assay. Hemolysates were made by adding 2.40 ml of distilled water to the packed cells, mixing vigorously and freeze-thawing prior to NMR analysis. Some phosphate influx samples were prepared in the same manner to test for the equivalence of the colorimetric and NMR determinations. The two methods gave identical results within error. The NMR spectrometers were a Nicolet 360 NB using a 10 mm Broad-Band probe operating at 146 MHz and a General Electric GN 500 using a 10 mm High-Band probe operating at 202 MHz for ^{31}P. The spectra were obtained at room temperature. Samples were spun at 8–12 Hz to improve signal resolution. Spectra were collected into either 4K or 8K data points using quadrature detection and signal averaging. 45 degree excitation pulses were used with short delay times to maximize the signal to noise. Under these conditions the phosphorous resonances experienced partial saturation. Hence, calibration of NMR integrals was accomplished using reference samples of known phosphorous content and hemoglobin/electrolyte content identical to that of the experimental samples. The acquisition parameters for the reference and experimental samples were identical.

Analysis of data. Influx rates were determined from the intracellular concentration of anion, the initial volume of cells and the influx times selected. For the more rapidly transported phosphite and hypophosphite

at higher temperatures the intracellular concentrations were measured at three or four different time points at a given temperature, and an exponential fit of concentration versus incubation time was made using nonlinear least-squares analysis. The true initial rate was estimated from the slope of the fit at zero time. Otherwise, under conditions of slow transport duplicate points were taken at the same incubation time and the initial influx rate was estimated as the quotient of the average intracellular concentration of anion and the influx time. This linear approximation was found to provide a good estimate of the initial transport rate provided that the intracellular concentration was less than 20% of its expected equilibrium value. The rates were expressed in terms of ions/s per carrier using the value of 10^6 carriers/cell [9] and a cell water volume of 70% of the initial cell volume [10].

Arrhenius diagrams were prepared by plotting the natural log of the rate versus $1000/T$. To determine whether a two-line fit of the data was warranted it was necessary to find the best temperature at which to separate the two regression fits. This temperature was selected as the one at which the multiple correlation coefficient of a two-line fit was a maximum. Linear regression was then used to obtain a fit for all data below this temperature and a second fit for all data above this temperature. The validity of the two-line fit was determined using a t-test for the difference between the slopes of the two regression lines.

The temperature at which the phase transition occurred, T_c, was found by the intersection of the two linear regression fits. The method used to determine the confidence interval of this point of intersection was that of Filliben and McKinney [11]. It is interesting to note that the midpoints of the T_c and r_c confidence intervals are not equal to the best estimate for T_c and r_c.

Results

Description of anions studied

Phosphate and phosphite are divalent anions that titrate from monoionized to diionized species near physiological pH, with pK_a values of 6.85 and 6.4, respectively [7]. Hypophosphite is a monovalent anion which is completely ionized at pH 7.0. These anions are tetrahedral in shape [12] and larger than the physiological substrates of AE1, namely Cl^- and HCO_3^-. The transport rates of phosphite and hypophosphite are about two orders of magnitude more rapid than that of phosphate [6].

Temperature dependence of phosphate influx into Cl^--loaded cells

The temperature dependence of phosphate influx was studied from 4.5°C to 52.0°C at an extracellular pH of 7.00 ± 0.15. Control experiments, described previously, were done to detect possible non-AE1 mediated phosphate influx due to thermally induced membrane damage at the high temperatures employed. For the rate determination at 52.0°C the cells were incubated at this temperature for only 5 s, and no significant non-AE1 mediated leak was found. Glibowicka et al. [3] have determined that the Cl^- binding site of AE1 remains intact at 60°C for longer than 5 s.

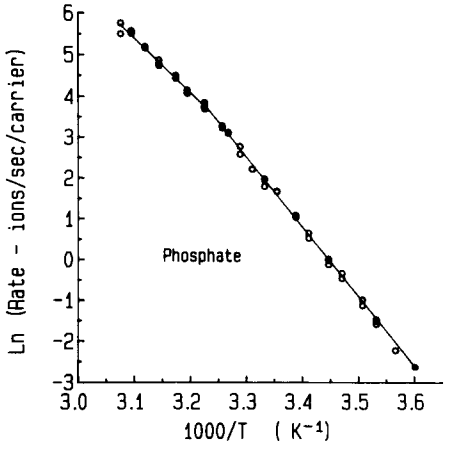

Fig.1. Arrhenius plot of the temperature dependence of phosphate influx into Cl^- loaded human erythrocytes at an extracellular pH of 7.00 ± 0.15. The E_a values given for the two linear regions are in kcal/mol. The ordinate represents the natural log of the influx rate in terms of ions/s per carrier. The correlation coefficients for the regressions fits are $r = 0.999$ for the low-temperature fit and $r = 0.994$ for the high-temperature fit. The slopes of the two regression lines are significantly different at $P = 0.005$.

The temperature dependence of phosphate influx is shown in Fig. 1. The data represent three independent experiments with a total of 52 points. Influx rates ranged over three orders of magnitude from about 0.1 ions/s per carrier at 4.5°C to 300 ions/s per carrier at 52.0°C. There are two linear regions in the plot. There was a significant difference between the slopes of the two linear regression fits at $P = 0.005$. The best fit for the phase transition temperature, T_c, was 36.4°C (34.6–38.1°C, $P = 0.05$). The thermodynamic data are given in Tables I and II. The activation energy, E_a, was 34.0 ± 0.3 kcal/mol below T_c and 26.1 ± 0.7 kcal/mol above T_c. The influx rate at which the phase transition occurred, r_c, was 40 (29–50, $P = 0.05$) ions/s per carrier.

Temperature dependence of phosphite and hypophosphite influx into Cl^--loaded cells

The temperature dependencies of phosphite and hypophosphite transport were measured from 2.0°C to 32.0°C at an extracellular pH of 7.00 ± 0.15 (Fig. 2). The data represent two independent experiments with a total of 23 points for phosphite and one experiment with 10 points for hypophosphite. The influx rates for

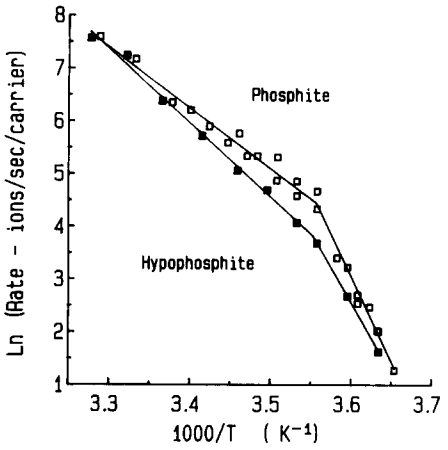

Fig.2. Arrhenius plots of the temperature dependences of phosphite (open symbols) and hypophosphite (solid symbols) influx into Cl^- loaded human erythrocytes at an extracellular pH of 7.00 ± 0.15. The E_a values given for the two linear regions are in kcal/mol. The ordinate represents the natural log of the influx rate in terms of ions/s per carrier. The correlation coefficients for the regression fits are $r = 0.985$ and $r = 0.997$ for the low-temperature region for phosphite and hypophosphite, respectively, and $r = 0.997$ and $r = 0.999$ for the high-temperature region for phosphite and hypophosphite, respectively. The slopes of the two regression lines are significantly different at $P = 0.005$ for both phosphite and hypophosphite.

both anions varied from about 1 ion/s per carrier at 2.0°C to 2000 ions/s per carrier at 32.0°C. The transport of these compounds above 32.0°C was too fast to be measured accurately by the methods employed. Distinct phase transitions for both phosphite and hypophosphite are present. For each compound there was a significant difference between the slopes of the two linear regression fits at $P = 0.005$. The thermodynamic data are given in Tables I and II. The T_c was 8.2°C (7.4–9.1°C, $P = 0.05$) for phosphite and 8.3°C (6.7–9.7°C, $P = 0.05$) for hypophosphite. For phosphite transport, E_a was 64.0 ± 3.3 kcal/mol below T_c and 22.8 ± 1.2 kcal/mol above T_c. For hypophosphite, E_a was 53.4 ± 0.4 kcal/mol below T_c and 27.8 ± 1.0 kcal/mol above T_c. The influx rates at T_c were 89 (64–102, $P = 0.05$) ions/s per carrier for phosphite and 45 (26–58, $P = 0.05$) ions/s per carrier for hypophosphite.

TABLE I

Phase transition temperatures (T_c) and transition rates (r_c)

Anion	T_c (°C)	r_c (ions/s per carrier)
PO_4^{3-}	(34.6–38.1) [a]	(29– 50) [a]
PO_3^{2-}	7.4– 9.1	(64–102)
PO_2^-	(6.7– 9.7)	(26– 58)
SO_4^{2-} (Ref. 3)	30–37	7
Cl^- (Ref. 1)	15	4000
Br^- (Ref. 1)	25	4000

[a] The values represent 95% confidence intervals.

Discussion

The temperature dependence of AE1-mediated influxes for the anions phosphate, phosphite and hypophosphite exhibit phase transitions. These transitions break the Arrhenius plots into two linear regions. The activation energies at temperatures above the transition assume smaller values than at temperatures below the transition. Although the temperature at which the transition occurs is much higher for phosphate than for the other anions, which are more rapidly transported, the rate at which the transition occurs only varies by a factor of two among all three anions. The T_c and r_c for the anions studied presently are compared to those previously published for the self-exchange of sulfate, Cl^- and Br^-, in Table I.

Thermodynamic analysis of the transport of phosphate, phosphite and hypophosphite

Using transition state theory [13] the activation enthalpy and entropy of this transport process can be calculated:

$$r = K_b T/h \, e^{(\Delta S^\dagger/R)} e^{(-\Delta H^\dagger/RT)} \quad (1)$$

TABLE II

Thermodynamic parameters for anion transport

Anion	Parameters (units: kcal/mol)							
	Below transition				Above transition			
	$\Delta G^\dagger$	$\Delta H^\dagger$	$T\Delta S^\dagger$	E_a	$\Delta G^\dagger$	$\Delta H^\dagger$	$T\Delta S^\dagger$	E_a
PO_4^{3-}	18	33	16	34	16	26	10	26
PO_3^{2-}	14	63	49	64	13	22	9	23
PO_2^-	15	53	39	53	13	27	14	28
SO_4^{2-} [a]	17	29	12	32	16	23	7	24
Cl^- [b]	13	29	15	30	12	19	7	20
Br^- [b]	14	31	17	32	12	21	8	22

[a] From Glibowicka et al. (1988) [3].
[b] From Brahm (1977) [1] and calculated by Glibowicka et al. (1988) [3].

where r is the rate in ions/s per carrier, K_b is the Boltzmann constant, T is the temperature in Kelvin, h is Planck's constant, R is the gas constant and $\Delta S^\dagger$ and $\Delta H^\dagger$ are the activation entropy and enthalpy. The Arrhenius activation energy, E_a, is related to $\Delta H^\dagger$ by:

$$E_a = \Delta H^\dagger + RT \tag{2}$$

At 25°C RT is equal to 0.6 kcal/mol so $\Delta H^\dagger$ and E_a are about equal to each other. From a plot of the $\ln(r)$ versus $1/T$, E_a can be determined from the slope of the line and the $\Delta S^\dagger$ from the Y intercept. The Gibbs free energy of activation, $\Delta G^\dagger$, is found by:

$$\Delta G^\dagger = \Delta H^\dagger - T\Delta S^\dagger \tag{3}$$

In Table II the thermodynamic parameters are listed for the influx of the anions studied along with those previously reported for the self-exchange of sulfate, Cl^- and Br^-. The parameters are provided at temperatures below and above the respective T_c for each anion.

The $\Delta H^\dagger$ and $T\Delta S^\dagger$ values for the transport of all the anions above their respective T_c values are fairly close. The E_a for the transport of all the anions above T_c varies only from 22–28 kcal/mol. Below their respective T_c values, however, the $\Delta H^\dagger$, $T\Delta S^\dagger$ and E_a are much larger for phosphite and hypophosphite than for the other anions. The $T\Delta S^\dagger$ is positive for all of the anions above and below T_c and quite large for transport below T_c.

It is clear that the phase transitions found in AE1 mediated transport are not related to thermally induced conformational changes in the protein or membrane lipid because of the wide range of T_c values found. In the present study the T_c values had a range of 8–36°C. Phase transitions related to thermally induced conformational changes would exhibit a single T_c independent of the transported anion.

The r_c values of the phase transitions appear to fall into two groups: halides having a critical rate around 4000 ions/s per carrier and non-spherical anions having critical rates of 7–80 ions/s per carrier. The rates among the three phosphate analogs are quite similar (ranging from 40–80 ions/s per carrier), while the rate found by Glibowicka et al. [3] for sulfate (7 ions/s per carrier) is lower. Although the sulfate r_c was determined under experimental conditions (self-exchange in erythrocyte ghosts at pH 6.5) that differed radically from the current study, the r_c value for sulfate exchange is nonetheless considerably closer to the values for the phosphate analogs than to Brahm's values for the halides [1]. We propose that the differences between the rates at which the phase transitions occur for the halides and for the nonspherical anions are due to the differences in their chemical structure.

The differences between the r_c values of the phosphate analogs and of the halides are not related to anion charge because the monovalent hypophosphite has an r_c equal to that of the divalent phosphate, but two orders of magnitude lower than the r_c of Cl^- and Br^-.

The hypothesis which we have proposed may be tested by an examination of the temperature dependence of the transport of another spherical anion such as I^-. The transport rate of I^- is similar to that of phosphite and more than 10^{-2} times slower than Cl^- [5]. If the r_c is related to the chemical structure of the anion being transported, then one would predict that I^- transport would exhibit an r_c near that of Cl^- and Br^- (4000 ions/s per carrier). Because the transport of I^- is much slower than that of Cl^- and Br^-, a fairly high temperature would be required to produce a rate of 4000 ions/s per carrier. Based on previously reported data [2] one would estimate a T_c near 40°C. At temperatures below 40°C our hypothesis predicts that the E_a of I^- transport would be similar to the E_a values for Cl^- and Br^- below their, respective transitions, about 32 kcal/mol [1], and also that no transition would occur at the slower rate where transitions are found for nonspherical anions.

Dalmark and Wieth have studied the temperature dependence of the equilibrium self-exchange of I^- from 0 to 38°C [2] and found a single E_a of 35.5 kcal/mol over this range. The self-exchange rate of I^- ranged from < 1 ion/s per carrier at 0°C to > 2000 ions/s per carrier at 38°C. That no transitions were found agrees well with our hypothesis that the phase transitions between 7 and 80 ions/s per carrier is limited to nonspherical anions. The E_a of I^- self-exchange is similar to that of Cl^- and Br^- below their respective phase transitions, suggesting that a phase transition should occur above 38°C. These data agree well with the latter two of the three predictions outlined above. Measurements of the temperature dependence of I^- transport at temperatures above 38°C will be required to verify the existence of the predicted phase transition near 4000 ions/s per carrier.

Kinetic implications

In the present study, conditions of hetero-exchange were employed with the influx of the test anion coupled to the efflux of Cl^-. Because all of the anions studied permeate the membrane much more slowly than Cl^-, Passow [14] has shown that the equation for r can be simplified to:

$$r = k_{in} N_{AE1} / (1 + Q) \tag{4}$$

where N_{AE1} is the total number of carriers, k_{in} is the rate of translocation of the outward facing test anion loaded carrier to the inside and Q is:

$$Q = k_{in} / k_{out(Cl)} * (1 + K_{d(P^-)} / [P^-]) \tag{4a}$$

where $k_{out(Cl)}$ is the rate of translocation of the inward facing Cl^--loaded transporter to the outside. $K_{d(P^-)}$ is the dissociation constant of the test anion and the outward facing transporter and $[P^-]$ is the concentration of the extracellular test anion. At 120 mM extracellular concentration of test anion, $k_{d(P^-)}/[P^-]$ is < 1 because the $k_{1/2}$ for the influx of all the anions studied is less than 100 mM [6]. $k_{in}/k_{out(Cl)}$ is $\ll 1$ due to the fact that Cl^- is transported much more rapidly than the anions studied. We can thus consider $Q \ll 1$ and Eqn. 4 reduces to:

$$r = k_{in} N_{AE1} \tag{5}$$

Transition state theory [13] predicts the temperature dependence of r to be:

$$r = k_{in}^{\circ} N_{AE1} e^{(\Delta S_{in}^{\dagger}/R)} e^{(-\Delta H_{in}^{\dagger}/RT)} \tag{6}$$

where k_{in}° is the temperature-independent portion of k_{in}. $\Delta S_{in}^{\dagger}$ and $\Delta H_{in}^{\dagger}$ are the activation entropy and activation enthalpy of k_{in}. Therefore, the temperature dependence contains only one rate constant associated with the translocation of the anion-loaded transporter from the outside of the membrane to the inside and is not dependent upon the Cl^- efflux rate. Hence, the transition observed at 15°C in Brahm's Cl^- self-exchange studies is not expected to produce a transition in our hetero-exchange studies with the phosphate analogs.

Glibowicka et al. [3] have proposed that the phase transition in the equilibrium self-exchange of sulfate results from a switch in the rate limiting step from that of outward translocation to that of inward translocation. This hypothesis however cannot explain the phase transitions for the phosphate analogs because the temperature dependence of the hetero-exchange with Cl^- is not a function of the outward Cl^- translocation rate. Under our conditions, the phase transition represents a change in the properties of the inward translocation step alone. Our results suggest that the inward translocation consists of more than one reaction step and that the phase transition represents a switch from one rate-limiting step to another during the inward translocation. The inward and outward translocations may or may not have symmetrical rate-limiting steps, and efflux studies with the phosphate analogs will be needed to determine the degree of similarity.

Relationship to physical models

The activation entropies of the transport of all the anions studied are positive and relatively large. Activation entropy is not a direct measure of conformational changes during the activation process, but does correlate well with activation volume [15] and thus is often used to provide inferences regarding the structure of the activation state [3,15,16]. It is thus not surprising that the activation volume for the equilibrium self-exchange of sulfate at 30°C has been found to be large and positive at 150 ± 30 ml/mol [17].

The entropies determined in this study are consistent with the assertion of Glibowicka et al., that AE1 undergoes major structural changes during the activation process. This would support the model of the transport process proposed by Kopito and Lodish [18] in which transmembrane segments slide across each other during the transport cycle. Our results would also be consistent with the model recently proposed by Krupka [5] which assumes that a large conformational change occurs when a polycationic binding pocket snaps tightly around a loosely bound anion. These data do not support 'zipper' models [19] in which only small localized movements are involved in the breaking and forming of 'salt bridges' between positive and negatively charged residues.

Our determination of the activation entropies for the influx of phosphate, phosphite and hypophosphite agree well with those values previously found for sulfate [3] and Cl^- [1] at temperatures above their respective T_c. Below T_c however, the activation entropies for phosphite and hypophosphite influx are twice as large as measured for the other anions. This suggests that the transition state for the inward translocation of the phosphite or hypophosphite-loaded carrier is structurally different than that of the carrier loaded with the other anions at low temperatures.

As mentioned earlier, studies on the transport rates of phosphate, phosphite and hypophosphite have shown that AE1-mediated transport has specificity for anions containing three oxygen atoms, presumable due to structural similarities to bicarbonate. The transition state for the inward translocation of the phosphite-loaded carrier may be similar to that of the bicarbonate-loaded carrier due to the structural similarity of bicarbonate and phosphite. It is possible that the difference between the activation entropies measured for Cl^- and phosphite reflect a similar difference between the activation states of the Cl^- and bicarbonate-loaded carriers. Thus the transition state of the bicarbonate-loaded carrier may be different than for the Cl^--loaded carrier. This would not be surprising based on the structurally dissimilarity of these physiologic substrates.

Concluding remarks

The temperature dependencies of the influxes of phosphate, phosphite and hypophosphite contain phase transitions. Comparison of these transitions to those reported for the self-exchange of sulfate, Cl^- and Br^- suggests that the transition rates are different for spherical and nonspherical anions. This indicates a difference in the interaction between the two classes of anions and the protein, consistent with a transporter that is highly specific for two dissimilar physiologic substrates.

This study was conducted under conditions of hetero-exchange with Cl^-. The measured influx rates were a function of the influx translocation rate, k_{in}, alone, suggesting the possibility of more than one step during the inward translocation process. The previous studies [1,3] were conducted under conditions of self-exchange, measuring the efflux of a radiolabeled anion. The measured transport rates in such self-exchange studies may be a function of the k_{in} or k_{out} alone, or a mixture of the two rate constants depending on their relative magnitudes. Thus while this study has shown the existence of phase transitions in the influx translocation of AE1, it is uncertain as to whether the phase transitions found in self-exchange studies represent transitions in the efflux or influx translocations (or both). Future efflux studies will help in determining whether the phase transitions found in AE1-mediated transport are specific to the influx translocation or also occur in the efflux translocation.

Above their respective T_c values the activation entropies and energies for Cl^-, Br^-, sulfate, phosphate, phosphite and hypophosphite are similar, suggesting structural similarities in the anion-loaded inward translocation activation states of all the anions considered. At temperatures below T_c, however, activation energy and entropy are dependent on the anion being transported. The phosphite or hypophosphite-loaded carrier has a transition state with an activation energy and entropy different than that of the carrier loaded with Cl^-. This is suggestive of differences between the activation state of the translocation of Cl^-- and bicarbonate-loaded carriers at lower temperature.

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