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The influence of alkali metals on the incorporation of labelled phosphate into ATP in red cell ghosts

The incorporation of $^{32}P_i$ into ATP by glycolytically inert erythrocyte ghosts is, under certain stringent conditions, partly inhibited by ouabain[1,2]. The necessary conditions are that the ghosts be rich in K^+ but low in Na^+, and that they be suspended in a high-Na^+ medium which is free of K^+. In this situation the movements of Na^+ and K^+ down their electrochemical gradients are also partly inhibited by ouabain[3–5]. The purpose of the present work was to test whether other alkali metals could replace Na^+ and K^+ in supporting the incorporation of $^{32}P_i$ into ATP and, particularly, whether replacement of K^+ by Rb^+, Cs^+ or Li^+ showed features similar to those observed for ATP hydrolysis in human red cell ghosts[6]. The methods employed were essentially similar to those described earlier[2].

In order to establish optimum conditions for incorporation, the requirement for Mg^{2+} and ATP was first checked. Fig. 1 shows the influence of Mg^{2+} concentration on incorporation when ATP, which acts as a source of ADP, is present at 1 mM. It is clear that although maximum total incorporation is achieved with Mg^{2+} at 1 mM, the contribution of the ouabain-insensitive (basal) component increases throughout the range of Mg^{2+} concentrations used (0–7 mM). Moreover, a similar experiment has shown that it is the internal, rather than the external, concentration of Mg^{2+} which is important. If the ouabain-sensitive (pump) component is taken as the difference between the two measured curves, as shown by the broken line, it is seen that maximum incorporation of $^{32}P_i$ into ATP through the reversed pump occurs when Mg^{2+}

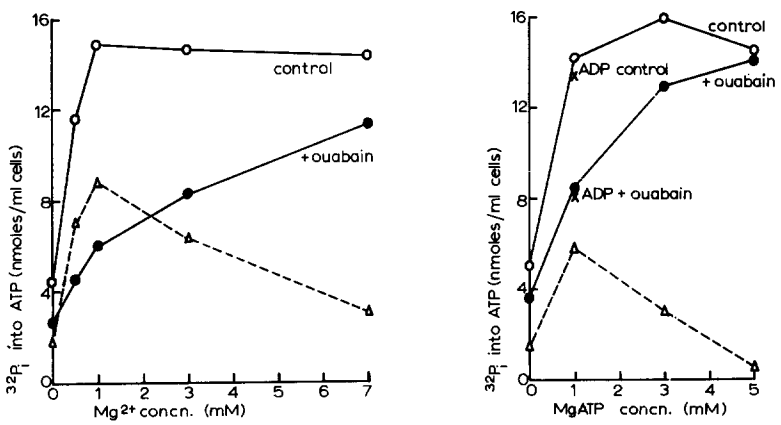

Fig. 1. Effect of Mg^{2+} concentration on incorporation of $^{32}P_i$ into ATP. Cells were lysed in a medium containing (mM): ATP, 1; [^{32}P]orthophosphate (pH 7.4), 5; Tris, 2; Na^+, 2; K^+, 11; F^-, 2; iodoacetate, 0.2, with $MgCl_2$ present at 0–7 mM. Isotonicity was restored with 3 M KCl and ghosts were resealed at 37° for 30 min. Washing and incubation media contained (mM): Tris (pH 7.4), 5; F^-, 2; iodoacetate, 0.2; Cl^-, 151–158; Na^+, 146–153; Mg^{2+}, 0–7. Ghosts equivalent to roughly 3 ml of original erythrocytes were suspended in 30 ml of medium with or without ouabain (0.1 mM) and incubated at 37° for 15 min.

Fig. 2. Effect of ATP concentration on incorporation of $^{32}P_i$ into ATP. Ghosts were prepared as described in Fig. 1 except that Mg^{2+} and ATP concentrations were varied in the range 0–5 mM. Incubation as in Fig. 1, the medium containing 1 mM Mg^{2+} throughout.

is at 1 mM (*i.e.* the Mg^{2+}:ATP ratio is 1). The same ratio was found for optimum ouabain-sensitive hydrolysis of ATP by fragmented erythrocyte membranes[7]. Other experiments have confirmed that the 'basal' component of $^{32}P_i$ incorporation occurs mainly during the preparative stages while the 'pump' component occurs only during the incubation period. Mg^{2+} appears to be a specific requirement for the 'pump' component of labelling, consistent with the observations that MgATP is the substrate for the $(Na^+ + K^+)$-ATPase (ATP phosphohydrolase, EC 3.6.1.3)[8].

Total incorporation of $^{32}P_i$ into ATP increases only slightly when ATP is raised above 1 mM with the Mg:ATP ratio kept at unity, but the 'basal' component rises substantially (Fig. 2). Hence, by difference, the 'pump' component is maximum with ATP at 1 mM. The decrease in this component with 5 mM ATP is the same when Mg^{2+} is at 5 mM or 1 mM showing that ATP itself is responsible for the inhibition. Adding 1 mM ADP with 1 mM Mg^{2+} gives the same amount of labelling of ATP as found when ATP is the nucleotide added (Fig. 2). However, it was found that when ITP, which does not support active transport[9], was added instead of ADP or ATP there was no ouabain-sensitive labelling and the 'basal' component was less than 10 % of that found with ATP.

Having established optimum conditions for labelling, the effects of the various alkali metals were studied. There is a considerable amount of evidence to show that, in ghosts or cells containing Na^+, the $(Na^+ + K^+)$-ATPase and Na^+ efflux are activated similarly by low concentrations of K^+ in the external medium[6, 10, 11]. Incorporation of $^{32}P_i$ into ATP by reversal of the Na^+ pump is completely inhibited by external K^+ (refs. 1, 2), and Fig. 3 shows the effectiveness of external K^+ and of other alkali metals in causing this inhibition. About 90 % of the incorporation of $^{32}P_i$ which occurs during the incubation period can be thought of as the 'pump' component. The concentrations of the ions which cause half-maximum inhibition are, K^+ and Rb^+ at 0.8 mM, Cs^+ at 6 mM and Li^+ at 8 mM. The curves in Fig. 3 cover only a short range of the concentrations tested; the ions have been added in increasing amounts until virtually the whole of the Na^+ (150 mM) has been replaced. This has shown that the maximum inhibition is the same with each ion and is achieved with K^+ or Rb^+ at 5 mM, Cs^+ at 30 mM and Li^+ at 50 mM, a pattern corresponding almost exactly with that observed for stimulation of ATP hydrolysis by $(Na^+ + K^+)$-ATPase[6]. When Na^+ is replaced by choline$^+$ the incorporation of $^{32}P_i$ into ATP decreases linearly with decrease in Na^+ concentration (Fig. 3, inset). This is interesting in relation to the observations that, in K^+-rich erythrocytes suspended in K^+-free medium, the ouabain-sensitive efflux of K^+ and ouabain-sensitive influx of Na^+ are both linearly related to external Na^+ concentration and, it is suggested, both associated with reversal of the sodium pump[12].

The effectiveness of Rb^+, Cs^+, Li^+ and choline$^+$ in substituting for internal K^+ is shown in Fig. 4. Incorporation of $^{32}P_i$ is the same with K^+, Rb^+ or Cs^+ as the major internal cation but Li^+ and choline$^+$ support very little incorporation. The nature of the 'basal' labelling was not studied in detail, but it was found that internal Li^+, in contrast to the other alkali metals, inhibited the 'basal' incorporation of $^{32}P_i$ by over 30 %, an effect which might be analogous to its inhibition of the non-specific ATP:ADP transphosphorylase of Electrophorus electric organ[13].

It appears from the present results that the requirement for external Na^+ is completely specific and that, because of their abilities to promote hydrolytic activity

by the $(Na^+ + K^+)$-ATPase, the other alkali metals exert an inhibitory influence over $^{32}P_i$ incorporation, in the following order of effectiveness: $K^+ = Rb^+ > Cs^+ > Li^+$.

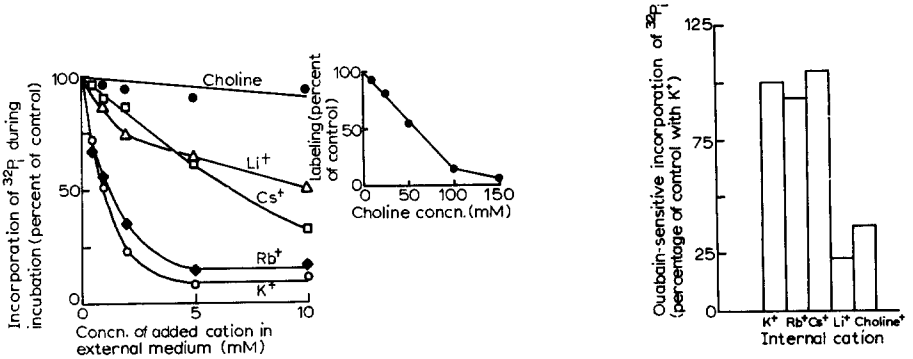

Fig. 3. Effect of alkali metal cations in external medium. Ghosts were prepared from a haemolysate containing 1 mM MgATP as in Fig. 1. Incubation as in Fig. 1, the medium containing 0–150 mM K^+, Rb^+, Cs^+, Li^+ or choline in place of an equivalent amount of Na^+.

Fig. 4. Effect of alkali metal cations inside the ghosts. Cells were lysed in the presence of 1 mM MgATP and isotonicity was restored with 3 M KCl, RbCl, CsCl, LiCl or choline chloride. Incubation as in Fig. 1, the medium containing 1 mM Mg^{2+} throughout.

This is the same order as for activation of erythrocyte $(Na^+ + K^+)$-ATPase[6], activation of a microsomal phosphatase from gastric mucosa[14], and for binding to certain antibiotics and cyclic polyethers which increase the permeability of biological membranes specifically to alkali metal cations[15]. It is also one of the eleven selectivity sequences found in both biological and non-biological membrane systems[16].

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