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REVERSIBLE TRANSPHOSPHORYLATION AMONG ATP, ADP, AND AMP IN THE PRESENCE OF METHYLATED CYCLODEXTRIN

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A transphosphorylation among AMP, ADP, and ATP in a neutral aqueous solution with $MgCl_2$ was examined in the presence of heptakis-(2,6-dimethyl)-β-cyclodextrin or heptakis-(2,3,6-trimethyl)-β-cyclodextrin. Adding methylated cyclodextrins was found to facilitate both forward and reversed reaction according to Eq.2 with an equilibrium constant of approximate unity.

The ATP formation *in vivo* can be classified into two pathways.[1] One is known as photophosphorylation or oxidative phosphorylation in which ATP was formed from a "energy rich phosphate".[2] These reactions can be described as Eq.1:

$$ADP + Pi \longrightarrow ATP \qquad (1)$$

The other is known as transphosphorylation[3] such as the reaction by adenylate kinase according to Eq.2:

$$2ADP \rightleftharpoons AMP + ATP \qquad (2)$$

We recently found a new nonenzymatic system to form ATP from ADP in moderate conditions with a phosphate buffer using β-cyclodextrin(β-CD).[4] Since both AMP and ATP formation from ADP was observed in this system, CD seemd to have an effect on the transphosphorylation in Eq.3:

$$2ADP \longrightarrow AMP + ATP \qquad (3)$$

However, as β-CD has poor solubility in water and the white precipitate containing phosphate, magnesium and CD was formed after a 60 h period among the previous ATP formation system, it could not been decided what was the exact kinetical behavior concering CD molecule in that system. In order to make sure of this point, we examined using methylated CDs that solved in water over 20 times higher than β-CD.

So that we found that the methylated CD had an effect on the reversible transphosphorylation according to Eq.2 in both directions as Eqs.3 and 4:

$$AMP + ATP \longrightarrow 2ADP \qquad (4)$$

Moreover, the equilibrium constants(K) which were calculated from the equilibrated concentrations of the reaction mixture were determined.

The kinetical run was carried out in test tubes capped with cotton. The test tubes were shaken in a water bath maintained at 37.0±1.0 °C. The reaction mixture consisted of adenosides, CD and $MgCl_2$ in the phosphate buffer of pH 7.00. The conversion of adenosides were confirmed by HPLC equipped with an ion-exchange column(Toyo Soda IEX-DEAE-2SW; ϕ:4x25 mm) eluted with 15% acetonitrile in 0.2 mol dm^{-3} phosphate buffer(pH 7). ATP formation was checked by ^{31}P-NMR spectroscopy detecting the β-phosphate of -20.6 ppm referred to H_3PO_4.

Figure 1 shows the typical time course of ATP formation in the presence and absence of heptakis-(2,6-dimethyl)-β-cyclodextrin(DM-β-CD). With DM-β-CD, the concentrations of ATP and AMP increased and the concentration of ADP decreased after a 20 h period of induction. After 150 h, the concentration of all three adenosides reached approximately to the same point. Until the final stage of this reaction, the white precipitate was not formed. It seems that the reaction mixture reached to a certain equilibrium point. Without DM-β-CD, no ATP formation occurred. Figure 1 also shows that there was no self-hydrolysis of adenosides. When DM-β-CD was replaced with heptakis-(2,3,6-trimethyl)-β-cyclodextrin(TM-β-CD), the reaction mixture also reached approximately to the same concentration without the white precipitate formation. The result suggested that cyclodextrin facilitated the transphosphorylation according to the Eq.2 and phosphate ion in the reaction solution was not essential. To confirm this assumption, the effect of DM-β-CD in the reversed courses of Eq.3 according to Eq.4 was observed. Adding DM-β-CD also effected the reaction process. The consumption of ATP and AMP and the formation of ADP were shown in Fig. 2. The reaction mixture reached to the same equilibrium mixture as the forward ATP formation after 150 h. In the presence of another CD, the same behavior was observed. When β-CD was used in reversed reaction, after 100 h, a white precipitate was formed same as in ATP formation reaction. Only a little change of adenosides occurred without CD. The equilibrium constants, K, in both forward and reversed reaction were determined in the presence of three CDs (Table 1). The K values in forward reaction(according to Eq.3) and in reversed reaction(according to Eq.4) were almost the same each other. And they

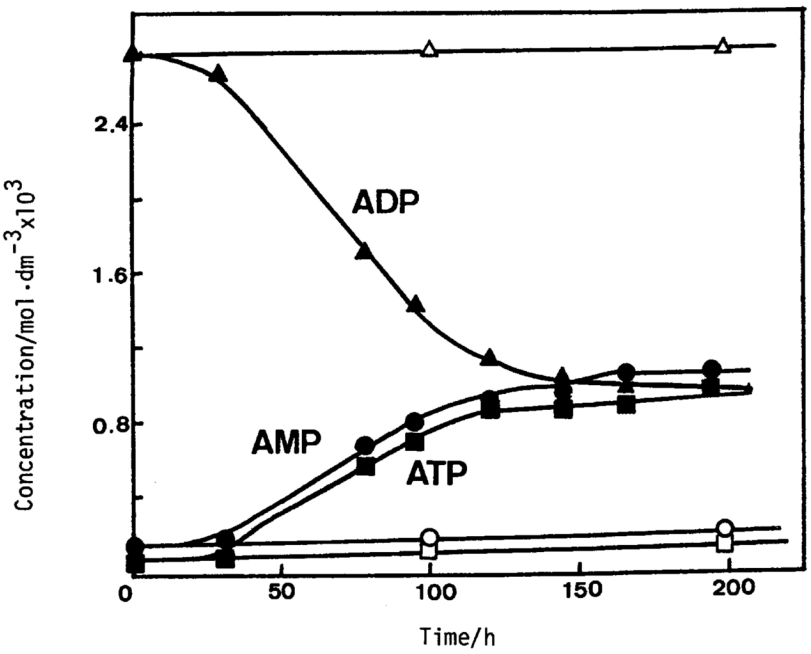

Fig. 1. Time course of ATP formation in the presence(●,▲, ■) and absence(○,△,□) of DM-β-CD in phosphate buffer (6.7×10^{-2}mol·dm^{-3}, pH 7.00) at 37 °C. [DM-β-CD]=1.00×10^{-2}mol·dm^{-3}, [$MgCl_2$]=2.5×10^{-3}mol·dm^{-3}, total adenosides concentration:2.5×10^{-3}mol·dm^{-3}.

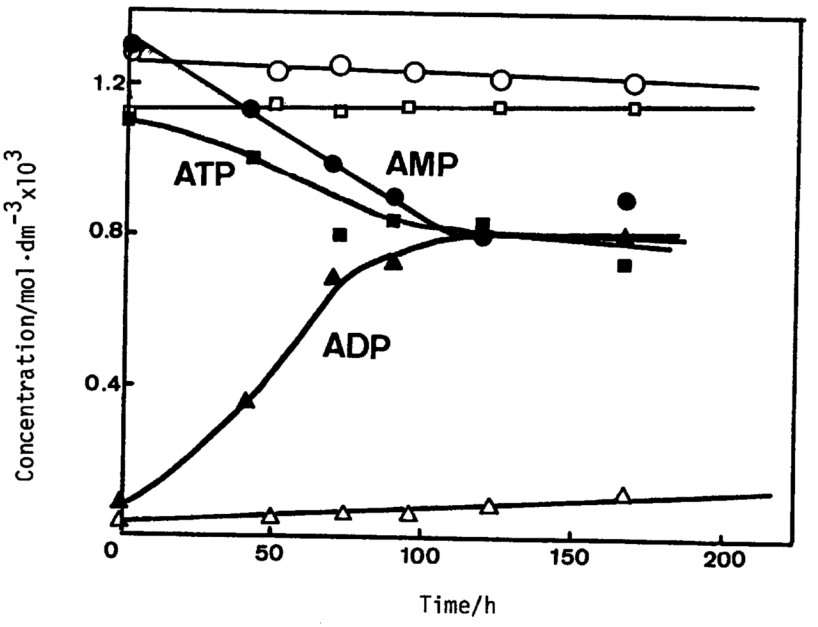

Fig. 2. The reversed course of ATP formation in the presence (●,▲, ■) and absence(○,△,□) of DM-β-CD in phosphate buffer(6.7×10^{-2}mol·dm^{-3}, pH 7.00) at 37 °C. [DM-β-CD]=1.00×10^{-2}mol·dm^{-3}, [$MgCl_2$]=2.5×10^{-3}mol·dm^{-3}, total adenosides concentration:2.5×10^{-3}mol·dm^{-3}.

Table 1. The ratio of adenosides concentrations in the equilibrated states[a)]

Cyclodextrins	K[b)]	
	Starting with ADP	Starting with AMP + ATP
β-CD	c)	1.1
DM-β-CD	0.9	1.1
TM-β-CD	0.9	1.3

a) $[\beta\text{-CD}]=[\text{DM-}\beta\text{-CD}]=[\text{TM-}\beta\text{-CD}]=1.00\times10^{-2}$ mol·dm^{-3}, pH 7.00, temperature: 37 °C, total concentrations of adenosides mixture were 2.5×10^{-3} mol·dm^{-3}.

b) These values were defined as fellows:

$$2\text{ADP} \underset{}{\overset{K}{\rightleftharpoons}} \text{AMP} + \text{ATP} \qquad K=\frac{[\text{AMP}][\text{ATP}]}{[\text{ADP}]^2}$$

c) The value was not calculated because of a white precipitate formation.

were very near to the reported equilibrium constants for the natural adenylate kinase 0.8 to 1.0.[3)] From these results, we would suggest an equilibrium reaction as in Eq.2 in the presence of a CD compound. The phosphate ion in the buffer solution may not be essential to ATP formation in the present CD's system. The increase of ATP and AMP and the decrease of ADP were also observed in the tris-HCl buffer solution in the presence of CD. This system was inhibited by adding cyclohexanol. And circular dichroism spectra showed the evidence of inclusion complex formation between CD and ADP or ATP at the adenyl moiety. DM-β-CD has only seven hydroxyl groups at C-3 position on glucose ring and TM-β-CD has not any active groups. The role of CD in this transphosphorylation system seemes to afford not a catalytic site but the conformational effector in the transition state. Complex formation with CD facilitated the approach of two adenosides and transphosphorylation occurred without self-hydrolysis. At this moment, this new type of the phosphorylation can be drawn as the equilibrium reaction and seems to be a model reaction similar to adenylate kinase.

References

1) A.L.Lehninger, "Bioenergetics," W.A.Benjamine, Inc., New York (1965).

2) F. C. Young and T. E. King, Biochem.Biophys.Res.Commun., 47, 380 (1972).

3) L. Noda, "The Enzymes," Academic Press, New York (1972), Vol.8, p. 279.

4) K.Hattori, K.Takahashi, and K.Sasao, J.Inclusion Phenomena, 2, 693 (1985).

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