STUDIES ON THE $Na^+ + K^+$ ACTIVATED ATP HYDROLYSING ENZYME SYSTEM

THE ROLE OF SH GROUPS

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There is increasing evidence that the $Na^+ + K^+$ activated ATP hydrolysing enzyme system which was primarily isolated from peripheral crab nerves (Skou, 1957) and since then from a number of different tissues is part of or the mechanism responsible for the active linked transport of Na^+ and K^+ across the cell membrane (for ref. see Skou, 1962b).

The hydrolysis of ATP involves at least one intermediary step, viz. a transfer of an energy-rich phosphate bond from ATP to the enzyme system (Skou, 196o); this transfer requires Mg^{2+}. The hydrolysis of the phosphorylated compound requires $Na^+ + K^+$. In order to understand how this enzyme system can transport cations it is, however, necessary to have a more detailed knowledge of the components of the system and of the individual steps in the transfer of ~ P and of the hydrolysis of the phosphorylated compound. In this paper, some of the results of an investigation on the role of SH groups are presented. A more detailed report will be given elsewhere.

The enzyme was prepared from ox brain and the activity measured as previously reported (Skou, 1962a).

P-chloromercuribenzoate (PCMB) inhibits the enzyme activity, table 1. The effect of PCMB is reversed by cysteine.

Table 1.

The effect of PCMB and of NEM on the enzyme activity

Enzyme incubated with	minutes	μMPi/h/mgN Mg	μMPi/h/mgN Mg+Na+K	Activity Ratio Mg+Na+K/Mg
Control		118	770	6.5
PCMB 10^{-5}M	1	55	278	5.1
- -	10	54	269	5.0
- -	1			
+ Cysteine 10^{-2}M	9	118	770	6.5
Control		181	845	4.7
NEM 10^{-3}M	1	86	608	7.1
- -	10	51	257	5.0
- -	60	32	80	2.5
NEM 10^{-3}+ ATP $3x10^{-3}$M	1	72	696	9.7
- - -	10	51	623	12.2
- - -	60	25	469	18.7
NEM 10^{-3}+DPNH $2x10^{-3}$M	1	86	673	7.8
- - -	10	48	367	7.6
- - -	60	29	88	3.1

Incubated at 37°C in Tris 30 mM, pH 7.6. After incubation, 0.1 ml of enzyme solution was added to 0.9 ml test solution: Tris 30 mM, pH 7.6, ATP 3 mM with Mg 3 and Mg 6 Na 100 K 20 mM, respectively.

N-ethyl maleimide (NEM)which is another SH group inhibitor (Friedmann, 1952) also inhibits the enzyme activity. The effect of NEM on the activity of the enzyme with Mg^{2+} differs from the effect on the activity with $Mg^{2+}+Na^{+}+K^{+}$ in the test medium, table 1. After a short time of incubation, 1 minute, the percentage decrease of the activity with Mg^{2+} is higher than the percentage decrease with $Mg^{2+}+Na^{+}+K^{+}$, i.e. the activity ratio increases. After a longer time of incubation, the percentage decrease of the $Mg^{2+}+Na^{+}+K^{+}$ activity is of the same order or higher than the percentage decrease of the activity with Mg^{2+}, i.e. the ratio decreases to the same or a lower level than without incubation with NEM.

ATP protects, at least to some extent, against the effect of NEM on the $Mg^{2+}+Na^{+}+K^{+}$ activity, while it does not protect against the effect of NEM on the Mg^{2+} activity. The activity ratio with NEM + ATP in the incubation medium therefore increases, table 1. ADP but not ITP has the same effect as ATP.

Table 2.

The effect of NEM on the DPNH-cyt.c reductase activity of the submicroscopic particle

Enzyme incubated with	minutes	cyt.c reduced μM/min./mgN	% of control
Control	o	1.9o	1oo
-	1o	1.55	82
DPNH $2x1o^{-3}M$	1o	2.27	12o
NEM $1o^{-3}M$	1o	o.47	24
- - +DPNH $2x1o^{-3}M$	1o	2.24	118
- - +ATP $3x1o^{-3}M$	1o	o.77	41
- - + - - +DPNH $2x1o^{-3}M$	1o	2.31	122

Incubated at 37°C in Tris 3o mM, pH 7.6. After incubation, o.1 ml of enzyme solution was added to test solution: Tris 3o mM, pH 7.6, cyt.c o.o16 mM, DPNH o.o2 mM, KCN o.1 mM, total 3.o ml. The reduction of cyt.c was measured at 55o mu.

The submicroscopic particle which contains the enzyme system also contains a system which can transfer electrons from DPNH to cytochrome c (cyt.c) and to dyes. This is true not only for the submicroscopic particle isolated from brain, but the author has made the same observation in enzyme preparations from other tissues in which it has been looked for, viz. in preparation from red blood cell membranes, kidney and cardiac muscle.

Both the DPNH-cyt.c reductase, table 2, and the diaphorase activity is inhibited by NEM. DPNH protects against this effect of NEM.

ATP protects but to a lesser extent than DPNH against the effect of NEM on the DPNH-cyt.c reductase activity, table 2, and DPNH seems to decrease the rate by which NEM inhibits the enzyme activity with $Mg^{2+}+Na^{+}+K^{+}$ in the medium, table 1, while DPNH has no influence on the effect of NEM on the activity with Mg^{2+} in the medium.

The experiments with PCMB indicates that SH groups are necessary for the enzyme activity. If one assumes that NEM reacts only with SH groups, the experiments with NEM seem to indicate that the submicroscopic particle contains at least three different types of SH groups:

1. A type of SH group which is necessary for the $Na^{+}+K^{+}$ activation. This group reacts slowly with NEM and may therefore be partly "masked". This group is partly protected against NEM by ATP.

2. A type of SH group which is necessary for the DPNH-cyt.c reductase activity, and which may or may not be of some importance for the $Na^{+}+K^{+}$ activation. This group reacts fast with NEM and is protected against the effect of NEM by DPNH.

3. A type of SH group which is necessary for the activity with Mg^{2+} alone. This group also reacts fast with NEM. It is not protected against NEM by ATP or DPNH.

These three types of SH groups are in their reactivity with NEM and in the effect of DPNH on the NEM inhibition similar to the three types of SH groups found by Strittmatter (1959) in the partly purified microsomal DPNH-cyt.c reductase.

The effect of ATP on the NEM inhibition of the DPNH-cyt.c reductase activity and of DPNH on the NEM inhibition of the

enzyme activity with $Na^+ + K^+$ in the medium may suggest some kind of relationship between the two systems.

The $Mg^{2+}+Na^+ + K^+/Mg^{2+}$ activity ratio varies in preparations from different tissues, and in different preparations from the same tissue (for ref. see Skou, 1962b).

The present results suggest three possible explanations why there is both an activity with Mg^{2+} and with $Mg^{2+}+Na^+ + K^+$ in the medium, and why the activity ratio may vary.

1. It is two different, completely independent, enzymes.

2. A free SH group on the submicroscopic particle which is not necessary for the $Mg^{2+}+Na^+ + K^+$ activity is localized in such a way that it influences the partly "masked" SH group of the $Mg^{2+}+Na^+ + K^+$ activated enzyme, and the effect of this group on the "masked" SH group leads to a hydrolysis of the phosphorylated compound without $Na^+ + K^+$ in the medium.

3. The Mg^{2+} activated enzyme is the same as the $Mg^{2+}+ Na^+ + K^+$ activated, but during the preparative procedure the partly "masked" SH group of some of the $Mg^{2+}+Na^+ + K^+$ activated enzyme has been changed into a free SH group. By this change the specific effect of $Na^+ + K^+$ has been partly or completely lost. The enzyme can still catalyse the transfer of phosphate from ATP to the system when there is Mg^{2+} in the medium, but the phosphorylated compound is spontaneously hydrolysed without the addition of $Na^+ + K^+$.

On the basis of the experiments it cannot be concluded which of these possibilities is correct.

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