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EVIDENCE FOR A NATURALLY OCCURRING ATPase-INHIBITOR IN *ESCHERICHIA COLI*

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Summary

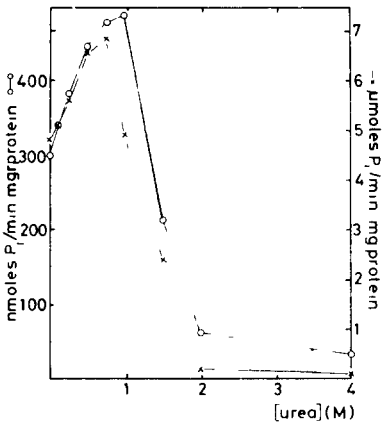
Stimulation of the *Escherichia coli* ATPase activity by urea and trypsin shows that there is a latent ATPase activity in particles and in a crude coupling factor of *E coli*. Moreover, crude coupling factor, completely dissociated by treatment with 7 M urea, can inhibit the ATPase activity of the crude coupling factor It is suggested that the latency of the ATPase activity of the coupling factor is due to the presence of a protein, the ATPase-inhibitor.

Both in mitochondria [1] and chloroplasts [2] the presence of a naturally occurring ATPase-inhibitor has been demonstrated. Ernster et al. [3] and Van de Stadt et al. [4] have presented evidence that the mitochondrial inhibitor plays a regulatory function in oxidative phosphorylation A similar regulatory function has been postulated for the chloroplast inhibitor [5] If such an inhibitor is an essential component of the system catalysing oxidative phosphorylation, it may be expected that it is also present in bacteria

Several bacteria are known that possess a latent ATPase activity (ref. 6, see ref. 7 for review) which can be unmasked by trypsin. It therefore seems logical to assume that this is due to a naturally occurring bacterial ATPase inhibitor analogous to that in mitochondria and chloroplasts The present study was undertaken to put this assumption to a test

Subbacterial particles and a crude coupling factor from *Escherichia coli* A 428 were isolated as described previously [8]. ATPase activity was assayed by measurement of inorganic phosphate [9] liberated from ATP Protein was determined by the method of Lowry et al. [10], with bovine serum albumin as standard.

As can be seen in Fig.1, high concentrations of urea completely inhibit



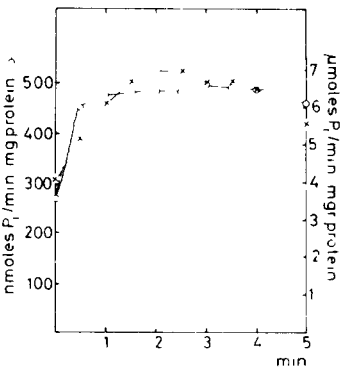


Fig 1 Effect of urea on the ATPase activity of *E coli* particles and crude coupling factor The crude coupling factor (16 µg) and particles (200 µg) were incubated for 5 min at 37°C with the indicated amounts of urea in 50 mM Tris•HCl and 2 5 mM $MgCl_2$ (pH 7 8), in a total volume of 1 ml After this incubation the ATPase activity was tested by addition of 5mM ATP X——X, crude coupling factor, O——O, particles

Fig 2 Stimulation of the ATPase activity of *E coli* particles and crude coupling factor by trypsin Crude coupling factor (16 µg) and particles (200 µg) were incubated with 250 µg TPCK-trypsin (Worthington) in 100 mM Tris•HCl and 5 mM $MgCl_2$ (pH 7 8) in a total volume of 0 5 ml for the indicated time Then the TPCK-trypsin was inhibited by adding 800 µg trypsin-inhibitor (Soybean, Worthington) and the volume brought to 1 ml with water The ATPase activity was tested in 1 ml by addition of 5 mM ATP X——X, crude coupling factor, O——O, particles

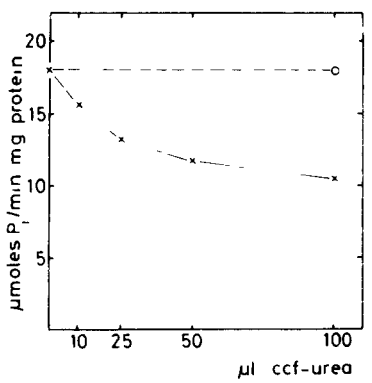


Fig 3 Inhibition of the ATPase activity of *E. coli* crude coupling factor by a completely dissociated crude coupling factor The crude coupling factor was purified over DEAE-Sephadex A (50 to 18 units mg protein) 'Crude coupling factor-urea' (ccf-urea) Crude coupling factor (1 mg/ml, 4—5 units/mg) was treated for 5 min with 7 M urea, then dialysed at 4°C for 3 × 1 h against 20 mM Tris•HCl (pH 7 5) to remove most of the urea X——X, the DEAE-purified crude coupling factor was incubated with the indicated amounts of 'ccf-urea' in 100 mM Tris•HCl, 5 mM $MgCl_2$ (pH 7 8) in a final volume of 0 5 ml. After 10 min the volume was brought to 1 ml with H_2O and ATPase activity was tested O——O, 100 µl 'crude coupling factor-urea' was pre-treated with TPCK-trypsin (250 µg) (Worthington) After 5 min the reaction was stopped by adding trypsin-inhibitor The inhibitory effect of this 100 µl was tested

the ATPase activity of both membrane-bound and solubilized ATPase. This inhibition is irreversible and probably due to splitting of the ATPase into subunits However, at lower concentrations, where the ATPase is not yet degraded, a stimulation by urea of the ATPase activity is found. A similar stimulation is illustrated in Fig 2, which shows the trypsin activation of the solubilized and membrane-bound ATPase. Apparently, in both cases there is a latent ATPase activity present. Through the proteolytic digestion of trypsin this activity is unmasked, which indicates that a protein is involved in the ATPase inhibition

Nelson et al. [2] have shown that a 7 M urea extract of chloroplast ATPase (CF_1) inhibits the activated CF_1 and that this inhibition is due to the ϵ subunit of the CF_1, the ATPase-inhibitor. Fig.3 shows a similar experiment with a crude solubilized ATPase from *E coli*.

It can be seen that the ATPase activity of the coupling factor is inhibited by 40%. If we activate the crude coupling factor with trypsin, as in Fig.2, the extra ATPase activity was inhibited 80% by 100 μl of crude coupling factor-treated with urea as described in the legend to Fig.3.

Further experiments have indicated that the interaction of the *E coli* ATPase with the inhibitor is different from that in mitochondria. Passage of *E coli* particles over a Sephadex G-50 column, according to Racker and Horstmann [11], which depletes submitochondrial particles of inhibitor, has no effect on the ATPase activity of *E coli* particles. Also, sonication of *E coli* cells in a medium containing EDTA (2 mM) at high pH (9 2) gives no enhancement of the ATPase activity.

These results indicate that the interaction of the *E coli* ATPase with its postulated inhibitor resembles more the interaction of CF_1 and its inhibitor than that of the mitochondrial ATPase. Pyridine treatment of the purified *E coli* ATPase according to Nelson et al [2] resulted in isolation of a fraction enriched in inhibitory activity and containing a higher proportion of a component of molecular weight 12 000, as determined by gel electrophoresis. Work is in progress to isolate a pure *E. coli* ATPase inhibitor.

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