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 FoF₁ ATPase in rat liver mitochondria. The FoF₁ 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 ºC and 37 ºC. 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 ºC and 37 ºC 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Activity (nmole O₂/min/mg protein)</th>
<th>Activity Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>One week</td>
<td>Control</td>
<td>34.24 ± 1.12</td>
<td>59.55 ± 3.94</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>11.15 ± 0.43\textsuperscript{a}</td>
<td>17.66 ± 0.92\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Diabetic + Insulin</td>
<td>28.79 ± 0.48\textsuperscript{a,§}</td>
<td>54.26 ± 1.16\textsuperscript{§}</td>
</tr>
<tr>
<td>One month</td>
<td>Control</td>
<td>31.35 ± 1.48</td>
<td>53.21 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>18.21 ± 1.26\textsuperscript{a}</td>
<td>26.39 ± 1.21\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Diabetic + Insulin</td>
<td>14.21 ± 0.18\textsuperscript{a,ψ}</td>
<td>23.24 ± 0.29\textsuperscript{a,ψ}</td>
</tr>
</tbody>
</table>

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.

\(\text{a, p}<0.001\) compared to the corresponding control.

\(\psi, \text{p}<0.05\) and \(\§, \text{p}<0.001\) 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

B. 45 °C

C. 45 °C

D. Log V vs. 1000/T
E. Log V vs. 1000/T
F. Log V vs. 1000/T
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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Energy of activation (KJ/mole)</th>
<th>Phase transition temperature Tᵢ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( E_H )</td>
<td>( E_L )</td>
</tr>
<tr>
<td>One week</td>
<td>Control</td>
<td>42.98 ± 2.31</td>
<td>105.7 ± 3.76</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>29.30 ± 1.14 (^d)</td>
<td>48.85 ± 1.37 (^d)</td>
</tr>
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<td></td>
<td>Diabetic + Insulin</td>
<td>43.12 ± 2.00 (^b)</td>
<td>71.24 ± 1.84 (^d)</td>
</tr>
<tr>
<td>One month</td>
<td>Control</td>
<td>40.37 ± 2.64</td>
<td>98.45 ± 4.83</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>32.83 ± 1.43 (^a)</td>
<td>79.38 ± 2.73 (^b)</td>
</tr>
<tr>
<td></td>
<td>Diabetic + Insulin</td>
<td>34.09 ± 0.75 (^a)</td>
<td>81.29 ± 2.34 (^b)</td>
</tr>
</tbody>
</table>

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.

\( \psi, p<0.05 \) 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 $T_1$ 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 sphingomyelin (SPM) showed a strong negative correlation with $E_l$ ($r = -0.735$). On the other hand $T_1$ 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, $T_r$ decreased in diabetic and insulin treated groups.

The changes in $E_H$ correlated negatively with SPM component.
References


