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## Irreversible inactivation of ($Na^+$-$K^+$)-dependent ATPase and $K^+$-dependent phosphatase by fluoride

Fluoride causes an increase in permeability and decrease in active ion transport at the cell membrane[1,2]. It is also known that fluoride inhibits the ($Na^+$-$K^+$)-dependent ATPase[2,3] which is considered to participate in active ion transport, but the nature of the inhibitory effect of fluoride has not yet been investigated. On the other hand, $K^+$-dependent phosphatase has been reported by us and other investigators to have many properties in common with the ($Na^+$-$K^+$)-dependent ATPase[4-9]. Accordingly, the effects of fluoride on the $K^+$-dependent phosphatase and the ($Na^+$-$K^+$)-dependent ATPase have been examined.

Guinea-pig brain microsomes were treated with NaI as described previously[4] and used in the following experiments.

The influence of anions on the $K^+$-dependent phosphatase was examined by replacing KCl in the reaction mixture by the potassium salts of $F^-$, $Br^-$, $I^-$, $SCN^-$, $NO_3^-$ and $SO_4^{2-}$. With fluoride as anion in the reaction medium, no $K^+$-dependent phosphatase activity was observed, though with the other anions similar degrees of $K^+$-dependent phosphatase activity were observed with chloride. These findings agree with the report on the effects of anions on the ($Na^+$-$K^+$)-dependent ATPase by OPIT, POTTER AND CHARNOCK[3]. The inhibitory effect of fluoride was observed at a concentration as low as 0.3 mM and the degree of inhibition increased with the reaction period. However, the inhibition by fluoride was not significantly influenced by raising the concentration of $Mg^{2+}$ from 2 mM to 10 mM or by addition of $P_i$ or *p*-nitrophenol. Thus, the possibility was examined that the enzyme might be progressively inactivated by incubation with fluoride at 37°.

The enzyme was pretreated at 37° for various periods with and without fluoride

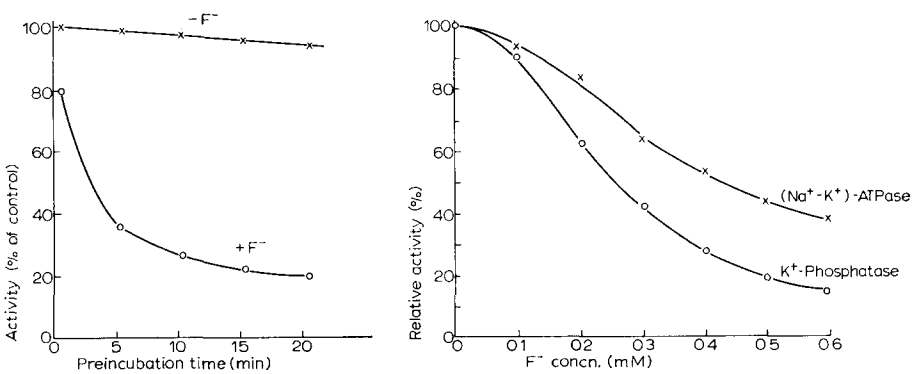


Fig. 1. Time course of inactivation of $K^+$-dependent phosphatase on preincubation with fluoride. Preincubation was carried out in 2 ml of a solution containing enzyme (about 1.5 mg protein), 40 mM of Tris buffer (pH 7.8), 5 mM of $MgCl_2$ and 10 mM of KCl at 37°. At the end of the preincubation 5 ml of ice-cold distilled water were added and the mixtures were centrifuged at 100 000 × *g* for 20 min at 0°. The precipitates were washed again with cold water and used as pretreated enzyme. The activity of $K^+$-dependent phosphatase was then estimated[4].

Fig. 2. Inactivation of ($Na^+$-$K^+$)-dependent ATPase and $K^+$-dependent phosphatase on preincubation with various concentrations of fluoride. Enzyme suspension containing 40 mM Tris buffer (pH 7.8), 5 mM $MgCl_2$ and 10 mM KCl was preincubated at 37° for 10 min.

TABLE I

INACTIVATION OF $K^+$-DEPENDENT PHOSPHATASE AND ($Na^+$-$K^+$)-DEPENDENT ATPase BY PREINCUBATION WITH FLUORIDE UNDER VARIOUS CONDITIONS

| *Preincubation mixture*** | *ATPase activity** | | | *Phosphatase activity*** | | |
|---|---|---|---|---|---|---|
| | *$Mg^{2+}$-ATPase* | *($Na^+$-$K^+$)-ATPase ($Mg^{2+}$-$Na^+$-$K^+$)-ATPase − $Mg^{2+}$-ATPase* | *% Activity of ($Na^+$-$K^+$)-ATPase* | *$Mg^{2+}$-phosphatase* | *$K^+$-phosphatase ($Mg^{2+}$-$K^+$)-ATPase − $Mg^{2+}$-ATPase* | *% Activity of $K^+$-phosphatase* |
| 1. Tris | 14.6 | 77.2 | 100 | 0.28 | 3.75 | 100 |
| 2. Tris, $Mg^{2+}$ | 10.9 | 75.6 | 98 | 0.23 | 3.72 | 99.0 |
| 3. Tris, $Mg^{2+}$, $K^+$ | 11.7 | 74.9 | 97 | 0.22 | 3.66 | 97.5 |
| 4. Tris, $F^-$ | 12.6 | 74.0 | 95.8 | 0.24 | 3.51 | 93.5 |
| 5. Tris, $Mg^{2+}$, $F^-$ | 11.8 | 61.4 | 79.5 | 0.21 | 2.21 | 58.9 |
| 6. Tris, $Mg^{2+}$, $K^+$, $F^-$ | 12.2 | 46.7 | 60.5 | 0.20 | 1.48 | 39.4 |
| 7. Tris, $K^+$, $F^-$ | 11.6 | 74.1 | 96.0 | 0.22 | 3.46 | 92.3 |
| 8. Tris, $Mg^{2+}$, $K^+$, ATP, $F^-$ | 11.4 | 76.4 | 99.0 | 0.25 | 3.53 | 94.0 |
| 9. Tris, $Mg^{2+}$, $K^+$, $Na^+$, $F^-$ | 12.4 | 70.4 | 91.2 | 0.24 | 2.77 | 73.8 |

* $\mu$moles $P_i$ liberated per mg protein per h.
** $\mu$moles *p*-nitrophenol liberated per mg protein per h.
*** Tris: Tris–HCl buffer (pH 7.8), 40 mM; $Mg^{2+}$: $MgCl_2$, 5 mM; $K^+$: KCl, 10 mM; $F^-$: KF, 0.3 mM; ATP: ATP–Tris, 2 mM; $Na^+$: NaCl, 100 mM. Preincubation was for 10 min at 37°.

and the activities of the $K^+$-dependent phosphatase and ($Na^+$-$K^+$)-dependent ATPase were estimated after washing the pretreated enzyme. As shown in Fig. 1, the $K^+$-dependent phosphatase activity decreased rapidly during the first 10 min of incubation with fluoride at 37°, though it was maintained at the initial level when the mixture was kept in an ice–water bath even if fluoride was added to the enzyme. This means that temperature affects the inactivation by fluoride. The results presented in Table I show that $Mg^{2+}$ is essential and $K^+$ is a stimulator for the inactivation of both the $K^+$-dependent phosphatase and the ($Na^+$-$K^+$)-dependent ATPase by fluoride. It was also shown that ATP and $Na^+$ strongly protect these enzymes against inactivation by fluoride. The findings suggest that $Mg^{2+}$ and $K^+$ may cause a change in structure of the two enzymes or alter the structure of the membrane in which the two enzymes are fixed. ATP and $Na^+$ may prevent the change in structure caused by $Mg^{2+}$ and $K^+$. Similar requirements and protectors were found in the inactivation of the ($Na^+$-$K^+$)-dependent ATPase by DFP, but the effective concentration of DFP was much higher[10]. The fact that the amount of incorporation of $^{32}P$ from [$^{32}P$]DFP into the ($Na^+$-$K^+$)-dependent ATPase was not related to the extent of inactivation of the enzyme[10] suggests that the processes of inactivation by DFP and fluoride may have the same fundamental mechanism and be related to the fluoride atom or ion. As the inhibition of ($Na^+$-$K^+$)-dependent ATPase by beryllium was also reported to be similar[11], it may be that in general the structural state which is promoted by $Mg^{2+}$ and $K^+$ and prevented by ATP and $Na^+$ is sensitive to inactivation processes.

As described above, the processes of inactivation of ($Na^+$-$K^+$)-dependent ATPase and $K^+$-dependent phosphatase were similar qualitatively, but differed quantitatively. The $K^+$-dependent phosphatase was apparently more sensitive to fluoride than the ($Na^+$-$K^+$)-dependent ATPase, as shown in Fig. 2.

The fluoride inactivation described here may serve as a unique tool to clarify the nature of ($Na^+$-$K^+$)-dependent ATPase and $K^+$-dependent phosphatase.

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