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Changes in the distribution of the control of the mitochondrial oxidative phosphorylation in regenerating rabbit liver

Takuya Inomoto [a], Akira Tanaka [a], Shigeto Mori [a], Maeng Bong Jin [a], Bunpei Sato [a], Nobuharu Yanabu [a], Atsuo Tokuka [a], Toshiyuki Kitai [a], Kazue Ozawa [b], Yoshio Yamaoka [a]

[a] *Second Department of Surgery, Faculty of Medicine, Kyoto University, 54 Kawaracho, Shogoin, Sakyoku, Kyoto 606, Japan*
[b] *Shiga University of Medical Science, Shiga, Japan*

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Abstract

Applying the metabolic control theory, inhibitor titration studies were carried out on Complex I, III, IV, ATP synthase, ATP/ADP carrier and P_i carrier of mitochondrial oxidative phosphorylation in normal and regenerating rabbit liver in order to examine the acceleration mechanism of mitochondrial oxidative phosphorylation. In regenerating rabbit liver the rate of state 3 respiration, respiratory control ratio and phosphorylation rate in the presence of 3 mM glutamate, 250 μM ADP and 3 mM inorganic phosphate increased significantly as compared with the control by 73%, 48% and 76%, respectively. The control of the rate of state 3 respiration in normal liver was exerted by Complexes I, IV and steps other than the aforementioned six steps, whose flux control coefficients were 0.317, 0.214 and 0.469, respectively. By contrast, in regenerating liver, the control was more evenly distributed among these steps in oxidative phosphorylation and the possibility is suggested that Complexes I, IV and steps other than the six steps are activated during regeneration. The activation of Complexes I and IV was attributed to their increased activity, since it was not accompanied by an increase in the amount of the enzymes.

Keywords: Mitochondrion; Oxidative phosphorylation; Liver regeneration; (Rabbit liver)

1. Introduction

Oxidative phosphorylation is an important reaction as the major source of ATP in aerobic organisms. Over the last few decades several investigations have been done concerning the means by which mitochondrial oxidative phosphorylation is controlled. Early studies tried to determine a single particular step which limits the rate of oxidative phosphorylation [1–5]. Since Kacser and Burns [6] and Heinrich and Rapoport [7] proposed the metabolic control theory, inhibitor titration studies have been carried out on mitochondrial oxidative phosphorylation. It has been demonstrated that the control of oxidative phosphorylation is distributed among several steps [8], and that the distribution of the control depends on the experimental condition: the concentrations of adenine nucleotides, phosphate, oxidized substrate and calcium [9–12]. In these studies, mitochondria were isolated from normal organs and the changes in the distribution of the control were observed in vitro by incubating the mitochondria with various media to change the rate of state 3 respiration. However, in order to carefully scrutinize the regulation of mitochondrial oxidative phosphorylation, it is necessary to elucidate the distribution of the control not only in normal mitochondria but also in pathological ones whose rate of respiration is altered in vivo, and to compare them because the distribution of the control can change significantly as a consequence of regulation of the activity of enzymes [13].

Partial hepatectomy induces the regeneration of the remnant liver [14]. In regenerating liver, ATP is consumed for active biosynthesis of components such as DNA and protein, causing an increased demand for energy [15]. Mitochondrial oxidative phosphorylation in regenerating liver is enhanced in order to meet the increased energy demand [16]. We have previously reported that mitochondrial oxidative phosphorylation is enhanced significantly in regenerating rabbit liver at

* Corresponding author. Fax: +81 75 7513099.

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24 h after 70% hepatectomy [17]. However, little is known concerning the acceleration mechanism of mitochondrial oxidative phosphorylation. In the present study, we performed inhibitor titration studies on two kinds of mitochondria, one from the normal liver and the other from the remnant liver at 24 h after 70% hepatectomy, to examine how the distribution of the control of the oxidative phosphorylation changed when the rate of mitochondrial oxidative phosphorylation increased in regenerating rabbit liver.

2. Materials and methods

2.1. Animals and operation

Male rabbits, weighing 2 kg and maintained on laboratory chow, were randomly assigned to one of two groups: sham-operated group (Group S) or 70% hepatectomy group (Group H). All rabbits were given access to water only for 12 h prior to operation. Operation was performed under anesthesia with thiobarbiturate at a dose of 15 mg/kg body weight. In Group S, simple laparotomy and mobilization of the liver were performed. In Group H, the left anterior, right anterior and right posterior lobes were resected according to the method of Higgins and Anderson [14]. After administration of 60 ml saline solution to maintain circulating blood volume, the rabbits were fasted for 24 h. Laparotomy was then carried out under anesthesia with a half dose of thiobarbiturate for sampling of liver tissue.

2.2. Preparation of mitochondria

Immediately after the removal of the liver, the tissue was minced into approximately 0.2 mm slices in chilled isolation medium containing 0.3 M mannitol and 0.1 mM EDTA and washed five times with the same medium to remove hemoglobin. Mitochondria were prepared by methods described elsewhere [18].

2.3. Measurement of mitochondrial respiration and the plot of the rate of respiration as a function of the concentrations of ADP and glutamate

Mitochondrial respiration was measured polarographically at 22°C in a reaction medium containing 0.3 M mannitol, 10 mM KCl, 10 mM Tris-HCl, 0.2 mM EDTA-Tris, 2 mM $MgCl_2$ and 3 mM phosphate buffer at pH 7.4. Mitochondrial respiration rate was measured with eight different concentrations of ADP from 3.9 μM to 500 μM in the presence of 3 mM glutamate to give a Michaelis constant (K_m) of state 3 respiration for ADP, and was measured with 15 different concentrations of glutamate from 0.015 mM to 15.05 mM in the presence of 250 μM ADP to give a K_m of state 3 respiration for glutamate. K_m of mitochondrial respiration was determined using Hanes-Woolf plot. In another set of experiments, mitochondrial respiration was measured with and without 2 mM malate, in the presence of 250 μM ADP and 3 mM glutamate. Mitochondrial protein was determined by the method of Lowry et al. [19].

2.4. Determination of flux control coefficients

State 3 respiration, which was initiated by the addition of 250 μM ADP in the presence of 3 mM glutamate, was inhibited by various concentrations of different specific inhibitors; rotenone, antimycin, cyanide, oligomycin, atractyloside and mersalyl for Complex I, III, IV, ATP synthase, ATP/ADP carrier and P_i carrier, respectively. Inhibitor titration curves were drawn for 0% to 100% inhibition. Flux control coefficients (FCC) for six steps were determined according to the equation proposed by Groen et al. [8]. Since inhibition by rotenone, antimycin, oligomycin and mersalyl is irreversible, FCCs of Complex I, III, ATP synthase and P_i carrier were calculated as the initial slope of the inhibition curve multiplied by the minimal amount of inhibitor required to obtain maximal flux inhibition (I_{max}).

For the inhibition of the ATP/ADP carrier, atractyloside was used, since carboxyatractyloside, an irreversible inhibitor of ATP/ADP carrier, cannot be purchased in Japan. Although the inhibition of ATP/ADP carrier by atractyloside has been called competitive, Klingenberg et al. revealed that it is far from being a clear competitive relationship and is,in fact, closer to an irreversible relationship, hence they have defined it instead as a semicompetitive inhibitor [20]. In this study, the FCC of ATP/ADP carrier was calculated assuming that atractyloside was an irreversible inhibitor. The actual value of the FCC of ATP/ADP carrier might be somewhat higher in hepatectomized rabbit. In the case of Complex IV, FCC was calculated as the initial slope of the inhibition curve multiplied by the dissociation constant (K_i) of 16 mM for cyanide, since cyanide is a noncompetitive inhibitor [21].

2.5. Measurement of tissue and intramitochondrial adenine nucleotide

Tissue and intramitochondrial adenine nucleotides were extracted with 6% perchloric acid and neutralized with K_2CO_3 and triethanolamine from the liver tissue freeze-clamped with tongs precooled in liquid nitrogen and isolated mitochondria, respectively. The concentrations of ATP, ADP and AMP were analyzed by HPLC [22].

2.6. Measurement of intramitochondrial cytochromes

The intramitochondrial concentration of cytochromes was determined spectrophotometrically in a medium containing 0.3 M mannitol, 10 mM Tris-HCl, 10 mM KCl, 0.2 mM EDTA-Tris, 3 mM phosphate buffer and 2% sodium cholate at pH 7.4 by the method previously reported [23]. Extinction coefficient differences between fully oxidized and reduced state employed were 26.4 $mM^{-1}cm^{-1}$ at 605–630 nm, 17.9 $mM^{-1}cm^{-1}$ at 562–575 nm, and 19.0 $mM^{-1}cm^{-1}$ at 550–540 nm for cytochrome aa_3, b and $c + c_1$, respectively.

2.7. Chemicals

Rotenone, antimycin, atractyloside, oligomycin and mersalyl were purchased from Sigma Chemical Co. and cyanide was from Wako Pure Chemical Industries.

2.8. Statistical analysis

All values are expressed as mean ± S.E. Analysis of variance and unpaired t-test were used for statistical analysis.

3. Results

3.1. Changes in oxidative phosphorylation activity in hepatectomized rabbit liver mitochondria and its dependence on the concentration of ADP and glutamate

Table 1 shows an increase in mitochondrial oxidative phosphorylation activity of the regenerating liver at 24 h after 70% hepatectomy, as measured using glutamate as an oxidized substrate. In hepatectomized rabbit the rate of state 3 respiration, respiratory control ratio and phosphorylation rate increased significantly as compared with the control by 73%, 48% and 76%, respectively. In the presence of 2 mM malate, the rate of state 3 respiration also increased significantly from 42.1 ± 2.2 nanoatom O consumed/min per mg of the control ($n = 4$) to 64.1 ± 3.0 ($n = 4$) in hepatectomized rabbit ($P < 0.05$).

Table 1
Mitochondrial oxidative phosphorylation activity of control and hepatectomized rabbit liver

	Control ($n = 17$)	Hepatectomy ($n = 17$)
ST3 (nanoatom O consumed/min per mg)	28.8 ±2.4	49.8 ±2.2 *
RC	5.0 ±0.3	7.4 ±0.4 *
ADP/O Ratio	2.42±0.04	2.51±0.05
PR (nonoatom ATP synthesized/min per mg)	70.4 ±6.5	124.1 ±4.1 *

Mitochondrial respiration was measured polarographically at 22°C in a reaction medium containing 0.3 M mannitol, 10 mM KCl, 10 mM Tris-HCl, 0.2 mM EDTA-Tris, 2 mM $MgCl_2$, 3 mM phosphate buffer and 3 mM glutamate at pH 7.4. State 3 respiration was initiated by addition of 250 μM ADP. Abbreviations: ST3, state 3 respiration; RC, respiratory control ratio; PR, phosphorylation activity.
* Significantly different from the control with $P < 0.01$.

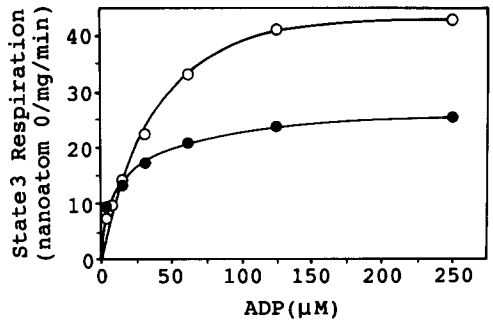

Fig. 1. The plot of the rate of the state 3 respiration as a function of the ADP concentration in control and regenerating rabbit liver. Mitochondrial respiration was measured polarographically at 22°C in the medium described in Table 1. State 3 respiration was initiated by addition of different concentrations of ADP, i.e., 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250 and 500 μM. The results obtained with 500 μM ADP were omitted. A representative case of three experiments is shown. •, control; ○, regenerating rabbit liver.

Figs. 1 and 2 show the plot of respiration rate as a function of the concentration of ADP and glutamate, respectively. As seen in these figures, these reactions can be considered to obey Michaelis-Menten kinetics. The concentrations of ADP and glutamate used in the inhibitor titration studies, i.e., 250 μM and 3 mM, were high enough to obtain the maximal respiration rate. Table 2 shows reciprocal changes in K_m for ADP and

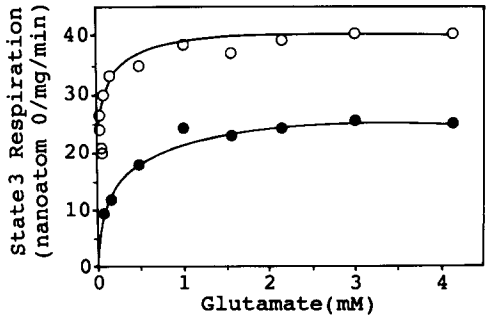

Fig. 2. The plot of the rate of respiration as a function of the glutamate concentration in control and regenerating rabbit liver. Mitochondrial respiration was measured polarographically at 22°C in the medium described in Table 1 except that the concentrations of glutamate were varied, i.e., 0.015, 0.025, 0.04, 0.05, 0.075, 0.15, 0.5, 1.0, 1.575, 2.15, 3, 4.15, 7.575, 9.04 and 15.05 mM. State 3 respiration was initiated by addition of 250 μM ADP. The results obtained with 7.575, 9.04 and 15.05 mM glutamate were omitted. A representative case of three experiments is shown. •, control; ○, regenerating rabbit liver.

Table 2
Michaelis constants of state 3 respiration for ADP and glutamate

	Control ($n = 4$)	Hepatectomy ($n = 4$)
ADP (μM)	26 ±5.1	53.8 ±4.4 *
Glutamate (mM)	0.237±0.012	0.040±0.003 **

Michaelis constants (K_m) for ADP and glutamate were determined using Hanes-Woolf plot.
*, ** Significantly different from the control with $P < 0.05$, $P < 0.01$, respectively.

glutamate as determined using Hanes-Woolf plot. After hepatectomy, the K_m value for glutamate decreased significantly from 0.237 ± 0.012 mM of the control to 0.040 ± 0.003 mM, while the value for ADP increased significantly from 26 ± 5.1 μM to 53.8 ± 4.4 μM.

3.2. Inhibitor titration studies

Fig. 3 shows the titration curves of the rate of state 3 respiration with specific inhibitors at the six steps of Complex I, III, IV, ATP synthase, ATP/ADP carrier and P_i carrier. In the titration curves of ATP synthase, ATP/ADP carrier and P_i carrier, approximately only an 80% reduction of state 3 respiration was achieved

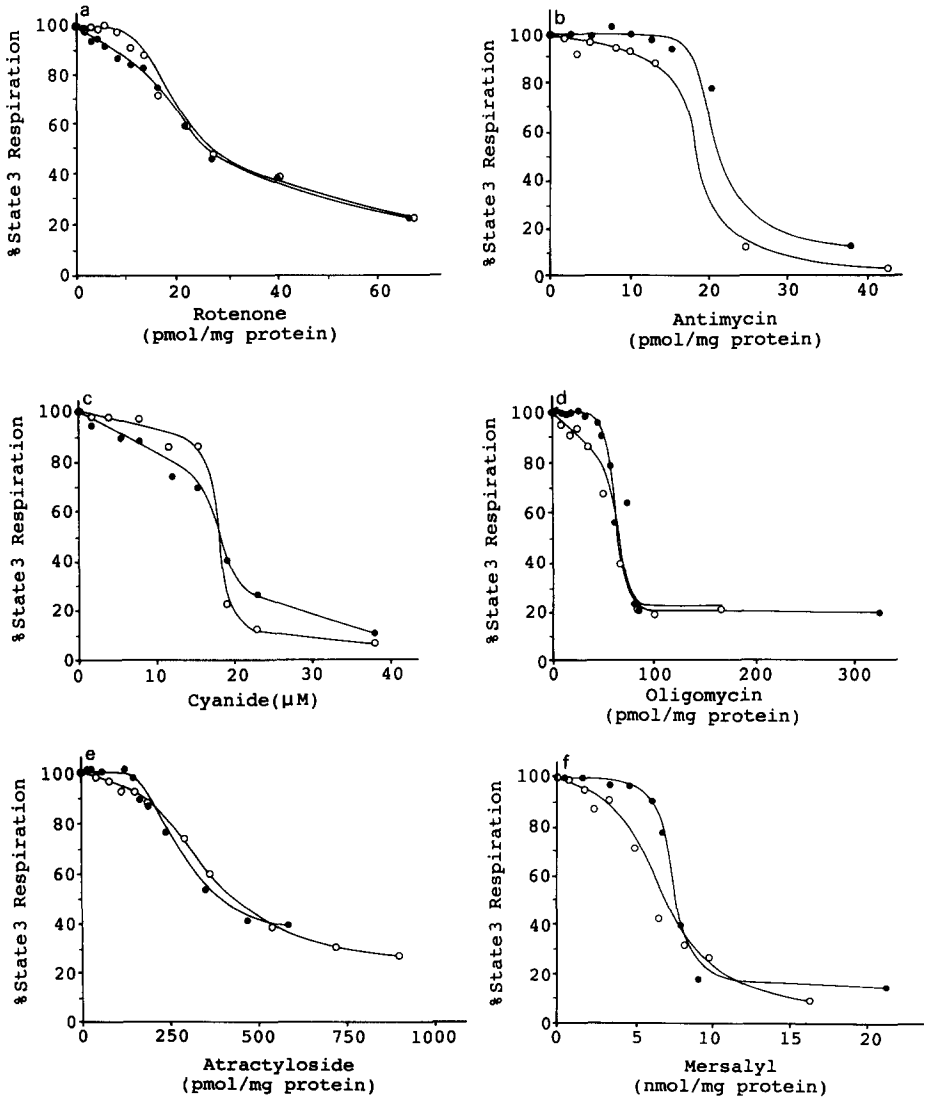

Fig. 3. Inhibitor titration curves. Mitochondria were incubated at 22°C in the medium described in Table 1 with the indicated concentrations of different specific inhibitors; (a) rotenone, (b) antimycin, (c) cyanide, (d) oligomycin, (e) atractyloside and (f) mersalyl for Complex I, III, IV, ATP synthase, ATP/ADP carrier and P_i carrier, respectively for 5 min because of the time dependence of inhibition by cyanide [40] and oligomycin [11]. State 3 respiration was initiated by addition of 250 μM ADP and measured polarographically. A representative case of three experiments is shown. The rates of state 3 respiration without an inhibitor, in both control and regenerating rabbit liver, were 26.6 ± 1.3 and 48.1 ± 4.0 nanoatom O consumed/min per mg protein for Complex I, 27.8 ± 1.9 and 53.4 ± 3.5 for Complex III, 29.4 ± 1.7 and 53.2 ± 3.9 for Complex IV, 28.5 ± 3.3 and 48.8 ± 3.2 for ATP synthase, 26.1 ± 0.9 and 54.1 ± 4.2 for ATP/ADP carrier and 25.4 ± 2.5 and 47.7 ± 4.3 for P_i carrier, respectively. ●, control; ○, regenerating rabbit liver.

Table 3
Flux control coefficient of the six steps in oxidative phosphorylation in control and hepatectomized rabbit

	Control ($n = 4$)	Hepatectomy ($n = 4$)
Complex I	0.317 ± 0.009	0 ± 0 *
Complex III	0 ± 0	0.139 ± 0.016 *
Complex IV	0.214 ± 0.047	0.077 ± 0.028
ATP synthase	0 ± 0	0.209 ± 0.045 *
ATP/ADP carrier	0 ± 0	0.266 ± 0.006 *
P_i carrier	0 ± 0	0.164 ± 0.03 *

* Significantly different from the control with $P < 0.01$.

because 100% inhibition brought the mitochondrial respiration into state 4 respiration.

Table 3 shows the changes in FCC of the six steps in oxidative phosphorylation calculated from the curves in Fig. 3. The FCCs of Complexes I and IV decreased from the control values of 0.317 and 0.214, and to 0 and 0.077 in hepatectomized rabbit, respectively. By contrast, the FCCs of Complex III, ATP synthase, ATP/ADP carrier and P_i carrier increased from the control values of 0 to 0.139, 0.209, 0.266 and 0.164 in hepatectomized rabbit, respectively.

3.3. Changes in tissue and intramitochondrial adenine nucleotide content

Table 4 shows that in hepatectomized rabbit liver, tissue ATP, ADP and total adenine nucleotide decreased significantly from the control value of 2.25, 0.81 and 3.32 to 0.82, 0.49 and 1.52 μmol/g liver, respectively. Tissue ATP/ADP ratio decreased significantly from the control value of 2.84 to 1.68. By contrast, intramitochondrial total adenine nucleotide did not decrease in hepatectomized rabbit and intramitochondrial ATP/ADP ratio increased from the control value of 0.62 to 1.27. Tissue adenine nucleotides are assumed to be cytosolic because the amount of cytosolic adenine nucleotides is far greater than that of intramitochondrial adenine nucleotides [24].

Table 4
Cytosolic and intramitochondrial adenine nucleotide contents in control and hepatectomized rabbit liver

	Control ($n = 4$)	Hepatectomy ($n = 4$)
Cytosol		
ATP (μmol/g liver)	2.25 ± 0.09	0.82 ± 0.17 **
ADP (μmol/g liver)	0.81 ± 0.07	0.49 ± 0.10 *
AMP (μmol/g liver)	0.26 ± 0.04	0.21 ± 0.04
TAN (μmol/g liver)	3.32 ± 0.14	1.52 ± 0.30 **
ATP/ADP ratio	2.84 ± 0.26	1.68 ± 0.17 *
Intramitochondria		
ATP (nmol/mg protein)	4.62 ± 1.27	7.36 ± 0.99
ADP (nmol/mg protein)	7.40 ± 0.88	5.74 ± 0.62
AMP (nmol/mg protein)	1.55 ± 0.17	0.93 ± 0.31
TAN (nmol/mg protein)	13.57 ± 1.97	14.03 ± 1.53
ATP/ADP Ratio	0.62 ± 0.14	1.27 ± 0.06 **

Cytosolic and intramitochondrial adenine nucleotides were extracted with 6% perchloric acid and neutralized with K_2CO_3 and triethanolamine from the liver tissue freeze-clamped with tongs precooled in liquid nitrogen and isolated mitochondria, respectively. Adenine nucleotides were analyzed by HPLC. TAN is the abbreviation of total adenine nucleotide.
*, ** Significantly different from the control with $P < 0.05$ and $P < 0.01$, respectively.

Table 5
Concentration of cytochrome aa_3, b and $c + c_1$ in isolated mitochondria of control and hepatectomized rabbit liver

	Control ($n = 4$)	Hepatectomy ($n = 4$)
Cytochrome aa_3 (pmol/mg protein)	98.0 ± 14.6	86.9 ± 8.0 NS
Cytochrome b (pmol/mg protein)	103.6 ± 13.3	93.4 ± 2.5 NS
Cytochrome $c + c_1$ (pmol/mg protein)	210.6 ± 24.6	187.9 ± 8.9 NS

Mitochondrial cytochromes were determined spectrophotometrically in a medium containing 0.3 M mannitol, 10 mM Tris-HCl, 10 mM KCl, 0.2 mM EDTA-Tris, 3 mM phosphate buffer, 3 mM ADP and 2% sodium cholate at pH 7.4. Sodium cholate was put into the medium to solubilize the mitochondria and lessen error due to light scattering. To reduce the influence of contaminated hemoglobin on the spectrophotometric measurement of mitochondrial cytochromes, the solutions were bubbled with 100% CO for 1 min. Extinction coefficient differences employed were 26.4 $mM^{-1}cm^{-1}$ at 605–630 nm, 17.9 $mM^{-1}cm^{-1}$ at 562–575 nm, 19.0 $mM^{-1}cm^{-1}$ at 550–540 nm for cytochrome aa_3, b and $c + c_1$, respectively. NS, not significantly different from the control.

3.4. Concentration of cytochromes

The concentration of cytochromes in isolated mitochondria of control and hepatectomized rabbit was determined in order to study whether the changes in FCC of Complexes III and IV were accompanied by any changes in the concentrations of cytochromes. Table 5 shows that the concentrations of cytochrome aa_3, b and $c + c_1$ did not change in hepatectomized rabbit as compared with the control.

4. Discussion

In inhibitor titration studies, it is necessary that concentrations of substrate and product be constant. The concentrations of ADP and glutamate used in the inhibitor titration studies, i.e., 250 μM and 3 mM, were sufficient to assume that the concentrations of the substrate and product are constant during the course of short-term measurement. In previous reports of inhibitor titration study in which glutamate was used as an oxidized substrate, malate was added into a reaction medium [21,25]. Malate is oxidized to form oxaloacetate and increases the rate of state 3 respiration of isolated liver mitochondria. In our study, the rate of

state 3 respiration significantly increased in regenerating rabbit liver whether or not malate was present in the reaction medium. Therefore, we omitted the malate and performed inhibitor titration studies with an endogenous oxaloacetate concentration.

After hepatectomy significant changes in K_m for ADP and glutamate were observed (Table 2). However, these changes seem to make no contribution to the increase in mitochondrial oxidative phosphorylation activity of the regenerating liver, since the concentrations of both ADP and glutamate in the cytosol are far higher than these K_m values [26].

The results of inhibitor titration studies (Table 3) indicate that in normal liver, the control of the rate of the oxidative phosphorylation was exerted by Complexes I and IV, whereas, in regenerating liver in which the rate of oxidative phosphorylation was increased, the control was more evenly distributed among the steps. Mitochondrial oxidative phosphorylation in this experiment involves many steps other than the six steps whose FCCs were calculated; for example, the glutamate-H^+ carrier and glutamate-aspartate exchanger for glutamate transport, glutamate dehydrogenase and oxidation steps from alpha-ketoglutarate to oxaloacetate in the citric acid cycle [21,27]. Since there are no specific inhibitors for them, the FCCs of these steps were estimated collectively by applying the summation theorem of control analysis, in which the sum of the FCCs of all steps included in a pathway are regarded as a unity [6–8]. The sum of their FCCs was 0.469 in normal liver and 0.145 in regenerating liver. This also indicates that in regenerating liver, the control of the oxidative phosphorylation was not exerted by particular steps and that there is no rate limiting step. FCCs in rat liver mitochondria have been reported previously. Gellerich et al. reported the FCC of ATP/ADP carrier to be between 0.39 and 0.44 [28]. Groen et al. reported that FCCs of Complex III, Complex IV and ATP/ADP carrier were 0.03, 0.17 and 0.29, respectively [8]. These values are quite different from those of rabbit liver mitochondria in our study. The distribution of the control depends on the experimental conditions [9–12]. In our study, the concentrations of adenine nucleotides, phosphate and glutamate are different from those used in previous reports. Furthermore, it was previously reported that the rate of state 3 respiration of rabbit liver mitochondria was less than half that of rat liver mitochondria [29] and the difference in mitochondrial respiration between rat and rabbit was suggested. The difference in FCCs is considered to be due to the different experimental conditions, and to the species difference in mitochondrial respiration.

Although inhibitor titration studies in this experiment have been performed in unphysiological conditions, these changes in the distribution of the control well explain phenomena in vivo and the efficacy of inhibitor titration studies are confirmed. The changes in cytosolic and intramitochondrial adenine nucleotide contents (Table 4) can be rationalized by the results of inhibitor titration studies. Cytosolic ATP and ADP decreased significantly with a parallel decrease in total adenine nucleotide as compared with the control because cytosolic ATP and ADP were consumed for liver regeneration. By contrast, in mitochondria, in which ATP synthesis from ADP is increased by an enhanced mitochondrial oxidative phosphorylation, ATP increased concomitant with a slight decrease in ADP as compared with the control. Intramitochondrial total adenine nucleotide was constant, which is considered to be due to the one-for-one exchange of ATP/ADP carrier [20]. As a result of these changes, ATP/ADP ratio showed a reciprocal change in cytosol and mitochondria. The decrease in cytosolic ATP/ADP ratio in regenerating liver is the result of utilizing ATP faster than it is supplied. The increase in intramitochondrial ATP/ADP ratio in regenerating liver is the result of changes in the activities of ATP/ADP carrier and ATP synthase. In hepatectomized rabbit liver mitochondria, the FCC of ATP/ADP carrier, which was the same as that of ATP synthase in normal liver mitochondria, was larger than that of ATP synthase. This suggests that ATP/ADP carrier became less active in regenerating liver mitochondria than ATP synthase. In regenerating liver mitochondria, ATP synthesis exceeded ATP–ADP exchange and the storage of ATP in mitochondria increased the intramitochondrial ATP/ADP ratio.

Westerhoff [30] stated that it is important not to confuse control with regulation in metabolic control analysis. The flux control coefficient of an enzyme indicates to what extent the flux would change if the activity of that enzyme were changed. It does not by itself indicate whether the organism actually modulates the activity of that enzyme to regulate the flux. In order to discuss regulation in more detail, the elasticity coefficient, which indicates to what extent the flux would change if the concentration of the substrate of an enzyme was changed, and actual change in the concentration of the substrate have to be taken into account. However, inhibitor titration studies still provide us with useful information as the first step in the investigation of the regulation of a metabolic pathway. The result of inhibitor titration analysis suggests the possibility that Complexes I, IV and steps other than the six steps are activated during regeneration because the increase in the rate of the oxidative phosphorylation in regenerating liver is only brought about with an activation of the steps which exert the control in normal liver. Nagino et al. followed up the rate of oxidative phosphorylation, enzymatic activities and cytochrome concentrations in isolated liver mitochondria for 7 days after 70% hepatectomy in rats [31]. They

concluded that the increase in oxidative phosphorylation from the second postoperative day onward was due to the increase in the amount of enzymes. However, they observed no increase in the amount of enzymes on the first day, in spite of the increase in the rate of oxidative phosphorylation. We likewise observed the constant concentrations of cytochromes at 24 h after 70% hepatectomy (Table 5). In addition to this fact, in inhibitor titration curves of rotenone and cyanide (Fig. 3), I_{max}, which corresponds to the amount of the enzymes [32], did not change in hepatectomized liver. These findings indicate that the activation of Complexes I and IV at 24 h after 70% hepatectomy is not due to an increase in the amount of enzymes, but to an increase in their activity.

In the past years, various studies have been done with regard to the regulation of the activities of steps in oxidative phosphorylation, i.e., NAD-linked dehydrogenase [12,33], Complex I [34], ATP/ADP carrier [35] and ATP synthase [36–39], and the role of Ca^{2+} has been discussed. Since these previous studies focused on each step separately, it was unknown, when the rate of oxidative phosphorylation is enhanced, how each step in oxidative phosphorylation is regulated and how they harmonized as a whole in vivo. Our study, which solved this problem and showed the changes in the distribution of the control in the regenerating liver, provides those who investigate the regulation of oxidative phosphorylation with a successful means of approach.

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