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크로마토포아와 NAD Kinase로 이루어진 공역반응계의 모형화와 수치모사

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Modeling and Simulation of the Coupled Reaction System Comprised of Chromatophore and NAD Kinase

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요 약

크로마토포아와 NAD kinase로 구성된 공역 반응계에 대하여 수학적 모형화와 콤퓨터 수치모사를 수행하였다. 크로마토포아에 대해서는 경험적인 반응 속도식을 사용하였으며 공역 반응계의 다른 촉매 성분에 대해서는 이론적으로 유도된 식들을 사용하였다. 크로마토포아와 NAD kinase로부터 완전히 분리하기가 어렵기 때문에 마이오 카이네이즈도 반응계의 구성 성분으로 포함시켰다.
반응계의 촉매 성분들의 상대적인 활성 비율이 NADP 생산에 미치는 영향도 조사되었다. 반응계의 여러 생화학 성분들의 시간에 따른 변화 추이도 수치모사 결과로 부터 알 수 있었다.
실험 데이타와 콤퓨터 수치모사 결과를 비교하여 보았다.

ABSTRACT

Mathematical modeling and computer simulation were carried out on the coupled reaction system compreised of chromatophore and NAD kinase. An empirical kinetic model was used for the chromatophore whereas analytically derived models were employed for othre catalytic components of the system. Myokinase was also included as a system component because of

the difficulty in its complete separation from the chromatophore and NAD kinase.
The effect of the relative ratio of the catalytic activities of the system on the overall production of NADP was studied. The time course behaviour of the biochemical species of the system was also obtained from the simulation results.
Experimental data and the results of the computer simulation were compared.

Introduction

The ultimate objective of mathematical modeling is to obtain expressions that quantitatively describe the behaviour of the process under consideration. The generality of a model depends upon several factors which include the complexity and information available concerning the process. To have a mathematically precise and general model of a reaction is an inevitable prerequisite to the optimization or the more exact control on the process under consideration.

Reaction mechanism of NAD kinase was investigated by previous workers and was shown to follow the "Ping Pong Bi Bi" model.[1] However modeling effort on the photophosphorylation by chromatophore is almost non-existent. In this report we tried to have a model for chromatophore in consideration of its deactivation phenomena under various assumptions. In addition, we considered the effect of myokinase activity that might exist in the coupled reaction system using the well-known model established by other workers.[2] A report from an experimental study on this same coupled-reaction system will be published elsewhere.[6]

(1) Modeling of the Photophosphorylation by Chromatophore

The overall reaction of photophosphorylation by chromatophore can be expressed by the following equation;

$$ADP + P_i \rightleftharpoons ATP + H_2O$$

The exact reaction sequence of the elementary steps based upon the chemiosmosis theory is much more complex than this[5] and a precise mathematical expression is not yet established for the photophosphorylation by chromatophore. We set about modeling for the photophosphorylation by chromatophore under the assumption that the concentration of the inorganic phosphate is at the saturation level, and ATP and ADP react by the "Ordered Uni Uni" mechanism. For the "Ordered Uni Uni" mechanism, the following reaction rate equation can be obtained.

$$\frac{d\xi_1}{dt} = \frac{V_{max_C}([ADP] - [ATP]/K_{EC})}{K_{MDPC} + [ADP] + K_{TPC}[ATP]} \quad (1)$$

In the consideration of the inhibitory effect of NAD and NADP that are reactant and product of the NAD kinase reaction in the coupled reaction system, the above equation can be altered into the following form.

$$\frac{d\xi_1}{dt} = \frac{V_{max_C}([ADP] - [ATP]/K_{EC})}{K_{MDPC} + [ADP] + K_{TPC}[ATP] + K_{NDC}[NAD] + K_{NPC}[NADP]} \quad (2)$$

Here the allowance for the inhibitory effect by NAD and NADP in this particular form is rather arbitrary. Anyhow the inhibitory effects by these components are rather weak[1] and the way in which these inhibitory effects are accounted for in the kinetic equation

does not make much difference.

The deactivation phenomenon of chromatophore is known to be significantly affected by such factors as temperature. The mechanism of chromatophore deactivation include the death of a chromatophore as a whole, the deactivation of ATPase, the deactivation of the proton motive force generating system through the deactivation of the electron transport chain, and the deactivation of the energy transfer component. Quite often the oxidation of the reaction system may also contribute to the lower activity of the overall chromatophore reaction. The death of a chromatophore as a whole will affect the maximum velocity of the photophosporylation through the reduction in the level of the catalytic entities. If Nc denotes the number of chromatophore at an appropriate time, the death rate of chromatophore can be expressed as in the following equation:

$$\frac{dN_C}{dt} = -K_P N_C \tag{3}$$

With the initial condition:

$$N_C = N_{C_0} \text{ at } t = 0 \tag{4}$$

the above equation can be integrated to give

$$N_C = N_{C_0} e^{-kt} \tag{5}$$

Other possible mechanisms of choromatophore deactivation, namely the deactivation of ATPase complex, the deactivation of the proton motive force generating system through the deactivation of bacteriochlorophyll or the components of the electron transport chain, and the deactivation of the energy ransfer component, can influence not only $V_{max,c}$ but also the equilibrium constant via the lowering of the proton motive force. Although other kinetic parameters will also be certainly affected, here we consider $V_{max,c}$ and the equilibrium constant, K_{EC}, as the two major parameters changing with time to any great extent and employ an empirical model for the time dependence of the equilibrium constant as

$$K_{EC} = K_{e_0} e^{-k} e^{t} + K_f \tag{6}$$

Now the final form of the kinetic equation for the photophosphorylation by chromatophore can be written as

$$\frac{d\xi_1}{dt} = \frac{V_{max,c_0} e^{-kt} \{[ADP] - [ATP]/(K_{e_0} e^{-kt} + K_f)\}}{K_{MDPC} + [ADP] + K_{TPC}[ATP] + K_{NDC}[NAD] + K_{NPC}[NADP]} \tag{7}$$

(2) Modeling of the NAD Phosphorylation by NAD kinase

Based upon the kinetic data for NAD kinase isolated from Brevibacterium ammoniagenes, Aiba et al.[1] showed that this particular enzyme follows the "Ping Pong Bi Bi" mechanism and the rate equation they obtained according to this mechanism is

$$\frac{d\xi_2}{dt} = \frac{V_{max,N}([ATP][NAD] - [ADP][NADP]/K_{EN})}{\{K_{TPN}[ATP] + K_{NDN}[NAD] + [ATP][NAD] + K_{DPN}[ADP] + K_{NPN}[NADP] + K_{NDNPN}[NAD][NADP] + K_{DPTPN}[ADP][ATP] + K_{DPNPN}[ADP][NADP]\}} \tag{8}$$

It is usually not so easy to obtain preparation of chromatophore and NAD kinase completely free of myokinase as well as other contaminating enzymes and the myokinase may exist as a third component of the coupled reaction system consisting of chromatophore and NAD kinase. Sometimes myokinase may even serve a useful purpose as in pumping AMP back to the high energy component ATP.

Through repeated washing the myokinase can be removed completely from the coupled reaction system. However, substantial portions of the activities of chromatophore and/

or NAD kinase may be lost during washing steps. Thus to retain high activity levels of chromatophore and NAD kinase one must accept the coexistence of myokinase with the other components of the coupled reaction system.

Myokinase carries out the AMP pumping reaction represented by the stoichiometry:

$$ATP + AMP \rightleftharpoons 2\,ADP \qquad (9)$$

A complete reaction rate equation for myokinase based upon "Random Bi Bi" mechanism has been obtained by Colton et al.[2] and can be written as follows:

$$\frac{d\xi_3}{dt} = \frac{V_{max,M}(K_1[AMP][ATP] - \left\{K_3[AMP]\left\{K_4\left(1 + \frac{K_5[AMP]}{K_8(1 + \frac{K_2[ADP]^2/K_{EM})}{(1 + K_6[ATP])}\right)}{K_7[AMP]}\right) + K_9[ADP] + K_{10}(K_{11}[AMP][ATP] + K_{12}) + K_{12}) + K_{13}[ATP](1 + K_{14}[ADP]) + K_{15}(K_{16}[ADP] + K_{17}[ADP]^2)\right\} \qquad (10)$$

Dimensionless groups defined as follows are introduced to simplify the system equations and the interpretation of the simulation results.

$$\alpha = \frac{[AMP]_o + [ADP]_o + [ATP]_o}{[NAD]_o + [NADP]_o}$$

$$X_{AMP} = \frac{[AMP]}{[AMP]_o + [ADP]_o + [ATP]_o}$$

$$X_{ADP} = \frac{[ADP]}{[AMP]_o + [ADP]_o + [ATP]_o}$$

$$X_{ATP} = 1 - X_{AMP} - X_{ADP}$$

$$X_{NAD} = \frac{[NAD]}{[NAD]_o + [NADP]_o}$$

$$X_{NADP} = 1 - X_{NAD}$$

$$\xi_i = \frac{\xi_i}{[AMP]_o + [ADP]_o + [ATP]_o}$$

$$\tau = t/\theta_F$$

$$P_C = V_{max,co} \cdot \theta_F / K_{MDPC}$$

$$P_N = V_{max,N} \cdot \theta_F / K_{TPN}$$

$$P_M = V_{max,M} \cdot \theta_F / K_3$$

Using the above dimensionless groups, reaction rate equations of chromatophore, NAD kinase, and myokinase can be written as follows.

For chromatophore

$$\frac{d\bar{\xi}_1}{d\tau} = \frac{P_C e^{-\bar{k}} p^r \{X_{ADP} - X_{ATP}/}{1 + C_{DPC}X_{ADP} + C_{TPC}X_{ATP}} \qquad (11)$$
$$\frac{(K_{eo}e^{-\bar{k}}e^r + K_f)\}}{+ C_{NDC}X_{NAD}/\alpha + C_{NPC}X_{NADP}/\alpha}$$

For NAD kinase

$$\frac{d\bar{\xi}_2}{d\tau} = \frac{P_N(X_{ATP}X_{NAD} - X_{ADP}X_{NADP}/K_{EN})}{(X_{ATP} + C_{NDN}X_{NAD}/\alpha + C_{NDTPN} X_{NAD}X_{ATP}/\alpha + C_{DPN}X_{ADP} + C_{NPN}X_{NADP}/\alpha + C_{DPNPN}X_{ADP}X_{NADP}/\alpha + C_{DPTPN}X_{ADP}X_{ATP} + C_{NDNPN}X_{NAD}X_{NADP}/\alpha^2)} \qquad (12)$$

For myokinase

Table 1. Values of Normalized Constants

$\bar{k}_p = 1.0$	$\bar{k}_e = 1.0$	$K_{EO} = 1.0$	$K_f = 1.0$
$C_{DPC} = 33.33$	$C_{TPC} = 1.0$	$C_{NDC} = 1.0$	$C_{NPC} = 1.0$
$C_{NDN} = 0.148$	$C_{NDTPN} = 0.617$	$C_{TPN} = 1.0$	$C_{NDN} = 1.0$
$C_{DPN} = 4.0$	$C_{DPNPN} = 1.0$	$C_{DPTPN} = 4.0$	$C_{NDNPN} = 1.0$
$K = 0.646$	$K_2 = 0.08$	$K_{EM} = 0.282$	$K_{EN} = 1.0$
$C_1 = 2.56$	$C_2 = 0.255$	$C_3 = 0.027$	$C_4 = 0.011$
$C_5 = 9.260$	$C_6 = 5.694$	$C_7 = 0.070$	$C_8 = 0.904$
$C_9 = 0.800$	$C_{10} = 2.644$	$C_{11} = 3.015$	

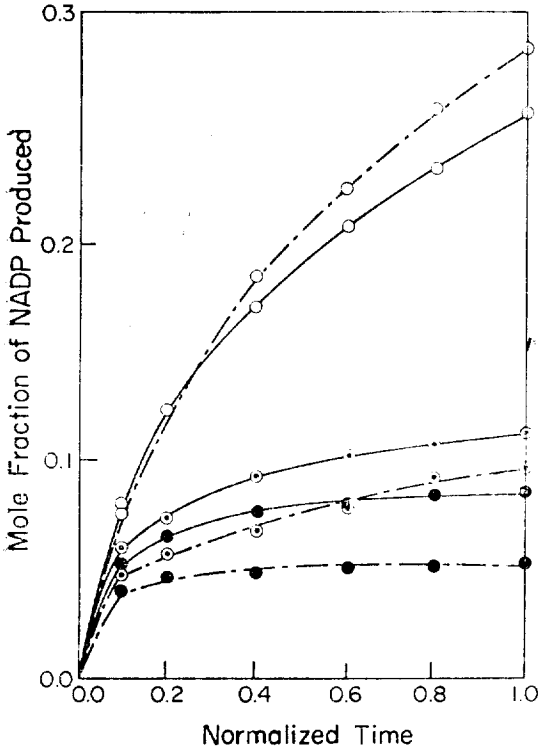

Fig. 1. Simulation Results on the Time Course Behaviour of X_{NADP}.
$P_N = 100.0$ ○—○ : $\beta = 10.0$
⊙—⊙ : $\beta = 1.0$
●—● : $\beta = 0.1$
– – – : $\gamma = 0.0$
——— : $\gamma = 1.0$

$$\frac{d\xi_3}{d\tau} = \frac{P_M(K_1X_{AMP}X_{ATP} - \dfrac{K_2X^2_{ADP}/K_{EN}}{X_{AMP}\left\{\left(C_1 + \dfrac{X_{AMP}(C_3 + C_4X_{ATP})}{1 + C_2X_{AMP}}\right) + C_5X_{ADP} + (C_6X_{AMP}X_{ATP} + C_7) + X_{ATP}(C_8 + C_9X_{ADP}) + (C_{10}X_{ADP} + C_{11}X^2_{ADP})\right.}}{} \quad (13)$$

Parameters, β, β' and γ defined in the following paragraph, are introduced for convenience.

$\beta = P_C/P_N$, $\beta' = P_N/P_C$, $\gamma = P_M/P_N$

(3) Simulation Results and Discussion

Three differential equations obtained from the above section were solved numerically by Runge-Kutta-Gill method.

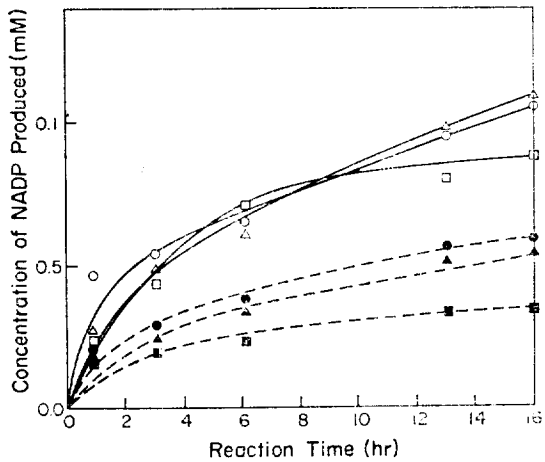

Fig. 2. Time Course Behaviour of NADP Production for the Various Concentration of Chromatophore in the Reaction Mixture
Reaction Temperature : 30°C
Light Intensity : 20 klux
○—○ : 0.2 ml NAD Kinase, 112 μM Bchl.
△—△ : 0.2 ml NAD Kinase, 84 μM Bchl.
□—□ : 0.2 ml NAD Kinase, 28 μM Bchl.
●– –● : 0.05 ml NAD Kina e, 112 μM Bchl.
▲– –▲ : 0.05 ml NAD Kinase, 84 μM Bchl.
■– –■ : 0.05 ml NAD Kinase, 28 μM Bchl.

Values of some constants used in the above equations were obtained from literatures[1)-3)] and others were selected arbitrarily. These are listed in *Table 1*.

In *Fig. 1* the time course behaviour of NADP production is shown for the case where β, i.e. P_C/P_N, varies via the change of chromatophore activity, P_C, with the NAD kinase activity, P_N, held constant. Here the NADP production tends to increase with increasing values of β. This simulation result shows trends similar to the experimental data shown in *Fig. 2*[4)] although the absolute values of the NADP production differed from each other. In the experimental data with

higher NAD kinase activity there exists an optimal chromatophore concentration that gives maximum NADP production but the simulation result shows no such maximum point. This may be due to the incompleteness of the model for chromatophore in that it lacks the consideration for the light shielding effect by the chromatophore itself. At higher NAD kinase activity there will be more rapid ADP formation from the usage of ATP in the conversion of NAD to NADP and hence a higher load on chromatophore for it to regenerate ATP from ADP as compared with the case of lower NAD kinase activity. Thus the relative light shielding effect of chromatophore on itself may be more pronounced and this may be why one has the optimal chromatophore concentration at the higher NAD kinase activity whereas no such peak-behaviour is observed at lower NAD kinase concentration.

The existence of myokinase in the simulation reaction system results in different effects on the NADP production depending upon the magnitude of the parameter, β. For small values of β, the existence of myokinase increases the NADP production. On the contrary, for large value of β, the existence of myokinase exhibits a rather inhibitory effect on NADP production. One can explain this result as follows: when β is small, low chromatophore activity does not allow the conversion of ADP, produced simultaneously with NADP by NAD kinase, back to ATP at a sufficient rate, and due to the consequent high cocentration of ADP accumulated, myokinase carries out the reverse reaction that results in the production of ATP and AMP. On the other hand, for large values of β, ADP, produced simultaneously with NADP, is rapidly converted to ATP by

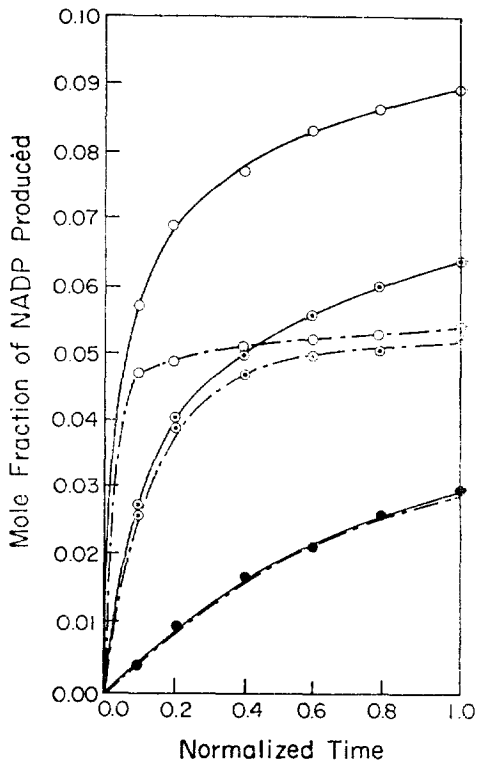

Fig. 3. Simulation Results on the Time Course Behaviour of X_{NADP} Constant Chromatophore Activity
$P_C = 10.0$, ○—○ : $\beta = 10.0$
⊙—⊙ : $\beta = 1.0$
●—● : $\beta = 0.1$
— — — : $\gamma = 0.0$
——— : $\gamma = 1.0$

high chromatophore activity and due to the consequent high concentration of ATP myokinase carries out the forward reaction that results in the production of ADP, thus resulting in the relatively lower phosphorylating potential. This particular phenomenon at higher value of β appears during the later reaction time period. The behaviour in early phase follows that of the case with lower value of β, which is attributed to myokinase action. That is there exists a time point at which an inversion in the relative amounts of NADP produced occurs.

Fig. 3 shows the time course behaviour of NADP production when the values of P_N are changed while keeping the value of P_C constant. NADP production increases as the value of P_N increases and this result is compatible with the experimental data shown in *Fig. 4.*[4] The existence of myokinase increases the NADP production and this is due to the ATP regenerating effect of myokinase. NADP production at final time is calculated and plotted against β as in *Fig. 5* by changing the values of P_C while keeping the values of P_N constant. At higher values of β, one gets higher NADP production and this is in general consistent with the experimental data shown in *Fig. 6*,[4] except that one observes the peakbehaviour at higher NAD kinase level as noted earlier.

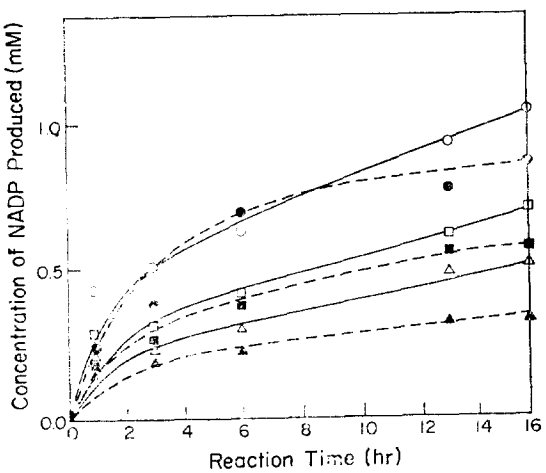

Fig. 4. Time Curse Behaviour of NADP Production for the Various Volume of NAD Kinase Added to the Reaction Mixture.
Reaction Temperature : 30°C
Light Intensity : 20 klux
○——○ : 0.2 ml NAD Kinase, 112 μM Bchl.
□——□ : 0.1 ml NAD Kinase, 112 μM Bchl.
△——△ : 0.05 ml NAD Kinase, 112 μM Bchl.
●- - -● : 0.2 ml NAD Kinase, 28 μM Bchl.
■- - -■ : 0.1 ml NAD Kinase, 28 μM Bchl.
▲- - -▲ : 0.05 ml NAD Kinase, 28 μM Bchl.

Fig. 7 shows the dependence of NADP production at final time on the value of β' for the constant value of P_C. NADP production increases as the value of β' increases and this is also in general agreement with the experimental data shown in *Fig. 8.*[4]

Fig. 9 shows the time course behaviour of X_{AMP} for various values of β and γ while keeping the value of P_N constant. As shown in *Fig. 9*, values of X_{AMP} decreases with the increment of β value under constant P_N and γ. As the value of β increases, P_C value also increases and thus ADP is rapidly converted back to ATP. The resultant ATP drives the myokinase reaction forward that

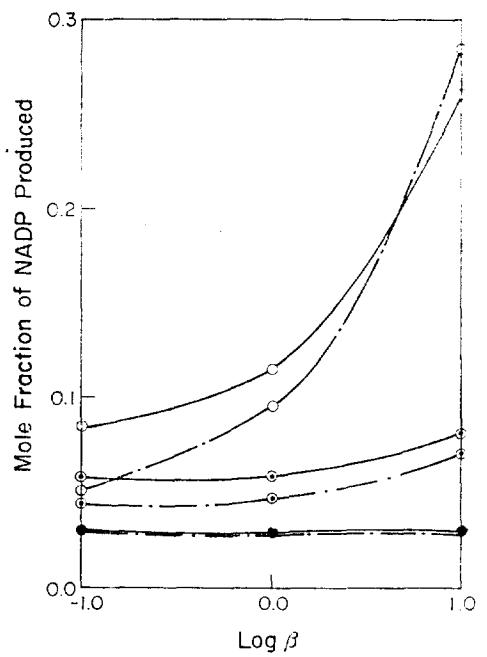

Fig. 5. Simulation Results on the Dependence of X_{NADP} at $\tau = 1.0$ on β(Chromatophore Activity/NAD kinase Actvity) for the fixed Values of NAD kinase Activity Denoted by P_N.
○——○ : $P_N = 100.0$
⊙——⊙ : $P_N = 10.0$
●——● : $P_N = 1.0$
- - - : $\gamma = 0.0$
—— : $\gamma = 1.0$

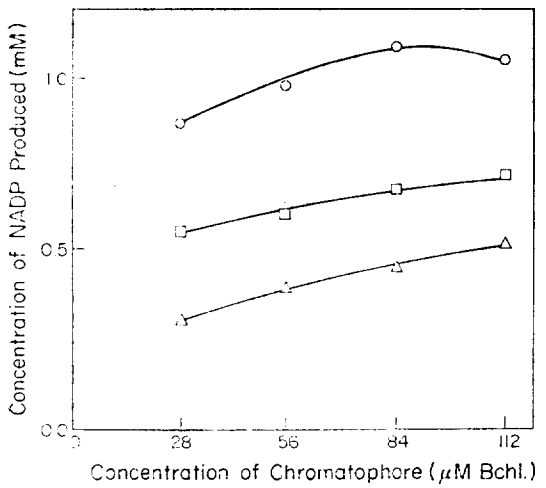

Fig. 6. Dependence of NADP Production on the Concentration of Chromatophore in the Reaction Mixture Reaction Temperature : 30°C
Light Intensity : 20 klux
Reaction Time : 16 hrs
○—○ : 0.2 ml NAD kinase
□—□ : 0.1 ml NAD kinase
△—△ : 0.05 ml NAD kinase

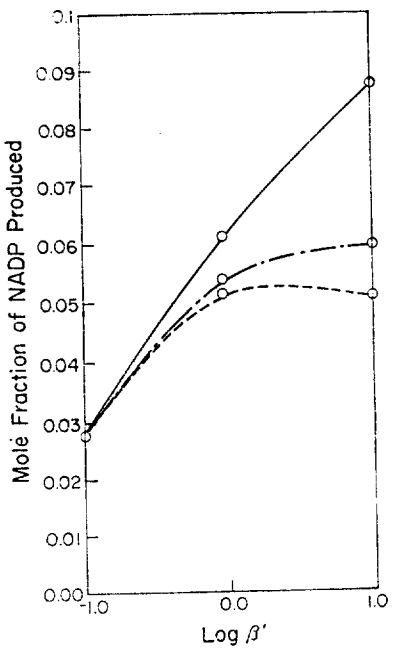

Fig. 7. Simulation Results on the Dependence of X_{NADP} at $\tau = 1$ on β'(NAD kinase Activity/Chromaphore Activity) for the Fixed Values of Chromatophore Activity Denoted by P_C $P_C = 10.0$
——— : $\gamma = 1.0$
– – – : $\gamma = 0.05$
– · – · – : $\gamma = 0.0$

produces ADP from AMP and ATP thus contributing to the AMP consumption. One can also observe the monotonic behaviour with respect to time for higher values of β and γ, which may be explained by the higher ATP production by chromatophore together with the faster disappearance of AMP by myokinase.

Time course behaviour of X_{ADP} is shown in *Fig. 10* for the case when the values of β and γ are changed while holding the value of P_N constant. The value of X_{ADP} is greater for smaller β than that for larger β value when the values of P_N and γ are kept constant. This is due to the slow conversion of ADP to ATP by insufficient activity of chromatophore. In case of constant values of P_N and β, the value of X_{ADP} is lower for larger value of γ than that for smaller value of γ. This seems to be due to the reverse reaction catalyzed by myokinase and coincides with the results shown in *Fig. 9*. Here again one observes a non-monotonic behaviour for certain set of parameter values. This behaviour can be explained by the early production of ADP by NAD kinase followed by the later regeneration of ATP from this ADP via chromatophore. This non-monotonic behaviour becomes more pronounced as one increases myokinase level.

In *Fig. 11*, the time course behaviour of the value of X_{ATP} is shown for the case when the values of β and γ are changed while keeping the values of P_N constant. In

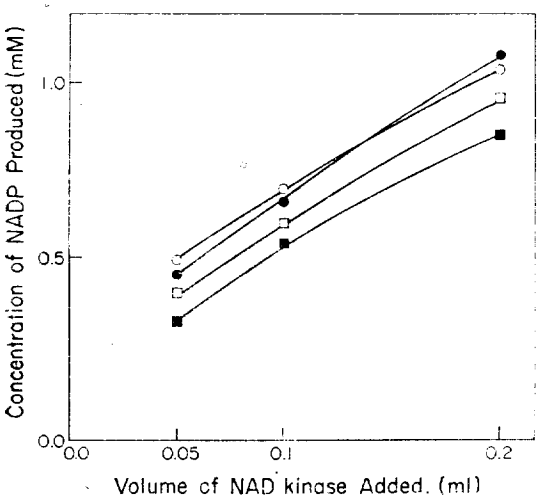

Fig. 8. Dependence of NADP Production on the Volume of NAD kinase added to the Reaction Mixture
Reaction Temperature : 30°C
Light Intensity : 20 klux
Reaction Time : 16 hrs
○—○ : 112 μM Bchl.
●—● : 84 μM Bchl.

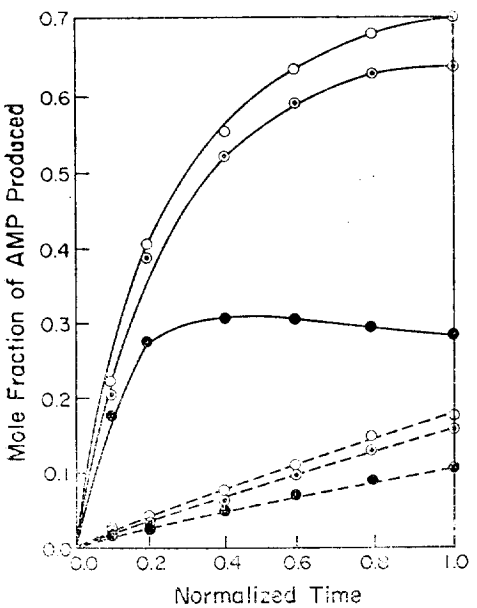

Fig. 9. Simulation Resuls on the Time Couse Beahviour of X_{AMP}. Constant NAD kinase Activity
$P_N = 100.0$ ●—● : $\beta = 10.0$ ⊙—⊙ : $\beta = 1.0$
○—○ : $\beta = 0.1$ —— : $\gamma = 1.0$
---- : $\gamma = 0.05$

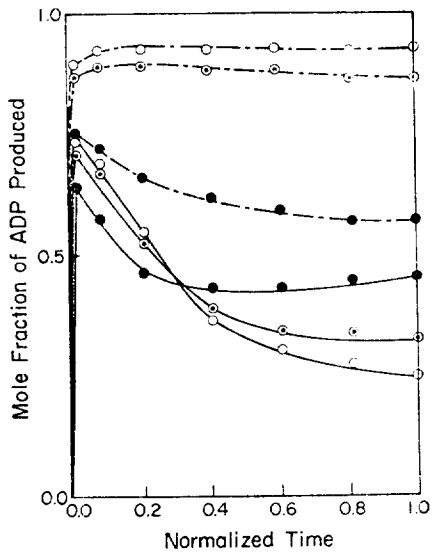

Fig. 10. Simulation Results on the Time Course Behaviour of X_{ADP}. Cnostant NAD kinase Activity
$P_N = 100.0$ ●—● : $\beta = 10.0$ ⊙—⊙ : $\beta = 1.0$
○—○ : $\beta = 0.1$ — — — : $\gamma = 0.0$
—— : $\gamma = 1.0$

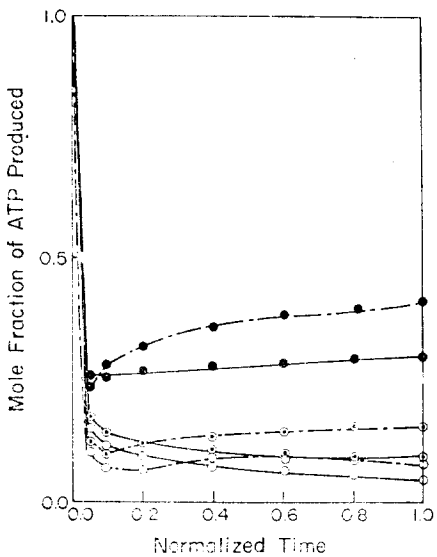

Fig. 11. Simulation Results on the Time Course Behaviour of X_{ATP} Constant NAD kinase Activity
$P_N = 100.0$ ●—● : $\beta = 10.0$ ⊙—⊙ : $\beta = 1.0$
○—○ : $\beta = 0.1$ — — — : $\gamma = 0.0$
—— : $\gamma = 1.0$

the case of constant valus of P_N and γ, the value of X_{ATP} is greater for larger β than that for smaller β. This is due to the ATP regeneration by the large activity of chromatophore. The non-monotonic behaviour appearing in this case can be explained by the same reasoning as in *Fig. 10.*

Ackno ledgment

This research was conceived during the three months' stay in Professor Aiba's laboratory of one of authors(Choi) under the inspiration by Professor Aiba and the financial support by the Korea Science and Engineering Foundation and the Japan Society for the Promotion of Science, for which the authors would like to express their sincere gratitude.

Nomenclature

ξ_1 Extent of reaction for chromatophore

ξ_2 Extent of reaction for NAD kinase

ξ_3 Extent of reaction for myokinase

$V_{max,C}$ Maximum velocity for chromatophore

$V_{max,N}$ Maximum velocity for NAD kinase

$V_{max,M}$ Maximum velocity for myokinase

K_{EC} Equilibrium constant for the reaction by chromatophore

N_C Number of chromatophore at appropriate time

$\tau = t/\theta_F$ Dimensionless variable for time

θ_F Final reaction time

X_i Mole fraction of the component i

$P_C = \frac{V_{max,Co}}{K_{MDPC}}\theta_F$ Dimensionless variable for the chromatophore activity

$P_N = \frac{V_{max,N}}{K_{TPN}}\theta_F$ Dimensionless variable for the NAD kinase activity

$P_M = \frac{V_{max,M}}{K_3}\theta_F$ Dimensionless variable for the myokinase activity

$\alpha = ([AMP]_o + [ADP]_o + [ATP]_o)/([NAD]_o + [NADP]_o)$

β = (Chromatophore activity)/(NAD kinase activity) $= P_C/P_N$

β' = (NAD kinase activity)/(Chromatophore activity) $= P_N/P_C$

γ = (Myokinase activity)/(NAD kinase activity) $= P_M/P_N$

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