Chapter 5

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

Introduction

From Chapter 2 it is clear that, the insulin status plays a significant role in altering the kinetic behavior of FoF_1 ATPase in rat liver mitochondria. The FoF_1 ATPase plays a significant role in energy coupling and has restricted micro-domain where it is localized but is not a component of the electron transport chain (E. T. C.) (1,2). As against this, enzyme succinate oxidase spans a major portion of the electron transport chain from succinate dehydrogenase (SDH) to cytochrome oxidase. Evaluation of its kinetic properties as influenced by insulin status can give broad based deeper insights in to the regulatory role of membrane lipids.

Materials and Methods

Chemicals

Sodium salt of ethylenediaminetetraacetic acid (EDTA) and bovine serum albumin (BSA) fraction V were purchased from Sigma Chemical Co., St. Louis, MO, U. S. A. Sodium salt of succinic acid was purchased from SRL, Mumbai. NPH insulin (40 IU/ml) was obtained from Lilli, France S.A.S. All other chemicals were of analytical-reagent grade and were purchased locally.

Details 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

Measurement of succinate oxidase activity was carried out polarographically using a Clarke-type oxygen electrode. The assay medium (final volume 1.6 ml) consisted of: 50 mM potassium phosphate buffer pH 7.4, containing 0.4 mM each of $CaCl_2$ and $AlCl_3$ (3), and saturating amount of sodium succinate (10 mM). The measurements were carried out over the temperature range from 5 to 53 °C with an increment of 4 °C at each step. The activity (v) is expressed as nmole O_2/min/mg protein.

Results and Discussion

Results on body weight, liver weight and diabetic parameters are similar as detailed in Chapter 2 of the Thesis.

In the preliminary study the alloxan-diabetes and insulin treatment on succinate oxidase activity. Measurements were carried out at 25 °C and 37 °C. Data are given in Table 1. Thus, in one week diabetic group enzyme activity decrease by 67 to 70 % respectively at two temperatures. Treatment with insulin almost restored the activity to normality. At the end of one month of diabetic state, the activity decrease by 42 and 50 % respectively at the two temperatures. However, in long-term diabetic animals, insulin treatment failed to restore the activity. Also, measurements at 25 °C and 37 °C indicated that the increment in the activity with temperature was differential and of lesser magnitude in the diabetic groups.

Table 1. Effect of alloxan-diabetes and insulin treatment on succinate oxidase activity in rat liver mitochondrial

Group	Treatment	Activity (nmole O_2/min/mg protein)		Activity Ratio
		25 °C	37 °C	
One week	Control	34.24 ± 1.12	59.55 ± 3.94	1.74 ± 0.08
	Diabetic	11.15 ± 0.43[a]	17.66 ± 0.92[a]	1.58 ± 0.09
	Diabetic + Insulin	28.79 ± 0.48[a,§]	54.26 ± 1.16[§]	1.88 ± 0.12
One month	Control	31.35 ± 1.48	53.21 ± 2.18	1.70 ± 0.13
	Diabetic	18.21 ± 1.26[a]	26.39 ± 1.21[a]	1.45 ± 0.09
	Diabetic + Insulin	14.21 ± 0.18[a,ψ]	23.24 ± 0.29[a,ψ]	1.64 ± 0.08

The experimental details are given in the text.

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

The results are given as mean ± SEM of 6-8 independent experiments in each group.

a, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.05$ and §, $p<0.001$ compared to the corresponding diabetic.

These observations prompt to investigate in detail the temperature-dependence of the enzyme activity under different experimental conditions. The typical activity versus temperature curves and corresponding Arrhenius plots for the one week and the one month groups respectively are shown in Fig. 1 and 2. As can be noted the activity versus temperature curves (Fig. 1 and 2) are consistent with the data in Table 1. Thus, the activities at any given temperature were low in the diabetic groups (Fig. 1 and 2, panels A, B and C). Insulin treatment restored the activities in one week diabetic but not in one month diabetic group. However, the most interesting feature was shift in optimum temperature in the diabetic or insulin treated animals. Thus, the optimum temperature for the control group was 37 °C which increased to 41-43 °C in diabetic groups and remained elevated at 45 °C in insulin treated diabetic animals. The differences in the profiles of activity versus temperature were clearly evident in the corresponding Arrhenius plots (Fig. 1 and 2, panels D, E and F).

The values of energies of activation derived from Arrhenius plots are given in Table 2. The values of E_H and E_L, respectively, were around 42 and 100 KJ/mole for the controls with phase transition occurring of around 20 °C. In diabetic animals the energies of activation E_H and E_L decreased significantly in one week diabetic group. In one month diabetic animals similar trend was noted although the extent of decrease was not as appreciable. In both the groups, phase transition temperature T_t decreased significantly. Treatment with insulin in one week diabetic group completely restored E_H with partial restoration in E_L; in one month diabetic animals insulin treatment was ineffective in this respect. The phase transition temperature T_t values were not restored to control levels and remained lower than the control values. The low values of T_t under both the

Figure 1

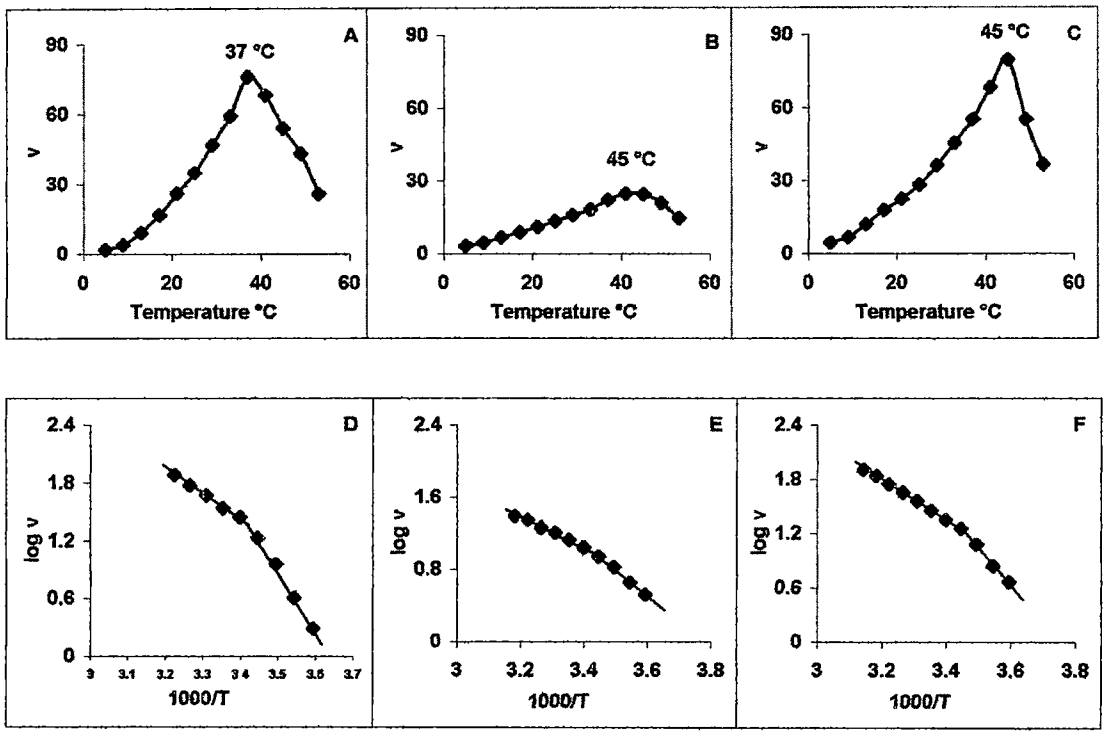
A
37 °C
v
Temperature °C
B
45 °C
v
Temperature °C
C
45 °C
v
Temperature °C
D
log v
1000/T
E
log v
1000/T
F
log v
1000/T

Figure 2

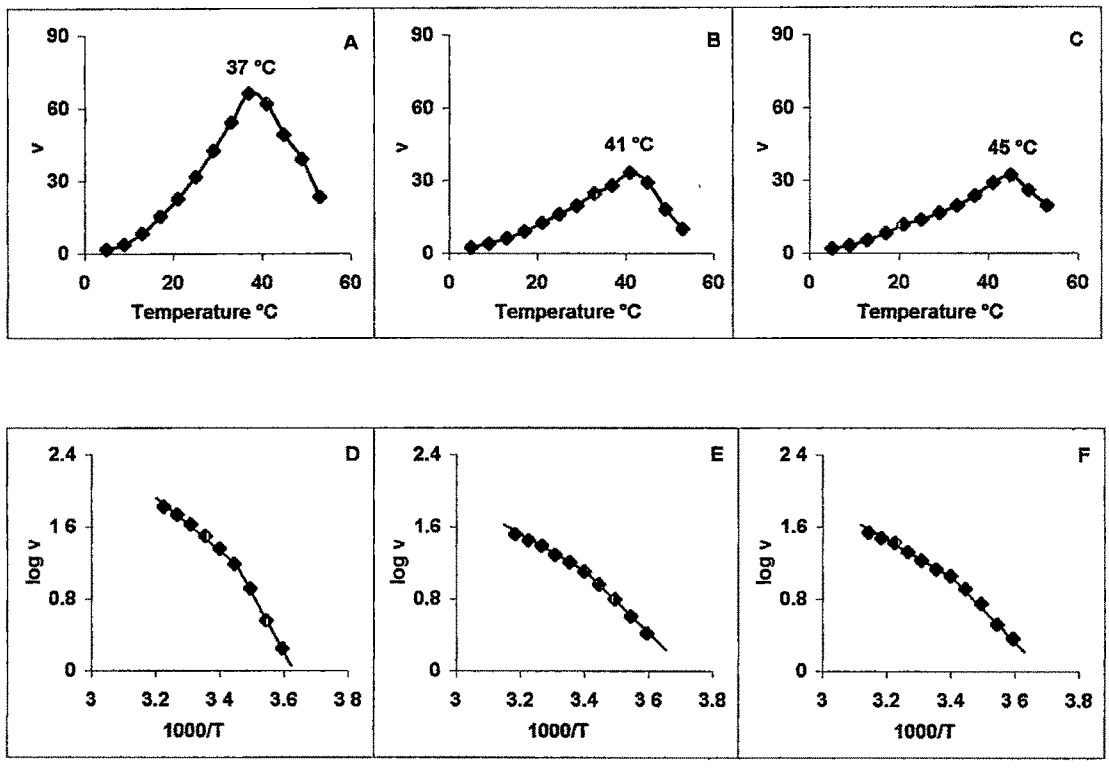

Figure 1. Typical plots depicting dependence of enzyme activity on temperature and the corresponding Arrhenius plots for one week groups. In the temperature curves, the enzyme activity v, on ordinate is plotted versus temperature (°C) on abscissa. A, B and C represent to the control, diabetic and insulin-treated 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 to 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 on temperature and the corresponding Arrhenius plots 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 to 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 to the control, diabetic and insulin treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

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

		Energy of activation (KJ/mole)		Phase transition temperature T_t (°C)
Group	Treatment	E_H	E_L	T_t
One week	Control	42.98 ± 2.31	105.7 ± 3.76	19.48 ± 0.642
	Diabetic	29.30 ± 1.14 [d]	48.85 ± 1.37 [d]	17.13 ± 0.544 [a]
	Diabetic + Insulin	43.12 ± 2.00 [§]	71.24 ± 1.84 [d,§]	15.72 ± 0.396 [d]
One month	Control	40.37 ± 2.64	98.45 ± 4.83	21.05 ± 0.696
	Diabetic	32.83 ± 1.43 [a]	79.38 ± 2.73 [b]	15.55 ± 0.675 [d]
	Diabetic + Insulin	34.09 ± 0.75 [a]	81.29 ± 2.34 [b]	17.60 ± 0.481 [c, ψ]

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

a, $p< 0.05$; b, $p< 0.01$; c, $p< 0.002$ and d, $p<0.001$ compared to the corresponding control.

ψ, $p< 0.05$ and §, $p<0.001$ compared to the corresponding diabetic.

experimental conditions i.e. diabetic and insulin treated diabetic groups seems to be paradoxical especially in view of the fact that the fatty acid desaturase activity and unsaturation index decrease in diabetes (4,5). It may hence be suggested that the observed changes in lowered values of E_H and E_L as well as T_t may represent compensatory mechanisms to improve catalytic efficiency of the enzyme system under experimental conditions. Since energies of activation and phase transition temperature showed significant insulin-status-dependant changes, it was of interest to find out if a correlation with lipid/phospholipids make-up existed. Regression analysis across the groups indicated that sphinghomyelin (SPM) showed a strong negative correlation with E_1 ($r = -0.735$). On the other hand T_t showed positive correlation with the total phospholipids (TPL) / phosphatidylinositol ($PI_,$) and TPL / phosphatidylserine (PS) ($r = +0.628$ and $+0.626$ respectively). As can be noted from the Chapter 2 of the Thesis, the SPM, PI and PS components increased in diabetic animals and remained elevated even after insulin treatment. However, the succinate oxidase activity by itself did not seem to be correlated with any of the lipid/phospholipids classes. This is consistent with earlier reported observation that the enzyme succinate oxidase has a non-specific requirement for phospholipids in general (6). Therefore, the bulk membrane lipids seem to meet the general requirements (6) and SPM, PI and PS emerged as modulatory factors.

The enzyme SDH which is responsible for initiating the process of electron transfer is a rate-limiting step in succinate oxidase activity (33). The enzyme is activated by several physiologic activators which include ATP, NADH, Co Q and Pi (33). Cytochromes of the

E. T. C. are other rate limiting step. Insulin-status-dependent changes in the contents of Co Q and cytochromes in mitochondria have been demonstrated (7-9).

The enzyme SDH is made up of two subunits, both of which are coded by nuclear DNA (10,11). As is evident from the data presented, the succinate oxidase activity was low in diabetic animals and could not be restored by insulin treatment in one month diabetic group. Based on these observations, it may be suggested that in the diabetic state, besides insulin, other hormones may be involved in the expression of subunits of SDH. A parallel relationship between insulin and thyroid hormones has been demonstrated. Thus, the thyroid hormones could be an additional regulatory factor. We have earlier noted thyroid-status-dependent alterations in mitochondrial lipid/phospholipid profiles, membrane fluidity and enzyme kinetics parameters (12,13).

In conclusion, results of our present studies besides demonstrating the regulatory role of specific phospholipids also emphasize that regulation of succinate oxidase in diabetes is a complex process which may involve hormonal interplay.

Summary

Diabetic state lowered the SO activity; insulin treatment was effective in restoring the activity only in one week diabetic rats.

The energies of activation in high and low temperature ranges (E_H and E_L) decreased significantly in diabetic animals; once again insulin treatment was partially effective only in one week diabetic group.

The phase transition temperature, T_t decreased in diabetic and insulin treated groups.

The changes in E_H correlated negatively with SPM component.

References

1. Pedersen P L, Amzel L M. ATP synthase: structure, reaction center, mechanism, and regulation of one of nature's most unique machines. J. Biol. Chem. 1993; 268:9937-9940.

2. Noji H, Yoshida M. The rotary machine in the cell, ATP synthase. J. Biol. Chem. 2001; 276:1665-1668.

3. Katyare S S, Fatterpaker P and Sreenivasan A. Effect of cytochrome c on oxidation of succinate in rat liver mitochondria. Ind. J. Biochem. Biophys. 1971; 8:130-132.

4. Kuwahara Y, Yanagishita T, Konno N and Katagiri T. Changes in microsomal membrane phospholipids and fatty acids and in activities of membrane-bound enzyme in diabetic rat heart. Basic Res. Cardiol. 1997; 92: 214-222.

5. Coste T, Pierlovisi M, Leonardi J, Dufayet D, Gerbi A, Lafont H, Vague P and Raccah D. Beneficial effects of gamma linolenic acid supplementation on nerve conduction velocity, Na+, K+ ATPase activity, and membrane fatty acid composition in sciatic nerve of diabetic rats. J. Nutr. Biochem. 1999; 10:411-420.

6. Daum G. Lipids of mitochondria. Biochim. Biophys. Acta 1985; 822:1-42.

7. Ferreira F M, Seica R, Oliveira P J, Coxito P M, Moreno A J, Palmeira C M and Santos M S. Diabetes induces metabolic adaptations in rat liver mitochondria: role of coenzyme Q and cardiolipin contents. Biochim. Biophys. Acta 2003; 1639:113-120.

8. Satav J G and Katyare S S. Effect of streptozotocin-induced diabetes on oxidative energy metabolism in rat liver mitochondria - A comparative study of early and late effects. Ind. J. Clin. Biochem. 2004; 19:26-36.

9. Katyare S S and Satav J G. Effect of streptozotocin-induced diabetes on oxidative energy metabolism in rat kidney mitochondria. A comparative study of early and late effects. Diab. Obes. Metabol. 2005; 7:555-562.

10. Singer, T P, Gutman, M, and Massey V. 1973. Succinate dehydrogenase, in: Iron-sulfur proteins (Ed. W. Lovenbarg) Vol 1, pp 227-254, Academic Press, Inc. New York.

11. Poyton, R O and McEwen J E. Crosstalk between nuclear and mitochondrial genomes. Annu. Rev. Biochem. 1996; 65:563-607.

12. Bangur C S, Howland J L and Katyare S S. Thyroid hormone treatment alters phospholipid composition and membrane fluidity of rat brain mitochondria. Biochem. J. 1995; 305:29-32.

13. Parmar, D V, Khandkar, M A, Pereira, L, Bangur, C S and Katyare, S S. Thyroid hormones alter Arrhenius kinetics of succinate-2,6- dichloroindophenol reductase, and the lipid composition and membrane fluidity of rat liver mitochondria. Eur. J. Biochem. 1995; 230; 576-581.