Chapter 6

Effect of Alloxan-Diabetes and Subsequent Treatment with Insulin on Kinetic Properties of Succinate Oxidase Activity from Rat Kidney Mitochondria

Introduction

Earlier observations suggest that insulin status significantly influenced the oxidative energy metabolism of rat kidney mitochondria. Under these conditions, one month diabetic animals the succinate dehydrogenase (SDH) activity increased by about 55% and could be partially controlled by insulin treatment; the contents of cytochromes changed only at the late stage of diabetes (1). Also from chapter 3 it can be noted that, alloxan-diabetes and subsequent treatment with insulin significantly changed the kinetics properties of FoF_1 ATPase in kidney mitochondria. The foregoing results thus point out that the electron transport system of kidney mitochondria is significantly influenced by insulin status. Insulin status influences the kinetic properties of succinate oxidase (SO) from liver mitochondria (Chapter 5). Thus, insulin-dependant changes in kinetics properties of SO were evaluated and the results are summarized in this Chapter.

Materials and Methods

Details of chemicals required as given in chapter 5. Procedure of induction of diabetes, insulin treatment, isolation of mitochondria, and data analysis are as described in Chapter 2 of the Thesis. Succinate oxidase assay as described in Chapter 5 of the Thesis.

Results and Discussion

Results of body weight and kidney weight are similar as given in Chapter 3 and that of diabetic parameters are as detailed in Chapter 2 of the Thesis.

The data in Table 1 summarize the effect of insulin status on SO activity in kidney mitochondria at early and late stages of diabetes. Measurements at 25 °C revealed that the SO activity had increased significantly in one week and one month diabetic groups (3.14 and 5.37 fold increase respectively). Treatment with insulin brought back the activity closer to the control values only in one month diabetic animals. When the measurements were carried out at 37 °C, increase in activity amounted to 1.47 and 2.12 fold respectively in one week and one month diabetic animals. Insulin treatment completely restored the activity in both the groups. The activity ratios remain low under all the experimental conditions compared to control (Table 1).

These temperature-dependent differential effects (Table 1) prompt to examine in details the temperature kinetics of the enzyme as influenced by the insulin status. The typical activity versus temperature plots and the corresponding Arrhenius plots for the one week and the one month groups are shown in Fig. 1 and 2.

Consistent with the data in Table 1, at any given temperature the activities were high in the diabetic groups (Fig. 1 and 2, Panels A, B and C). Insulin treatment had an apparent generalized restorative effect. However, the important and interesting feature was the shift in the optimum temperature. In control groups the optimum temperature was 45 °C which did not change at early stage of diabetes but decreased to 41 °C at the late stage. Insulin treatment shifted the optimum temperature to 49 °C in one week diabetic group whereas in one month diabetic animals it was restored to 45 °C. These differences in

Table 1. Effect of alloxan-diabetes and insulin treatment on succinate oxidase activity of rat kidney mitochondria

Group	Treatment	Activity		Activity Ratio
		25 °C	37 °C	
One week	Control	10.91 ± 0.41	56.09 ± 1.59	5.23 ± 0.29
	Diabetic	34.22 ± 1.80[a]	83.81 ± 2.81[a]	2.45 ± 0.12
	Diabetic + Insulin	25.90 ± 1.00[a,§]	54.09 ± 1.33[§]	2.09 ± 0.08
One month	Control	9.43 ± 0.82	54.49 ± 1.42	5.80 ± 0.22
	Diabetic	50.51 ± 2.62[a]	115.79 ± 7.58[a]	2.29 ± 0.11
	Diabetic + Insulin	14.20 ± 0.49[a,§]	51.38 ± 1.37[§]	3.62 ± 0.16

The experimental details are given in the text. The results are given as mean ± SEM of 6-8 independent observations in each group.

Activity ratio was calculated as: activity at 37 °C/activity at °C

a, $p<0.001$ compared to the corresponding control.
§, $p<0.001$ compared to the corresponding diabetic.

Figure 1

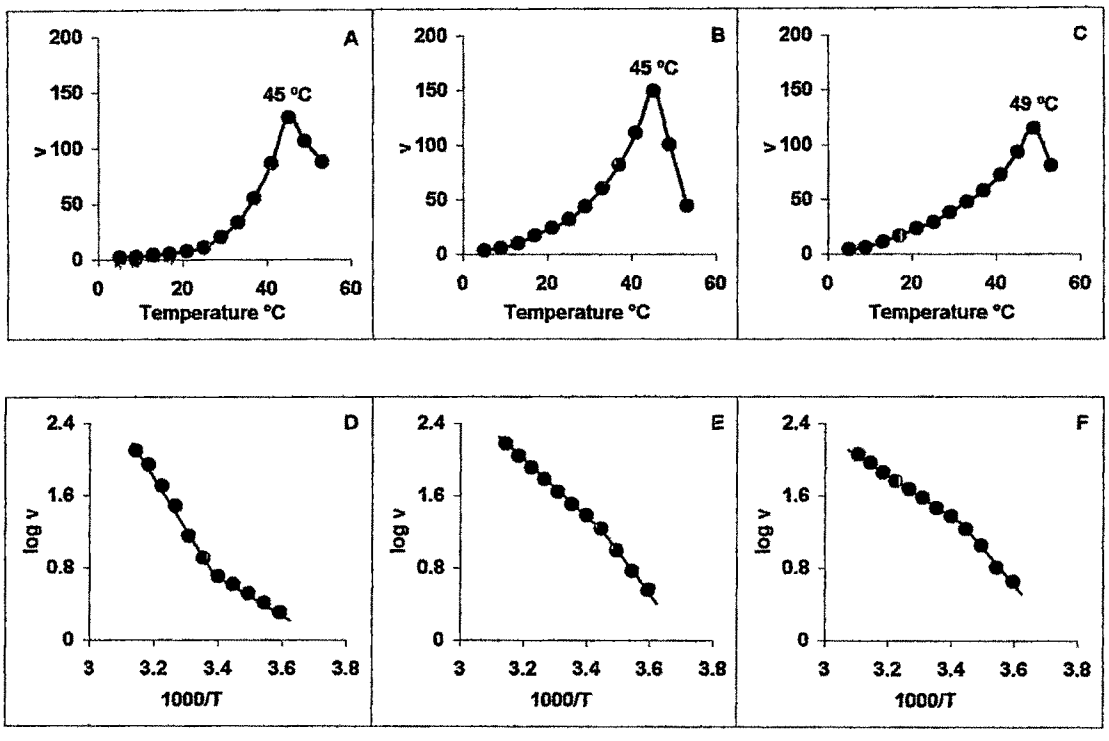
A
B
C
45 °C
45 °C
49 °C
200
150
100
50
0
v
0
20
40
60
Temperature °C
D
E
F
2.4
1.6
0.8
0
log v
3
3.2
3.4
3.6
3.8
1000/T

Figure 2

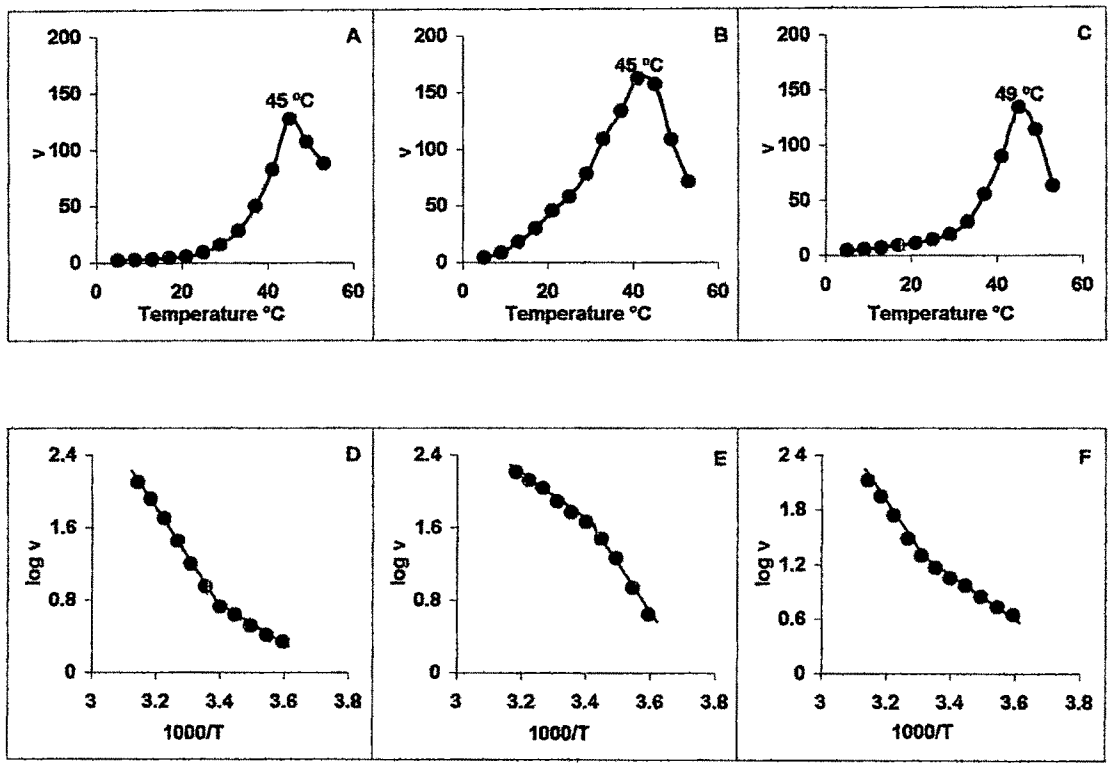

Figure 1. Typical plots depicting dependence of enzyme activity (Panels A, B and C) on temperature and the corresponding Arrhenius plots (Panels D, E and F) for one week groups. In the temperature curves, the enzyme activity v, on ordinate is plotted versus temperature (°C) on abscissa. A, B and C represent the control, diabetic and insulin-treated diabetic groups. In Arrhenius plots, log of v on ordinate is plotted against 1000/T on abscissa, where v and T represent respectively, the activity at corresponding absolute temperature T (temperature in ° Celsius + 273.18). D, E and F represent the control, diabetic and insulin-treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

Figure 2. Typical plots depicting dependence of enzyme activity (Panels A, B and C) on temperature and the corresponding Arrhenius plots (Panels D, E and F) for one month groups. In the temperature curves, the enzyme activity v, on ordinate is plotted versus temperature (°C) on abscissa. A, B and C represent the control, diabetic and insulin-treated diabetic groups. In Arrhenius plots, log of v on ordinate is plotted against 1000/T on abscissa, where v and T represent respectively, the activity at corresponding absolute temperature T (temperature in ° Celsius + 273.18). D, E and F represent the control, diabetic and insulin-treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

temperature kinetics properties became prominently evident when the data were transformed in the corresponding Arrhenius plots (Fig. 1 and 2, Panels D, E and F).

The interesting feature of the Arrhenius plots for the control group was that the energy of activation in the low temperature range (E_L) was low while that in the high temperature range (E_H) was high (Fig. 1 and 2, Panels D, E and F). Thus the pattern was opposite to what is commonly noted for most of the enzyme systems i.e. high values of E_L and low values of E_H (2, 3). Diabetic state reversed the pattern of Arrhenius plots (Fig. 1 and 2, Panels D, E and F). Insulin treatment had no effect on the pattern in one week diabetic group but in one month diabetic animals the features were restored to normality (Fig. 1 and 2, Panels D, E and F).

The values of energies of activation (E_H and E_L) and phase transition temperature (T_t) are given in Table 2. Thus in the control groups the values of E_L and E_H were around 52-55 and 102-111 KJ/mole with phase transition occurring at around 23-24 °C. In both the diabetic groups E_L almost doubled whereas E_H decreased to half of the control value. In the diabetic animals the phase transition temperature (T_t), decreased significantly with the effect being more marked in the early stage. Treatment with insulin in one week diabetic group partially lowered the E_L and brought about further decrease in E_H; in one month diabetic animals E_L decreased further while E_H became comparable to the control group. The phase transition temperature (T_t) values were not restored to control levels. In one week group insulin treatment elevated the T_t although it was still lower than that in the

controls. By contrast, in the one month group insulin treatment caused increase in T_t, beyond control. The low values of T_t in diabetic and insulin one week diabetic group

Table 2. Effect of alloxan-diabetes and insulin treatment on Arrhenius kinetics properties of succinate oxidase of rat kidney mitochondria

Group	Treatment	Energy of activation (KJ/mole)		Phase transition temperature T_t (°C)
		E_H	E_L	
One week	Control	101.9 ± 4.5	54.5 ± 3.0	22.9 ± 0.88
	Diabetic	58.8 ± 1.3[b]	97.8 ± 3.4[b]	15.8 ± 0.67[b]
	Diabetic + Insulin	46.4 ± 1.1[b,Ψ]	77.0 ± 3.0[b,Ψ]	18.8 ± 0.46[a,§]
One month	Control	111.4 ± 4.6	52.2 ± 2.3	24.4 ± 0.50
	Diabetic	51.5 ± 1.5[b]	108.6 ± 4.9[b]	19.0 ± 0.80[b]
	Diabetic + Insulin	101.9 ± 2.8[Ψ]	39.5 ± 1.5[b,Ψ]	28.0 ± 0.55[b,Ψ]

The experimental details are given in the text. The results are given as mean ± SEM of 6-8 independent observations in each group.

a, $p<0.002$ and b, $p<0.001$ compared to the corresponding control.
§, $p< 0.01$ and ψ, $p<0.001$ compared to the corresponding diabetic.

treated with insulin seem to be paradoxical especially in view of the fact that the fatty acid desaturase activity and unsaturation index decrease in diabetes (4).

The interesting feature of the present studies was increase in the SO activity in the diabetic animals and its suppression by insulin treatment. The enzyme SDH is responsible for initiating the process of electron transfer and is a rate-limiting step in SO activity (5). We have earlier noted that SDH activity increased marginally only in one month diabetic group and insulin treatment could only partly lower this activity (1). Therefore it is unlikely that the observed increase in SO in the diabetic or insulin treated diabetic animals is attributable to this factor. SDH is known to be activated by several physiologic activators which include ATP, NADH, Co Q and Pi (5). Insulin-status-dependent changes in the contents of Co Q and of cytochromes in mitochondria have been demonstrated (1,6). However, these changes do not correlate with observed changes in SO activities (1,6).

The other interesting feature of the present studies was the uncommon nature of the Arrhenius plots in the control animals and its reversal in the diabetic animals (Fig. 1 and 2). The net effect was that, in the diabetic animals the value of E_2 i.e. energy of activation in the high temperature range decreased substantially. As is evident, the phase transition temperature T_t was around 16-19 °C. This would mean that the high temperature range covered the physiologic temperature of 37 °C. Therefore the direct consequence would be that in the diabetic animals the energy barrier would decrease which would allow the enzyme system to function more efficiently. This could thus be a possible regulatory mechanism.

It has been reported that SO has requirement of bulk membrane phospholipids for its activity (7) Since energies of activation and T_t showed significant insulin-status-dependant changes (Table 2), it was of interest to find out if a correlation with lipid/phospholipids make-up existed. Regression analysis across the groups revealed that phosphatidylethanolamine (PE) showed a negative correlation with E_L ($r = - 0.650$) while phosphatidylcholine (PC) correlated positively with T_t ($r = + 0.614$). The SO activity, v by itself did not show any correlation with any of the lipid/phospholipids classes. The latter observation is consistent with and substantiates the earlier report that SO has a non-specific requirement for phospholipids (7). The bulk membrane lipids would thus seem to meet this requirement (7).

In conclusion, results of our present studies point out that regulation of SO in diabetes is a complex process. Additionally our studies also show that modulation od temperature kinetics properties may be a regulatory mechanism which improves the catalytic efficiency of the enzyme system in the diabetic state.

Summary

In the diabetic animals SO activity increased significantly and the increase was more pronounced at the late stage. Insulin treatment partially restored the SO activity. However, the effect was temperature-dependent.

In the diabetic animals the energy of activation in low temperature range (E_L) increased significantly while that in the high temperature ranges (E_H) decreased. The latter seems to be responsible for improving catalytic efficiency in diabetic state. Insulin treatment normalized E_H only in one month diabetic group.

The phase transition temperature, T_t decreased in diabetic animals. Insulin treatment caused increase beyond control value in T_t in one month diabetic animals.

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