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EFFECT OF THE SODIUM/POTASSIUM RATIO ON GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE INTERACTION WITH RED CELL VESICLES

ERIC T. FOSSEL [a] and A.K. SOLOMON [b]

[a,b] *Biophysical Laboratory, Harvard Medical School, Boston, MA 02115 and* [a] *Francis Bitter National Magnet Laboratory, Cambridge, MA 02139 (U.S.A.)*

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Summary

Binding of glyceraldehyde 3-phosphate to glyceraldehyde-3-phosphate dehydrogenase, the membrane protein known as Band 6, causes shifts in the ^{31}P nuclear magnetic resonance spectrum of the substrate (Fossel, E.T. and Solomon, A.K. (1977) Biochim. Biophys. Acta 464, 82—92). We have studied the resonance shifts produced by varying the sodium/potassium ratio, at constant ionic strength, in order to examine the relationship between the cation transport system and glyceraldehyde-3-phosphate dehydrogenase. Alteration of the potassium concentration at the extracellular face of the vesicle affects the conformation of glyceraldehyde-3-phosphate dehydrogenase at the cytoplasmic face, thus showing that a conformation change induced by a change in extracellular potassium can be transmitted across the membrane. Alterations of the sodium concentration at the cytoplasmic face also affect the enzyme conformation, whereas sodium changes at the extracellular face are without effect. In contrast, there is no sidedness difference in the effect of potassium concentrations. The half-values for these effects are like those for activation of the red cell ($Na^+ + K^+$)-ATPase. We have also produced ionic concentration gradients across the vesicle similar to those Glynn and Lew ((1970) J. Physiol. London 207, 393—402) found to be effective in running the cation pump backwards to produce adenosine triphosphate in the human red cell. The sodium/potassium concentration dependence of this process in red cells is mimicked by ^{31}P resonance shifts in the (glyceraldehyde 3-phosphate/glyceraldehyde-3-phosphate dehydrogenase/inside out vesicle) system. These experiments provide strong support for the existence of a functional linkage between the membrane ($Na^+ + K^+$)-ATPase and the glyceraldehyde-3-phosphate dehydrogenase at the cytoplasmic face.

Introduction

The membrane protein known as Band 6 has been shown to be glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) which binds to the cytoplasmic side of Band 3, the major transmembrane protein in human red cells [1—5]. Both McDaniel et al. [3] and Kant and Steck [4] have found the binding of glyceraldehyde-3-phosphate dehydrogenase to be dependent upon ionic strength. Fossel and Solomon [6] suggested that glyceraldehyde-3-phosphate dehydrogenase plays a role in the linkage of cation transport in the red cell to glucose metabolism.

We have used nuclear magnetic resonance (NMR) to examine the conformation of glyceraldehyde-3-phosphate dehydrogenase, using glyceraldehyde 3-phosphate as a probe whose interactions with the enzyme may be monitored by ^{31}P resonance shifts [6]. The basis for implicating glyceraldehyde-3-phosphate dehydrogenase in the cation transport system of human red cells is our observation that the specific $(Na^+ + K^+)$-ATPase inhibitor, ouabain, inhibits the resonance shift normally observed when glyceraldehyde-3-phosphate dehydrogenase interacts with the cytoplasmic face of inside out red cell vesicles. The use of inside out vesicles makes it possible to vary the cation concentration separately on both faces of the membrane while keeping the ionic strength constant. The dependence of the ^{31}P resonance shift on the sodium and potassium concentrations on each membrane face is consistent with a functional linkage between the conformation of glyceraldehyde-3-phosphate dehydrogenase and the red cell cation transport system.

Experimental Methods

Blostein and Chu [7] have shown that inside out red cell vesicles can be used to study the sidedness of the action of cations on the $(Na^+ + K^+)$-ATPase and Sze and Solomon [8] have measured the permeability of such vesicles to potassium. The permeation is slow enough that there is no appreciable concentration change within 5—10 min of placing the vesicles in their new environment so that the major requirement is to complete the NMR measurement within that time frame. Fortunately, the time needed to make the measurement is short enough that this requirement can be satisfied.

NMR experiments were either carried out on a JEOL FX-60 Fourier transform NMR spectrometer (JEOL Analytical Instruments, Inc., Cranford, NJ) operating at 24.16 MHz for ^{31}P in 10 mm (outer diameter) tubes, or on a Bruker HX-270 Fourier transform spectrometer (Bruker Instruments, Inc. Billerica, MA) operating at 109.3 MHz for ^{31}P in 10 mm, or occasionally 15 mm, sample tubes as previously described [6]. The 90° resonance frequency pulse width was 20 μs for the FX-60 and 21 μs for the HX-270. Pulse angles of 45—60° were routinely used. Spectral widths of 3 kHz were employed with 8 K data tables. The signal to noise ratio was improved by digital filtering (1.5 Hz line broadening on both the FX-60 and the HX-270). Broad band noise-modulated proton decoupling was used (1 kHz at 12.5 W on the FX-60 and 2 kHz at 1.5 W on the HX-270). In all cases, the spectrometer was locked on the deuterium signal of ^2H_2O.

Experimental reproducibility is highly critical in our experiments as some of the reported chemical shift changes are small. Multiple determinations of chemical shift values on the same sample were reproducible to one computer data point at 24.16 MHz (usually one data point equaled 0.25 Hz). This extremely good reproducibility is due in part to the fact that the peaks measured were very sharp, so that we were able to place 12—25 data points over the upper half of the peak and one data point clearly defined the maximum. At 109.3 MHz, the normal peak half-width for glyceraldehyde 3-phosphate is 3.5 Hz. We were able to place six data points over the top of the peak at the higher frequency and one data point clearly defined the maximum. Hence the resonance position was reproducible to 0.6 Hz on the HX-270. The presence of paramagnetic impurities can clearly be seen by peak broadening. The extensive dialysis used in the preparation of enzymes described below served to eliminate paramagnetic impurities and no broadening was observed, thus indicating that our dialysis procedure had been effective. Furthermore, 0.1 mM EDTA was occasionally added to the buffer in these experiments. The absence of any subsequent change in half-width confirmed that the preparations were free of paramagnetic impurities.

Multiple determinations of chemical shift values in the same sample were reproducible to one data point. However, when samples were prepared from different vesicle preparations on different occasions, differences, of about 0.2 Hz at 24.16 MHz were frequently observed. In one case there was a difference of 1 Hz (at 24.16 MHz) between results obtained in two different vesicle preparations; we have ascribed this to differences in the vesicles themselves. The reproducibility is much better when comparisons are made using the same vesicle preparation and the data for the control measurements in Table I are therefore reported as differences from reference solutions measured at the same time and under the same conditions. In practice, actual differences between experiments under different conditions could be small. The average difference between the results of the two duplicate experiments at 24.16 MHz shown in Fig. 1 was 0.2 Hz (when cytoplasmic Na^+ is altered at 10 mM and 5 mM K^+) though the experiments were done on different days and with different vesicle preparations. The duplicate experiments at 109.3 MHz in Fig. 3 were carried out on the same day with two different sets of solutions made from the same original vesicle preparation. This may account for the very small average difference of 0.1 Hz.

Inside out vesicles and right side out vesicles were produced from dated bank blood by the method of Steck and Kant [9]. The sidedness of the vesicles was routinely measured by the usual acetylcholinesterase assay. The inside out vesicles were 83 ± 7% (S.D., 10 expts.) inaccessible to acetylcholinesterase. We have previously shown [6] that glyceraldehyde 3-phosphate binding to right side out vesicles is about 10% of that to inside out vesicles and have therefore neglected the possible effect of the presence of right side out vesicles in the 17% fraction accessible to acetylcholinesterase. Vesicles were observed at a concentration of 0.7 ± 0.2 unit acetylcholinesterase/ml (S.D., 10 expts.) in NMR buffer: 150 mM NaCl, 5 mM KCl, 17 mM Tris, plus 20% ^2H_2O, pH 7.40. Concentrated glyceraldehyde-3-phosphate dehydrogenase solutions were dialyzed against NMR buffer prior to use. Solutions of glyceraldehyde 3-phosphate were

prepared in this buffer and pH was re-adjusted to 7.40. pH was occasionally checked at the close of experiments and found to remain at 7.40. All experiments were carried out at 24—25° C.

When sodium or potassium concentrations were changed, ionic strength was maintained by the addition of choline chloride. Vesicles were equilibrated with buffer of the selected internal cation concentration and were mixed with a solution of the selected external cation concentrations immediately before the NMR measurement. pH was adjusted to 7.40 in each case. Cation concentrations were checked on one occasion by flame photometry and found to be correct within 1%. The NMR measurements were completed within 7—10 min, so that the internal vesicle concentrations remained essentially at their initial values as discussed below. When ouabain was used in inside out vesicles, it was added during vesicle preparation as previously described [6]. All enzymes and phosphorus-containing molecules were purchased from Sigma Chemical Co., St. Louis, MO.

Results and Discussion

We had previously observed [6] that glyceraldehyde 3-phosphate interacted with the glyceraldehyde-3-phosphate dehydrogenase normally present on the external face of the inside out vesicle and that this interaction was suppressed by the addition of 10^{-6} M ouabain to the internal face of the vesicle. The present experiments follow from these initial observations. Before describing them it is necessary to make a critical examination of the premise that the glyceraldehyde 3-phosphate resonance shifts observed when the cation content of the medium is altered are an index of interactions of the 3-member complex (inside out vesicle/glyceraldehyde-3-phosphate dehydrogenase/glyceraldehyde 3-phosphate). Two control experiments at 24.16 MHz were carried out specifically to demonstrate the validity of this premise and incidentally to confirm the results of other experiments that had been carried out piecemeal in the course of the study. It is necessary to show: (1) that it is glyceraldehyde 3-phosphate dehydrogenase with which the glyceraldehyde 3-phosphate interacts, (2) that the resonance shift not only involves that specific couple, but is also mediated by interactions with the inside out vesicle, and (3) that the ouabain effect results from binding to the specific ouabain site on $(Na^+ + K^+)$-ATPase on the extracellular face of the cell.

The results of these control experiments are presented in Table I. The column headed inside out vesicle shows that the 1.2 Hz resonance shift observed when glyceraldehyde 3-phosphate is added to inside out vesicles is reduced to 0.9 Hz when 1 mM reduced nicotinamide adenosine dinucleotide is added to the sample and further reduced to 0.5 Hz when 1 mM adenosine triphosphate is added in addition. Kant and Steck [4] have shown that treatment with each of these reactants removes around 50% of the glyceraldehyde-3-phosphate dehydrogenase from red cell membranes. In the companion experiment, inside out vesicles were washed with fructose 1,6-diphosphate which Strapazon and Steck [10] had shown to be specific for removing aldolase from Band 3; the resonance shift remained at 1.4 Hz which is characteristic of the 1.2—1.4 Hz shift untreated vesicles. These experiments indicate that a substance which elutes from inside out vesicles or Band 3 with the characteristics of

glyceraldehyde-3-phosphate dehydrogenase is responsible, at least in part, for the observed resonance shifts. A further control strengthens this conclusion. Inside out vesicles were washed with 1 mM reduced nicotinamide adenosine dinucleotide plus 0.1 mM glyceraldehyde 3-phosphate, a process which Kant and Steck [4] had shown to be effective in removing 90% of the bound glyceraldehyde-3-phosphate dehydrogenase. In our hands, the effectiveness of glyceraldehyde-3-phosphate dehydrogenase removal is variable and only occasionally may reach as high a value as 90%. The results in the column headed inside out vesicle-(glyceraldehyde-3-phosphate dehydrogenase) show that addition of glyceraldehyde 3-phosphate to washed vesicles produces a resonance shift of 0.5 Hz, much smaller than the 1.2—1.4 Hz shift, characteristic of untreated vesicles. Upon addition of the couple (glyceraldehyde 3-phosphate/glyceraldehyde-3-phosphate dehydrogenase) the resonance shift increases to 0.8 Hz which is reduced first to 0.5 Hz and then to 0.3 Hz when reduced nicotinamide adenosine dinucleotide is added, first alone and then with adenosine triphosphate. These experiments show that glyceraldehyde-3-phosphate dehydrogenase is an essential component for the resonance shifts.

The evidence for the involvement of the inside out vesicle in the resonance shift is summarized in the bottom section of Table I, which emphasizes the relevant comparisons using the data from the top section of the table. The top comparison shows that there is a resonance shift of —0.3 Hz when glyceraldehyde 3-phosphate is added to glyceraldehyde-3-phosphate dehydrogenase in solution. However when glyceraldehyde 3-phosphate is added to inside out vesicles, the resonance shift becomes +1.2 Hz thus showing that the inside out vesicle is an essential component of the system. This conclusion is strengthened when inside out vesicles, washed to remove glyceraldehyde-3-phosphate dehydrogenase are used. The difference between the —0.3 Hz resonance shift observed for the couple (glyceraldehyde phosphate/glyceraldehyde-3-phosphate dehydrogenase) and the +0.8 Hz resonance shift observed when washed inside out vesicles are added to the couple shows that the presence of the membrane modifies the conformation of glyceraldehyde-3-phosphate dehydrogenase. In still another experiment, the half-width of the enzyme-substrate couple was measured at 109.3 MHz and found to be 3.5 Hz; addition of inside out vesicles increased the half-width to 8.5 Hz consistent with binding of the (glyceraldehyde 3-phosphate/glyceraldehyde-3-phosphate dehydrogenase) couple to the inside out vesicle.

The control experiments were also designed to show that ouabain produced its effect as a result of binding to the highly specific ouabain site on the extracellular face of the human red cell rather than through loose adherence to other parts of the cell surface. Human red cells were incubated at 22—24°C for 30 min with 10^{-6} M ouabain and then washed three times with ouabain free buffer to remove any loosely bound ouabain. Control cells were treated similarly but without ouabain. Inside out and right side out vesicles were prepared from these cells. The resonance shift for the ouabain-treated inside out vesicles was 0.0 Hz, that for both the right side out vesicle preparations was —0.2 Hz. These experiments show that binding of ouabain to its specific site on the extracellular human red cell face inhibits the glyceraldehyde 3-phosphate resonance shifts.

TABLE I

GLYCERALDEHYDE 3-PHOSPHATE RESONANCE SHIFTS IN CONTROL EXPERIMENTS

NADH, nicotinamide adenosine dinucleotide. The concentration of glyceraldehyde 3-phosphate was 5 mM and 5 mM Mg^{2+} was added with it. Glyceraldehyde-3-phosphate dehydrogenase was added to produce a concentration of 0.2 mg/ml. Ouabain was 10^{-6} M. NADH was 1 mM, as was ATP.

Compound added to vesicle suspension	Glyceraldehyde 3-phosphate resonance shifts (ΔHz)					
	No vesicle	Inside out vesicle	Inside out vesicle (+ouabain)	Inside out vesicle -(glyceraldehyde 3-phosphate dehydrogenase)	Right side out vesicle	Right side out vesicle (+ouabain)
Glyceraldehyde 3-phosphate	0.0	1.2 *	0.0	0.5	—0.2	—0.2
+NADH	0.0	0.9				
+NADH + ATP	0.0	0.5				
(Glyceraldehyde 3-phosphate/(Glyceraldehyde-3-phosphate dehydrogenase)	—0.3		0.2	0.8		
+NADH	—0.3			0.5		
+NADH + ATP	—0.3			0.3		

Comparisons	ΔHz
(Glyceraldehyde 3-phosphate)/(glyceraldehyde-3-phosphate dehydrogenase)	—0.3
(Glyceraldehyde 3-phosphate)/(inside out vesicle)	1.2
(Glyceraldehyde 3-phosphate)/(glyceraldehyde-3-phosphate dehydrogenase)	—0.3
(Glyceraldehyde 3-phosphate)/(glyceraldehyde-3-phosphate dehydrogenase)/(inside out vesicles-[glyceraldehyde 3-phosphate dehydrogenase])	0.8

* Inside out vesicles from a different preparation washed with fructose 1,6-diphosphate produce a resonance shift of 1.4 Hz.

These control experiments indicate that there is coupling between the (Na^+ + K^+)-ATPase which contains the ouabain site, Band 3 which contains the glyceraldehyde-3-phosphate dehydrogenase site, and the enzyme itself. The least interesting coupling mechanism would be for ouabain to disconnect glyceraldehyde-3-phosphate dehydrogenase from its site on Band 3 so that no functional interaction remained. This would be an all-or-none effect in which the enzyme was either connected to Band 3 or disconnected. However, the experiments whose results are shown in Fig. 1 indicate that the interaction is more subtle.

Fig. 1 demonstrates that whereas the (glyceraldehyde 3-phosphate/glyceraldehyde-3-phosphate dehydrogenase) resonance shift is essentially independent of the sodium concentration at the extracellular face (of the red cell membrane; the intravesicular face), the resonance shift depends strongly on the cytoplasmic sodium concentration (the extravesicular face). The sodium concentration was varied over the range from 5 to 150 mM and half the maximum effect was observed at a sodium concentration of 20—30 mM. The sodium-induced resonance shift was virtually independent of the potassium concentra-

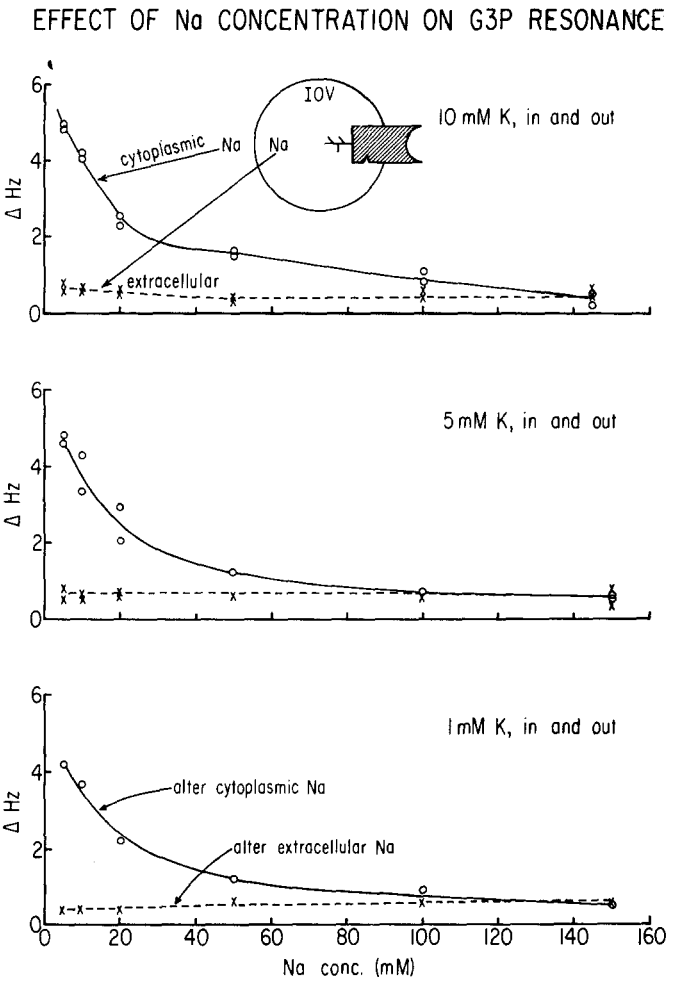

Fig. 1. The effect of Na^+ concentration on the resonance shift of glyceraldehyde 3-phosphate at 24.16 MHz. The K^+ concentrations were the same inside and outside the vesicle, at the values shown in the figure. When the Na^+ concentration was varied on one face of the vesicle, it was maintained at 150 mM on the other face (145 mM in the experiments at 10 mM K^+). This means that the two curves must converge at the highest Na^+ concentration. Two experiments at 10 and 5 mM K^+; one experiment at 1 mM K^+. The shaded symbol represents ($Na^+ + K^+$)-ATPase with the carbohydrate on the inside of the vesicle.

tion as it was varied from 1 to 10 mM, with no potassium gradient across the membrane. There is an internal control in these experiments in that the point at 150 mM sodium must have the same value for changes of either intra- or extravesicular sodium.

When potassium is added to the extracellular face (intravesicular) in the presence of 0—15 mM sodium, the resonance shifts smoothly with the potassium concentration at the extracellular face as shown in Fig. 2. Since the cytoplasmic face sodium and potassium concentrations were fixed and since the ionic strength at both membrane faces was maintained constant by the addition of choline chloride, these experiments demonstrate a functional linkage

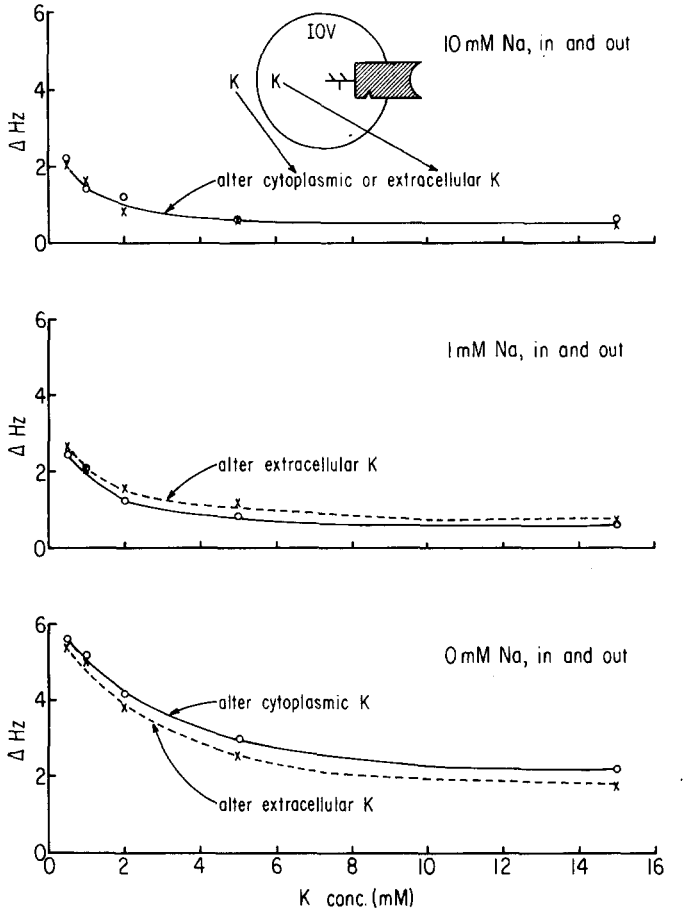

Fig. 2. The effect of K^+ concentration on the resonance shift of glyceraldehyde 3-phosphate at 24.16 MHz. The Na^+ concentrations were the same inside and outside the vesicles, at the values shown in the figure. When the K^+ concentration was varied on one side of the membrane, it was kept constant at 1 mM K^+ on the other face, which means that the point at 1 mM K^+ must have the same value, whether the cytoplasmic or the extracellular K^+ concentration was varied. One experiment, typical of two, at 10 mM; one experiment at the other concentrations.

between a potassium-sensitive site on the extracellular face of the red cell membrane and the conformation of glyceraldehyde 3-phosphate dehydrogenase on the cytoplasmic face. Taken in conjunction with the ouabain inhibition the present results strongly suggest that the ((Na^+ + K^+)-ATPase/Band 3/glyceraldehyde-3-phosphate dehydrogenase) linkage can convey information from outside of the cell to the inside.

This transmembrane effect on glyceraldehyde-3-phosphate dehydrogenase is in general agreement with the observations of Blostein and Chu [7] on the (Na^+ + K^+)-ATPase. They found that 2 mM potassium on the extracellular face of inside out vesicles stimulated ATP hydrolysis on the cytoplasmic side in the presence of 40 mM sodium. At low adenosine triphosphate and sodium concentrations Blostein and Chu [7] found that potassium on either face of the

membrane inhibited adenosine triphosphate hydrolysis. These results are not directly comparable to ours since Blostein and Chu's studies [7] were concerned with the detailed mechanism of the ($Na^+ + K^+$)-ATPase and required, for example, adenosine triphosphate which was absent in our system. Fig. 2 shows that alteration of the potassium concentration at the cytoplasmic face produces essentially the same effect as on the extracellular face.

The similarity of the effects of intra- and extravesicular potassium might be ascribed to leakiness of the vesicles. However Sze and Solomon [8] have shown that about 10% of the vesicle K^+ leaks out in about 10 min. Since the NMR experiments required 5 min of data accumulation plus 2—3 min for preparation, potassium permeation would not have been greater than, say 10%. Blostein and Chu's observation [7] that vesicle permeation by sodium and potassium is closely similar, makes it possible to check the sidedness of the vesicles used for the potassium experiments by using the same preparation for sodium experiments. Consequently, the experiments at 10 mM sodium in Fig. 2, which showed no sidedness, were carried out on the same preparation of vesicles used in the 5 mM potassium experiment in Fig. 1 which exhibited the usual sodium sidedness. This result means that the absence of a sidedness effect in the experiments in Fig. 2 is probably not to be ascribed to leaky vesicles.

The experiments in Fig. 2 also demonstrate an antagonism between sodium and potassium, since increases in the sodium concentration cause a decrease in the resonance shift, which is at a maximum in the absence of sodium. This is consistent with the antagonistic effects of sodium and potassium as shown, for example, in the observation of Post et al. [11] that high concentrations of extracellular sodium inhibit the activation of the cation pump by extracellular potassium. In these classical experiments of Post et al. [11] the half-maximal cytoplasmic sodium concentration for cation transport was about 20 mM; the half-maximal extracellular potassium concentration for potassium influx [12] was 2 mM. Post et al. [11] showed that the half-maximal cation concentrations for activating the ($Na^+ + K^+$)-ATPase were similar to the half-values for cation transport, being 24 mM sodium and 3 mM potassium. The potassium site is at the external face of the ($Na^+ + K^+$)-ATPase and the sodium site at the internal face (see ref. 13). The half-maximal values for the resonance shifts shown in Figs. 1 and 2 are very similar to those found by Post et al. [11] and the sidedness for sodium is the same as for the ($Na^+ + K^+$)-ATPase, notwithstanding our observation that the potassium-related resonance shift shows no sidedness. These similarities are consistent with a linkage between the glyceraldehyde-3-phosphate dehydrogenase conformation and the state of activity of the ($Na^+ + K^+$)-ATPase.

The cation transport system in the human red cell can be driven backwards to produce adenosine triphosphate by imposing a steep sodium concentration gradient in the reverse direction. Glynn and Lew [14] prepared red cells with an intracellular sodium concentration of 3.3 mM and a potassium concentration which we have estimated as being about 100 mM. Under these conditions they found that the incorporation of ^{32}P into adenosine triphosphate increased linearly with extracellular sodium concentration up to 120 mM in the absence of extracellular potassium. Addition of extracellular potassium decreased the incorporation hyperbolically with half-maximal activity at a potassium con-

centration of 1.3 mM, as shown in the bottom section of Fig. 3. We have reproduced their conditions in inside out vesicles and obtained the results shown in the top section of Fig. 3. Though the resonance shifts are not large, they are very consistent in the two experiments shown and reproduce the shape of Glynn and Lew's curves very well. Though the sodium activation is not quite linear, it is not far different; the half-value for potassium inhibition is 1.5 mM. 10^{-6} M ouabain suppresses the vesicle-induced glyceraldehyde-3-phosphate dehydrogenase resonance shift, as previously shown; it also suppresses ATP synthesis. This experiment provides convincing evidence that there is a functional linkage between the (Na^+ + K^+)-ATPase and glyceraldehyde-3-phosphate dehydrogenase.

When this conclusion is taken in conjunction with the effect of ouabain on the glyceraldehyde-3-phosphate dehydrogenase conformation and the evidence that glyceraldehyde-3-phosphate dehydrogenase binds to its own specific site

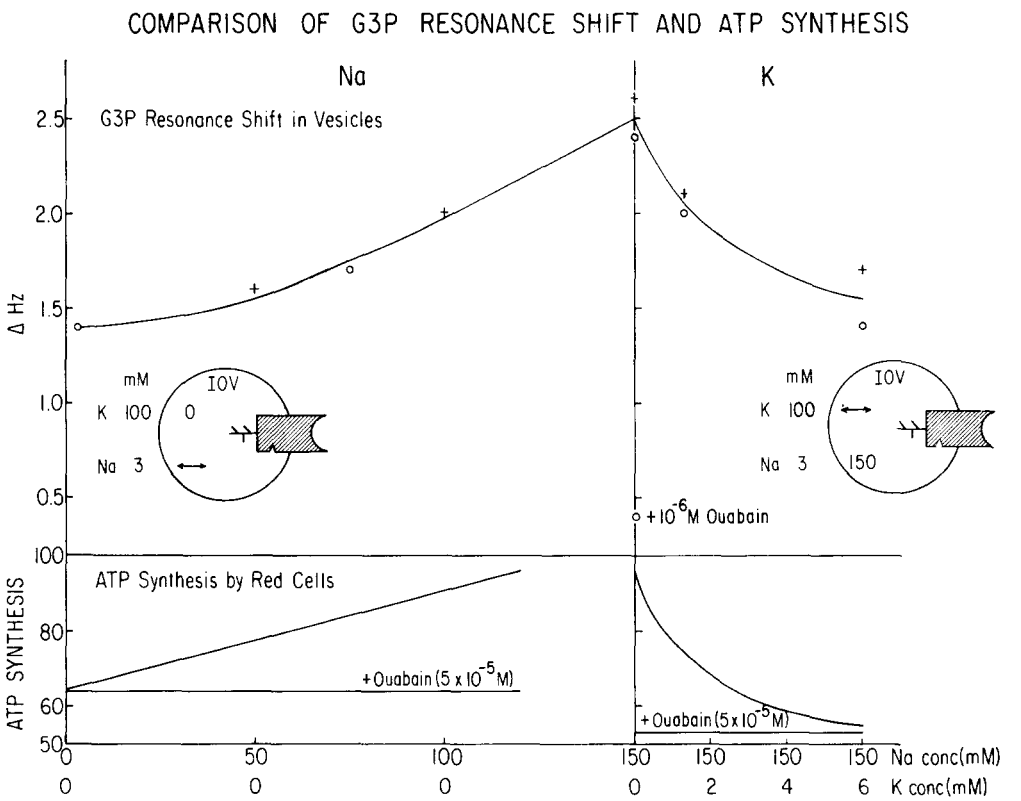

Fig. 3. Top: resonance shifts at 109.3 MHz for glyceraldehyde 3-phosphate (G3P) plus inside out vesicles; data from 2 experiments at 24—25° C, pH 7.4, typical of 4. The extravesicular solution (cytoplasmic face) contained 100 mM Na^+ and 3 mM K^+ plus 5 mM Mg^{2+} and 5 mM glyceraldehyde 3-phosphate. The intravesicular solution (extracellular face) was the NMR buffer with the cation compositions indicated on the abscissa. Bottom: ATP synthesis by red cells redrawn from Glynn and Lew [14]. The extracellular cation concentrations are indicated on the abscissa and the intracellular Na^+ and K^+ concentrations we have estimated as 3 mM Na^+ and 100 mM K^+, pH 7.4 at 37° C.

on Band 3, the evidence indicates a functional linkage: ($(Na^+ + K^+)$ATPase/ Band 3/glyceraldehyde-3-phosphate dehydrogenase). The case for a linkage between the first and third members of the assembly is very strong, as is the need for a membrane component to transmit the signal from one face to the other. It has still not been proved, though the evidence is suggestive, that the membrane component is Band 3. Since the $(Na^+ + K^+)$-ATPase is also a transmembrane protein, it would be possible for it to react with glyceraldehyde-3-phosphate dehydrogenase, though no binding sites for it have been suggested. Thus, further experiments will be required to determine what role Band 3 actually plays.

Glyceraldehyde-3-phosphate dehydrogenase sits in the metabolic chain astride a bifurcation in the glycolysis pathway. Its product, 1,3-diphosphoglycerate, can either by metabolised by phosphoglycerate kinase to produce 3-phosphoglycerate and adenosine triphosphate or travel on the shunt path and produce 3-phosphoglycerate in a series of bypass reactions that produce no high energy substrates. Evidence has been presented by us [6] and by Parker and Hoffman [15] and Proverbio and Hoffman [16] indicating that phosphoglycerate kinase is directly coupled to the $(Na^+ + K^+)$-ATPase involved in active transport. Hence it would be quite possible for glyceraldehyde-3-phosphate dehydrogenase to play a regulatory role and adopt conformations that would favor directing its product into either the energy-producing, transport-linked pathway or through the bypass. Since glyceraldehyde-3-phosphate dehydrogenase binding to its site on the inner membrane surface is dependent upon ionic strength as shown by Kant and Steck [4] and McDaniel et al. [3] and since its conformation depends upon the sodium and potassium concentrations in the medium as we have shown, it would seem that the necessary sensing apparatus to enable glyceraldehyde-3-phosphate dehydrogenase to play a regulatory role is already in place.

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References

1 Tanner, M.J.A. and Gray, W.R. (1971) Biochem. J. 125, 1109—1117
2 Shin, B.C. and Carraway, K.L. (1973) J. Biol. Chem. 248, 1436—1444
3 McDaniel, C.F., Kirtley, M.E. and Tanner, M.J.A. (1974) J. Biol. Chem. 249, 6478—6485
4 Kant, J.A. and Steck, T.L. (1973) J. Biol. Chem. 248, 8457—8464
5 Yu, J. and Steck, T.L. (1975) J. Biol. Chem. 250, 9176—9184
6 Fossel, E.T. and Solomon, A.K. (1977) Biochim. Biophys. Acta 464, 82—92
7 Blostein, R. and Chu, L. (1977) J. Biol. Chem. 252, 3035—3043

8 Sze, H. and Solomon, A.K. (1979) Biochim. Biophys. Acta 550, 393—406
9 Steck, T.L. and Kant, J.A. (1974) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 31, pp. 172—180, Academic Press, New York
10 Strapazon, E. and Steck, T.L. (1976) Biochemistry 15, 1421—1424
11 Post, R.L., Merritt, C.R., Kinsolving, C.R. and Albright, C.D. (1960) J. Biol. Chem. 235, 1796—1802
12 Streeten, D.H.P. and Solomon, A.K. (1954) J. Gen. Physiol. 37, 643—661
13 Schwartz, A., Lindenmayer, G.E. and Allen, J.C. (1975) Pharmacol. Rev. 27, 3—134
14 Glynn, I.M. and Lew, V.L. (1970) J. Physiol. London 207, 393—402
15 Parker, J.C. and Hoffman, J.F. (1967) J. Gen. Physiol. 50, 893—916
16 Proverbio, F. and Hoffman, J.F. (1977) J. Gen. Physiol. 69, 605—632