ATP AND AMP FORMATION FROM ADP IN THE PRESENCE OF CYCLODEXTRIN

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ABSTRACT. A new nonenzymatic formation of ATP from ADP was observed in the presence of cyclodextrin in a phosphate buffer of pH 7.00 at 37.0°C under at mospheric conditions. Time conversion curves were obtained in the presence of β-cyclodextrin and heptakis-(2,6-dimethyl)-β-cyclodextrin. The effect of adding β-cyclodextrin, $MgCl_2$, phosphate buffer and creatine was examined kinetically as well as the effect of cyclohexanol as an inhibtor.

Biomimetic simulation of ATP regeneration in connection with the natural respiratory chain in mitochondoria has been studied. A reconstruction of cytochrome c-cytochrome oxydase complex shows not only controlled oxidation of any external coupling factors but phospho-controlled oxidation of external coupling factors or phospholipids.(Ref. 1) The formation of ATP *in vivo* is known as a photophosphorylation or oxidative phosphorylation from ADP. Also, ATP regeneration *in vitro* has been investigated using immobilized enzymes,(Ref. 2,3) mitochondria,(Ref. 4) bacterial chromophores(Ref. 5,6) and dried yeast cells.(Ref. 7) Converting oxidation energy into the "energy rich" diphosphate bond has been tried with organic preparation methods in an organic medium.(Ref. 8,9) We would like to report a new nonenzymatic preparation method of converting ADP to ATP and AMP in the presence of cyclodextrins in a phosphate buffer of pH 7.00 at 37.0°C within 100 hrs.

Materials and Methods

The representative experiment was carried out in test tubes capped with cotton. The test tubes were shaken in a water bath maintained at 37.0° C. The reaction mixture consisted of 1.7 mM ADP, 0.8 mM AMP, creatine and β-cyclodextrin (2.5 mM respectively) in 4.0 ml of 1/15M phosphate buffer (Na_2HPO_4+KH_2PO_4) of pH 7.00 containing 2.5 mM $MgCl_2$. 20 µl of the reaction mixture was sampled at various intervals and analyzed with a HPLC apparatus equipped with an ion-exchange column of IEX-540 DEAE φ4x250 mm(Toyo Soda Co.) and eluted with a 0.1 M phosphate

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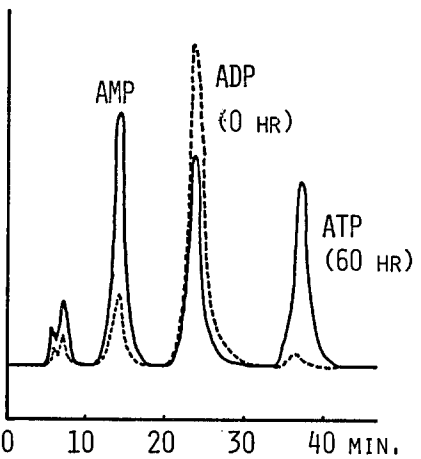
buffer of pH 7.00 containing 20% acetonitrile at a flow rate of 0.33 ml/min. Detection was 260 nm UV absorption at room temperature. The ATP formation was confirmed by the peaks at a retention time, Rt, of 28 min compared to ADP and AMP of Rt 20 and 15 min respectively. The fraction containing ATP by HPLC was checked by TLC with a solvent system of isobutyric acid:ammonia:water=66:1:33.

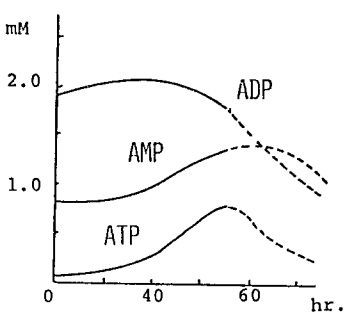
Results and Discussion

Fig. 1 HPLC Data of ATP Formation



Column:TSK-IEX-DEAE,4.0IDx25 cm
Eluent:0.1M phosphate buffer, pH 7.00, 20%-CH_3CN,
Flow rate: 0.33 ml/min.
Detection:UV 260 nm. Temperature:room temperature.

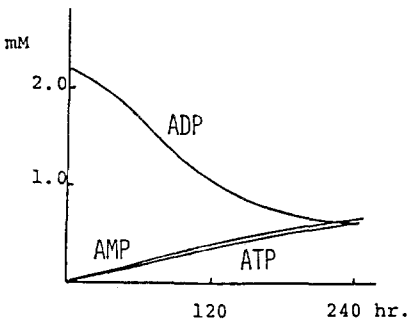
Fig. 2 Time Course of ATP Formation in the Presence of β-Cyclodextrin



REACTION CONDITION:[β-CD]=2.5mM, [creatine]=2.5mM, [$MgCl_2$]=2.5mM, [ADP]=1.7mM, [AMP]=0.8mM in 1/15M-phosphate buffer pH 7.00 at 37.0°C

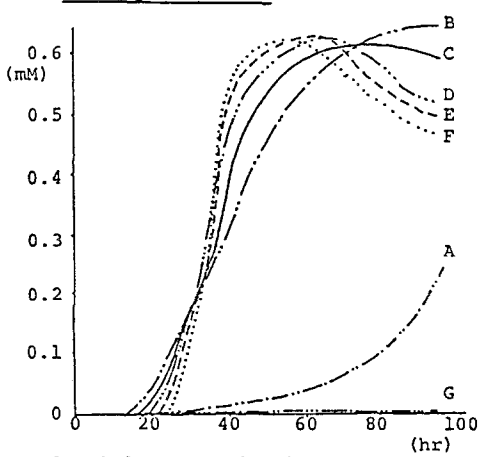
The formation of ATP from ADP was observed at a maximum of 41% at approximately 60 hrs as shown in Fig. 1. The time course of ATP formation is shown in Fig.2. In the initial stage, the induction period was as long as 20 hrs. Then ATP and AMP were increased and ADP was decreased. In the final stage after 60 hrs, a white precipitate was formed and simultaneously the decrease in the concentration of all three compounds occurred. The precipitates contained magnesium and adenosine residues. Another time course analysis clearly showed the modes of this reaction as shown in Fig. 3. When heptakis-(2,6-dimethyl)-β-CD was used in place of β-CD itself, there was no formation of a white precipitate.

Fig. 3 Time Course of ATP Formation in the Presence of Heptakis-(2,6-dimethyl)-β-cyclodextrin



REACTION CONDITION: [DM-β-CD]=2.5mM, [$MgCl_2$]=2.5mM, [ADP]=2.2mM, in 1/15M-phosphate buffer pH 7.00 at 37.0°C

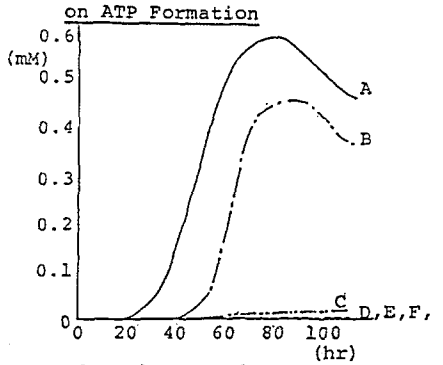
Fig. 4 Dependence of ATP Formation on β-Cyclodextrin



[$MgCl_2$]=2.5mM, [ADP]= 1.7mM, [AMP]=0.8mM [creatine]=2.5mM, [β-CD]=(A:10.00mM, B:5.00mM, C:2.50mM, D:1.25mM, E:0.625mM, F:0.3125mM, G:0.00mM)

This result indicates the disappearance of two molecules of ADP and the formation of one molecule each of AMP and ATP; they should reach an equilibrium. The dependence of ATP formation on the cyclodextrin concentration is shown in Fig. 4. At the higher cyclodextrin concentration, the induction period increased and the decrease in ATP concentration was depressed. ATP formation depended on the β-cyclodextrin concentration. With 1/5 to 3 times the equimolar amount of β-CD to ADP, the ATP formation was not so different but 6 mol excess β-CD caused slow formation of ATP. The addition of cyclohexanol caused a

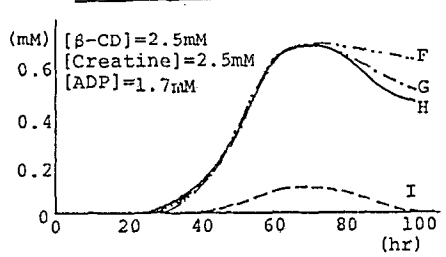
Fig. 5 Effect of Added Cyclohexanol on ATP Formation



[β-CD]=2.5mM, [$MgCl_2$]=2.5mM, [Creatine]=2.5mM, [ADP]=1.7mM, [AMP]=0.8mM, [cyclohexanol]=(A:0.0mM, B:1.0 mM, C:2.5mM, D:10.0mM, E:50.0mM, F:100.0mM)

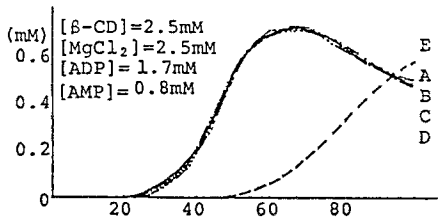
drastic drop in ATP formation as shown in Fig. 5. When the amount of cyclohexanol was more than the amount of cyclodextrin, ATP formation completely stopped. This was due to an inhibiting effect by cyclohexanol which occupied the hydrophobic cavity of cyclodextrin.
The interaction between cyclodextrin and ADP was examined by circular dichroism. In the presence of $MgCl_2$, an increase of β-cyclodextrin caused a decrease in the molar ellipticity at 258 and 210 nm. This suggested a complex formation between cyclodextrin and ADP with a magnesium ion involved in ATP formation. Moreover, the dependence of

Fig. 6 Dependence of ATP Formation on $MgCl_2$ Concentration



[$MgCl_2$]=(F:125.0mM, G:62.5mM:H: 25.0mM, I:0.00mM)

Fig.7 Dependence of ATP Formation on Creatine Concentration



[Creatine]=(A:250.0mM,B:125.0mM, C:25.0mM,D:2.50mM,E: 0.00mM)

ATP formation on creatine and $MgCl_2$ concentrations was also examined and the results are summarized in Fig. 6 and Fig. 7. Without creatine, ATP formation occurred later and more slowly but at the same high conversion level. The addition of excess creatine caused acceleration of ATP formation. Without creatine, the induction period took two times longer. As shown in the time conversion curve with heptakis-(2,6-dimethyl)-β-CD (DM-β-CD) without creatine, ATP formation occurred later and more slowly at the same conversion level. Besides these examinations, the effect of O_2, buffer solution and temperature were observed. Without O_2, the reaction did not proceed. Ionic strength and pH of the phosphate buffer and reaction temperature were optimum under the present conditions. The results obtained here showed the same kind of catalytic activity of the CD in the equilibrium between ADP and ATP in this scheme 1. This new type of transphosphorylation seems to be a

Scheme 1 Suggested Mechanism

$$2\ \text{ADP} \xrightarrow[\text{WITH } MgCl_2,\ O_2]{\text{CYCLODEXTRIN}} \text{ATP} + \text{AMP}$$

IN pH 7.0 PHOSPHATE BUFFER

AT 37.0°C, 60-120 HRS.

model reaction of the adenyl kinase reaction.(Ref. 10) It can be said that the cyclodextrin can produce a hydrophobic field for reaction in many cases.(Ref. 11,12) Also, cyclodextrins catalyze the hydrolysis of the phosphate,(Ref. 13-15) and creatine may play a role in forming an intermediate. Because the system is so simple and well-defined, the results thus obtained afforded a new approach to the mechanism of phosphorylation. The precise kinetic work concerning the mechanism is under investigation.

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