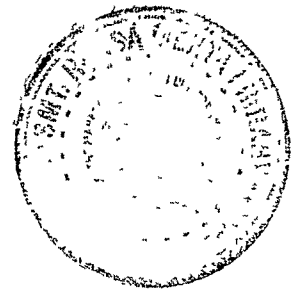


Chapter 3

Insulin-status-dependant modulation of FoF_1 ATPase activity in rat kidney mitochondria

Introduction

Diabetic nephropathy is a major cause of diabetes-related morbidity and mortality (1, 2). Diabetic nephropathy affects about 15-25 % of all type 1 diabetic patients and 20-40 % of all patients with type 2 diabetes (3, 4). Oxidative and carbonyl stress, and advanced glycation end products (AGEs) are involved in the pathogenesis of diabetic nephropathy (3, 5-9). AGEs can form glycoxidation products in peptide and protein structures and heterogeneous groups of proteins and lipids which finally modulate or influence biological reactivity (5-7). There is increased accumulation of reactive carbonyl compounds (RCOs) and AGEs in diabetes which leads to renal failure. RCOs and AGEs are implicated in uraemic toxicity (5) and also cause down regulation of nephrin which leads to increased proteinuria (10). Diabetic nephropathy results in damaged proximal tubules cells, thickening of glomerular basement membrane (GBM) and podocyte apoptosis which leads to altered filtration process (8-11). The filtration process is an energy dependant process and the energy in the form of ATP derived mainly from the mitochondria.

Earlier we have reported that the rates of oxidation of NAD^+-linked and other substrates were differentially affected in the kidney mitochondria from streptozotocin-diabetes (12). Thus the rate of oxidation of β-hydroxybutyrate was impaired whereas that of pyruvate + malate was significantly elevated. Likewise, the rate of succinate oxidation also increased. Interestingly, insulin treatment did not ameliorate the changes (12). In chapter 2 it has been stated that the FoF_1 ATPase (factor V) of mitochondria is localized in the inner membrane and has requirement for specific phospholipids for its activity (13).

In the light of the above, we examined the effects of alloxan-induced diabetes at early and late stages on the kinetic properties of rat kidney mitochondrial FoF_1 ATPase and lipid/phospholipid profile. Effects of insulin treatment were also evaluated. These findings are summarized in this chapter.

Materials and methods

Details of chemicals required, procedure of induction of diabetes, insulin treatment, isolation of mitochondria, ATPase assay procedure, extraction of mitochondrial lipids/phospholipids, estimation of cholesterol, determination of phospholipid profile, membrane fluidity and data analysis are as described in Chapter 2 of the Thesis.

Results

The kidney weight of the alloxan diabetic rats increased, by 12 % and 30 % respectively at the end of one week and one month. Treatment with insulin had partial corrective effect only in one month diabetic group (Table 1). The relative kidney weight increased significantly in diabetic condition and the effect was more pronounced in the one month diabetic animals; insulin treatment had partial restorative effects (Table 1). These results are consistent with our previously reported observations (12, 14).

Results of diabetic parameters are similar as detailed in Chapter 2 of the Thesis.

In preliminary studies, the effects of alloxan-diabetes and subsequent treatment with insulin on ATPase activity under different conditions were examined. Results of which

Table 1. Effect of alloxan diabetes and insulin treatment on body and kidney weight of the rats

Treatment Period	Group	Final body weight, g	Kidney weight	
			g	% of body weight
One week	Control	251.1 ± 5.6	1.76 ± 0.03	0.70 ± 0.011
	Diabetic	215.0 ± 6.9[c]	1.97 ± 0.03[d]	0.92 ± 0.013[d]
	Diabetic + Insulin	232.4 ± 8.7[a,§]	1.90 ± 0.06[c]	0.82 ± 0.025[c,¶]
One month	Control	284.7 ± 4.7	1.88 ± 0.04	0.66 ± 0.014
	Diabetic	173.7 ± 5.2[d]	2.45 ± 0.05[d]	1.41 ± 0.028[d]
	Diabetic + Insulin	278.5 ± 7.2[Ψ]	2.05 ± 0.03[d,¶]	0.74 ± 0.012[c,Ψ]

The results are given as mean ± S. E. M. of 6-8 independent observations.

a, $p<0.05$; b, $p< 0.01$; c, $p< 0.002$ and d, $p<0.001$; compared with control values.
§, $p<0.05$; ¶, $p< 0.01$ and Ψ, $p< 0.001$ compared with corresponding diabetic values.

are given in Table 2. As can be noted, in control addition of Mg^{2+} and DNP stimulated the enzyme activity to almost same extent and the maximum activity was noted in presence of both Mg^{2+} and DNP (Table 2). Alloxan-diabetes stimulate the basal (-Mg^{2+} and – DNP) activity whereas DNP and combination of Mg^{2+} and DNP stimulated activity were decreased. Insulin treatment had marginal restorative effects only in early stage whereas at late stage it resulted in hyper-stimulation in the enzyme activity in all the cases (Table 2).

In the next sets of experiments, ATPase activity was measured at 25 and 37 °C. The results are given in Table 3. As can be noted, in one week diabetic group the enzyme activity decreased by 33 and 25 % respectively, at the two temperatures. A similar change was also observed in the one month diabetic animals (23 and 21 % decrease). Insulin treatment resulted in hyper-stimulation of the activity in both the diabetic groups with the magnitude of increase being higher in one month group. The activity ratios were remain higher under all the experimental except in one month diabetic group compared to the control (Table 3).

Since the diabetic state and insulin treatment significantly influenced the enzyme activity, and activity ratio, in the next sets of experiments, the kinetic behavior of the enzyme as a response to change in the substrate i.e. ATP concentration and change in temperature were examined.

Table 2. Effect of alloxan-diabetes and subsequent treatment with insulin on FoF_1 ATPase activity in rat kidney mitochondria

Group	Treatment	Basal	+ Mg	+DNP	+Mg, + DNP
One week	Control	2.62 ± 0.11	12.27 ± 0.51	11.83 ± 0.78	17.45 ± 0.96
	Diabetic	4.42 ± 0.25^{c}	11.82 ± 0.42	6.22 ± 0.25^{c}	13.03 ± 0.30^{b}
	Diabetic + Insulin	3.09 ± 0.22§	12.17 ± 0.61	11.18 ± 1.03$^{\Psi}$	23.26 ± 0.67$^{c,\Psi}$
One Month	Control	2.46 ± 0.13	11.55 ± 0.48	11.14 ± 0.73	16.43 ± 0.90
	Diabetic	3.12 ± 0.25^{a}	11.04 ± 0.49	5.91 ± 0.30	12.57 ± 0.38
	Diabetic + Insulin	8.70 ± 0.25$^{c,\Psi}$	34.35 ± 1.42$^{c,\Psi}$	16.87 ± 0.83$^{c,\Psi}$	33.75 ± 1.14$^{c,\Psi}$

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

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

Table 3. Effect of alloxan diabetes and insulin treatment on FoF_1 ATPase activity in rat kidney mitochondria

Group	Treatment	Activity (μmole of Pi liberated / hr / mg protein)		Activity Ratio
		25 °C	37 °C	
One week	Control	9.04 ± 0.63	16.92 ± 1.04	1.87 ± 0.09
	Diabetic	6.09 ± 0.27[b]	12.75 ± 0.53[a]	2.09 ± 0.11
	Diabetic + Insulin	12.77 ± 0.92[a,§]	25.54 ± 1.72[c,§]	2.00 ± 0.08
One month	Control	9.19 ± 0.57	17.21 ± 0.94	1.87 ± 0.11
	Diabetic	7.06 ± 0.20[a]	13.64 ± 0.18[a]	1.93 ± 0.12
	Diabetic + Insulin	15.63 ± 0.58[c,§]	35.00 ± 0.80[c,§]	2.24 ± 0.11

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

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

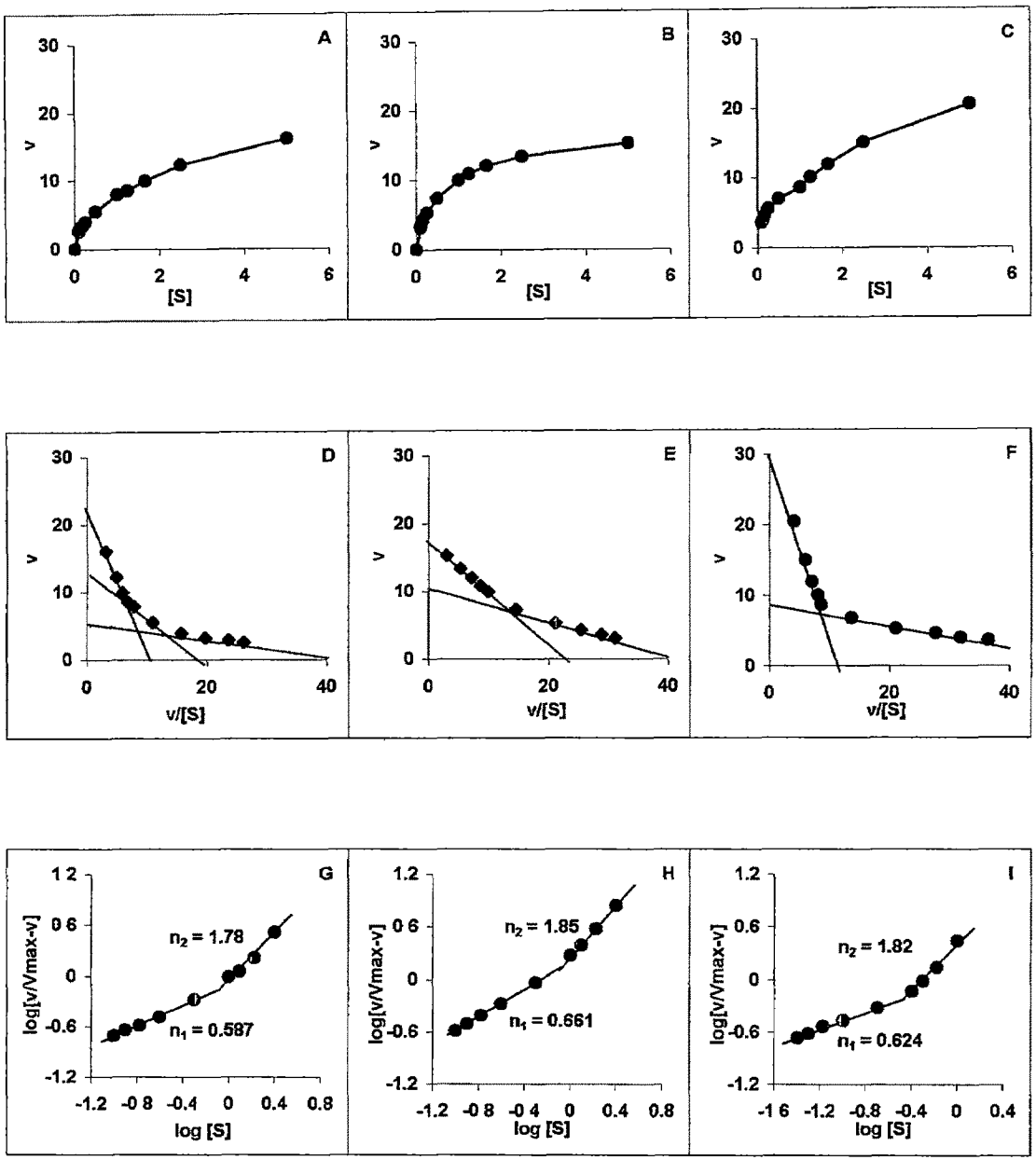
a, $p<0.01$; b, $p<0.002$ and c, $p<0.001$ compared to the corresponding control.
§, $p<0.001$ compared to the corresponding diabetic.

The data on substrate kinetics were analyzed as detailed in previous chapter 2. The typical substrate saturation curves and corresponding Eadie-Hofstee plots and Hill plots are shown in Fig. 1 and 2. As can be seen, in the control animals the enzyme activity resolved in three kinetically distinguishable components. In the diabetic group only first two components were present. Treatment with insulin resulted in restoring component III. However, component II was abolished following insulin treatment (e.g. see Fig. 1 and 2 Panels D, E and F and Table 4).

Data on Km and Vmax values of the three components as affected by insulin status are given in Table 4. Thus, in one week diabetic group the Km of component I increased by 1.75 fold in early diabetic state whereas in the chronic diabetic state the Km was somewhat lowered. Insulin treatment partially restored the Km value in one week diabetic animals whereas in one month diabetic animals the Km increased beyond the control values (Table 4). Diabetic state in general resulted in increased Vmax of component I with the increase being more pronounced in the one week diabetic group; insulin treatment resulted in substantial increase in the Vmax values in both the diabetic groups (Table 4).

The Km of component II increased only in one month diabetic animals while the Vmax value increased in both the diabetic groups (34 and 44 % increase) (Table 4). Component III was not discernible in the diabetic conditions. Insulin treatment resulted in lowering the Km in the early stage while at late stage the Km increased. Insulin treatment resulted

Figure 1


A
B
C
D
E
F
G
H
I
v
[S]
v/[S]
log[v/(Vmax-v)]
log [S]
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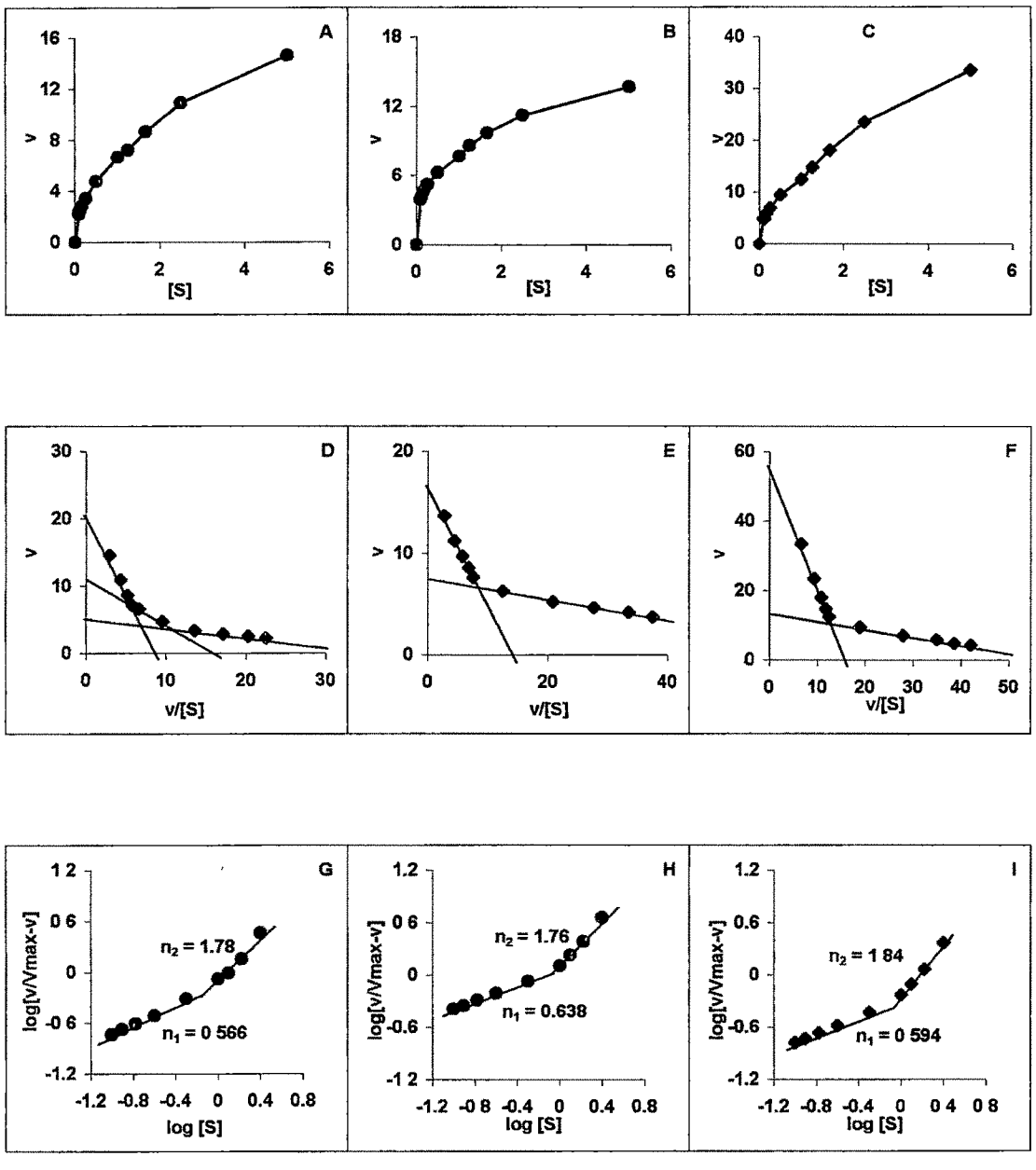
n1 = 0.624

Figure 2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n1 = 0 566
n2 = 1.76
n1 = 0.638
n2 = 1 84
n1 = 0 594

Figure 1. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for FoF_1 ATPase from liver mitochondria for one week group. In substrate saturation curve the enzyme activity v on abscissa is plotted versus [S] on ordinate v is the enzyme activity at the given ATP concentration [S]. A, B and C represent to the control, diabetic and insulin-treated diabetic groups. In Eadie-Hofstee plot the enzyme activity v on abscissa is plotted versus v/[S] on ordinate. D, E and F represent to the control, diabetic and insulin-treated diabetic groups. In Hill plot log (v/Vmax-v) on abscissa is plotted versus log [S] on ordinate. The Hill coefficients n_1 and n_2 depict number of ATP molecules bound for the given concentration range of ATP. G, H and I 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 substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for FoF_1 ATPase from liver mitochondria for one week group. In substrate saturation curve the enzyme activity v on abscissa is plotted versus [S] on ordinate v is the enzyme activity at the given ATP concentration [S]. A, B and C represent to the control, diabetic and insulin-treated diabetic groups. In Eadie-Hofstee plot the enzyme activity v on abscissa is plotted versus v/[S] on ordinate. D, E and F represent to the control, diabetic and insulin-treated diabetic groups. In Hill plot log (v/Vmax-v) on abscissa is plotted versus log [S] on ordinate. The Hill coefficients n_1 and n_2 depict number of ATP molecules bound for the given concentration range of ATP. G, H and I represent to the control, diabetic and insulin-treated diabetic groups The plots are typical of 6-8 independent experiments in each group.

Table 4 Effect of alloxan diabetes and insulin treatment on substrate kinetics properties of FoF_1 ATPase activity in rat kidney mitochondria

Group	Treatment	Component I		Component II		Component III	
		Km	Vmax	Km	Vmax	Km	Vmax
One week	Control	0.135 ± 0.004	5.61 ± 0.176	0.73 ± 0.024	11.95 ± 0.254	2.31 ± 0.082	21.21 ± 0.364
	Diabetic	0.236 ± 0.009[c]	9.53 ± 0.241[c]	0.77 ± 0.022	16.07 ± 0.335[c]	—	—
	Diabetic + Insulin	0.205 ± 0.011[c]	11.82 ± 0.356[c,§]	—	—	1.33 ± 0.050[c]	28.02 ± 0.725[c]
One month	Control	0.144 ± 0.004	5.23 ± 0.083	0.83 ± 0.040	11.32 ± 0.371	2.45 ± 0.126	20.05 ± 0.619
	Diabetic	0.109 ± 0.004[c]	6.96 ± 0.189[c]	1.07 ± 0.048[a]	16.34 ± 0.243[c]	—	—
	Diabetic + Insulin	0.200 ± 0.009[c,§]	12.26 ± 0.380[c,§]	—	—	3.51 ± 0.210[b]	51.34 ± 1.41[c]

The Km (mM) and Vmax (μmole of Pi liberated . hr^{-1}. mg $protein^{-1}$) values were calculated as described in the text. The experiments were carried out at 37 °C.

The results are given as mean ± SEM of 6-8 independent experiments. As indicated in the text, the kinetic components represent the potential and the response of the enzyme to increasing concentrations of the substrate.

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

in marginal (32%) increase in the Vmax of component III in one week diabetic animals whereas maximum stimulatory effect (2.5 fold increase) was seen in one month diabetic animals (Table 4).

In view of the observed changes in the Km and Vmax values under the different experimental conditions, it was of interest to find out if the relative efficiency of the enzyme was influenced by the insulin status. Thus we computed the relative efficiency of the FoF_1 ATPase in terms of Kcat / Km values (15). As described in the previous chapter 2 we normalized the Kcat / Km values and designated these values as ^{App}Kcat / Km. The data for ^{App}Kcat / Km are given in Table 5.

As is evident, diabetic state increased the efficiency of component I and the increase was more pronounced in the one month group; insulin treatment normalized the ^{App}Kcat / Km values (Table 5). Efficiency of component II also increased significantly in the diabetic animals. Interestingly; insulin treatment improved catalytic efficacy of component III (Table 5).

The observed changes prompt to analyze if the substrate binding properties of the enzyme were altered under different experimental conditions. The analysis of the substrate kinetics data by Hill plots indicated that up to 0.9 mM ATP concentration one ATP molecule was bound while beyond this concentration two molecules of ATP were bound

Table 5. Effect of alloxan diabetes and insulin treatment on apparent Kcat/Km values of the kinetic components of FoF_1 ATPase in rat liver mitochondria

Group	Treatment	^{App}Kcat/Km x 10^{11}		
		Component I	Component II	Component III
One week	Control	8.59 ± 0.36	3.41 ± 0.13	1.90 ± 0.08
	Diabetic	11.73 ± 0.30[b]	6.09 ± 0.16[b]	—
	Diabetic + Insulin	9.95 ± 0.59[Ψ]	—	3.56 ± 0.17[b]
One month	Control	7.96 ± 0.36	2.99 ± 0.19	1.87 ± 0.08
	Diabetic	21.35 ± 0.52[c]	5.23 ± 0.20[b]	—
	Diabetic + Insulin	8.05 ± 0.29[§]	—	2.22 ± 0.11[a]

The values of Kcat/ Km were computed as described in the text. The results are given as mean ± SEM of 6-8 independent experiments.

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

under all experimental conditions. Since the patterns were similar for all experimental groups a typical Hill plots are shown in Fig. 1 and 2 Panels G, H and I.

Since the insulin-status significantly altered the substrate kinetic properties of ATPase, the effect of alloxan diabetes and insulin treatment on the temperature-dependant-change in the enzyme activity were examined. The data were analyzed in terms of Arrhenius plots to find out energies of activation in high and low temperature ranges (E_H and E_L respectively) and phase transition temperature (T_t). The typical activity versus temperature curves and corresponding Arrhenius plots are shown in Fig. 3 and 4 respectively for one week and one month groups. As can be noted, in one week as well as one month groups the patterns of activity versus temperature and the corresponding Arrhenius plots for diabetic and insulin treated diabetic animals differed considerably from the control (Fig. 3 and 4).

From activity versus temperature curves it is evident that in the control groups the optimum temperature was around 49 °C. Diabetic state had no effect on optimum temperature in one week group but resulted in shift in the optimum temperature beyond 53 °C in one month group (Fig. 3 and 4). A reversed picture was observed after insulin treatment i.e. the optimum temperature increased to 53 °C in one week group while in the one month group it was normalized to control value (Fig. 3 and 4)

The Arrhenius plot in general followed a biphasic pattern (Fig. 3 and 4) depicting that the values of E_L were higher than those of E_H. However, one month diabetic group treated

Figure 3

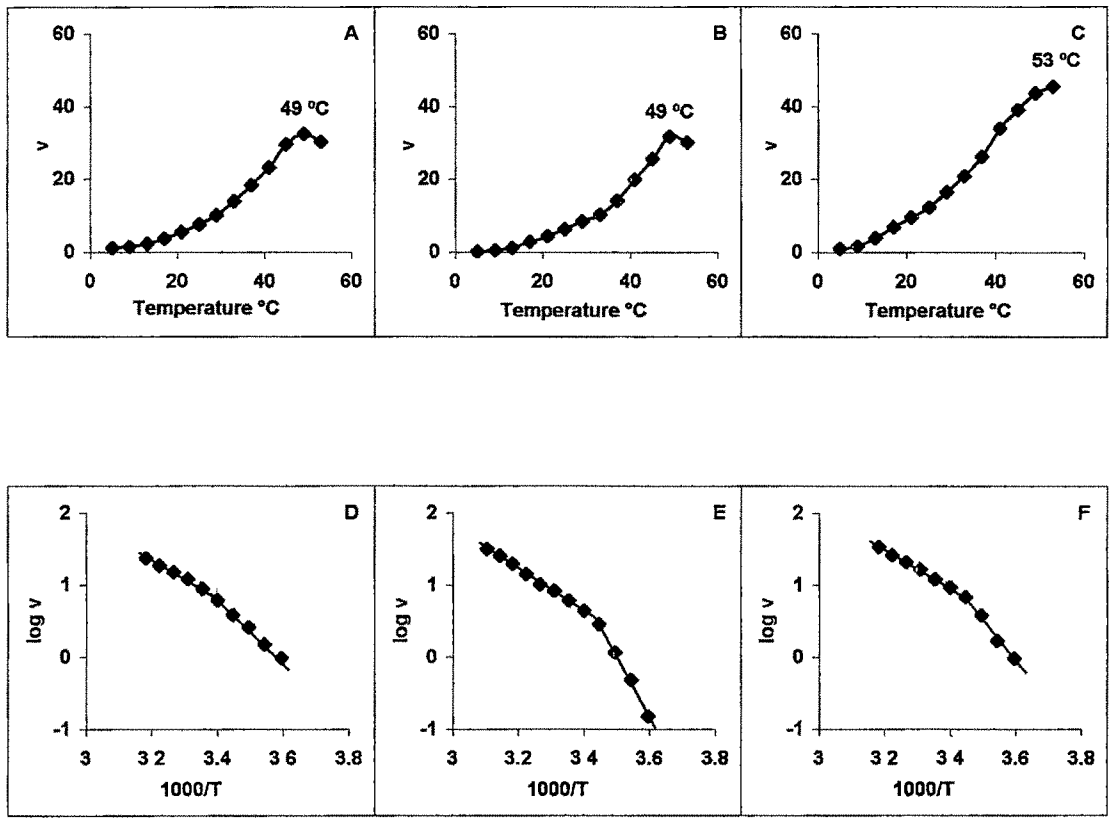
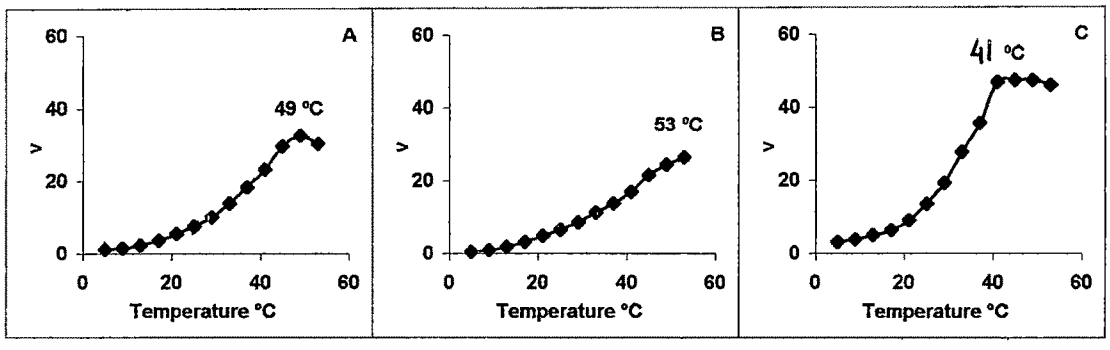


Figure 4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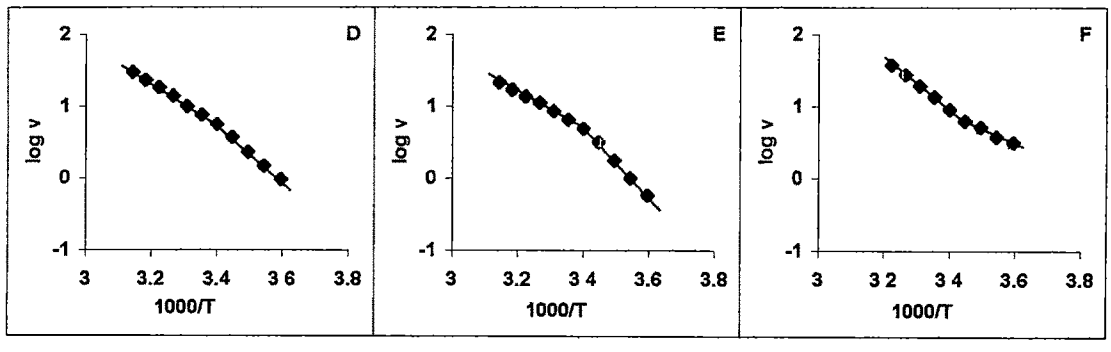
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3.6
3.8

Figure 3. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for one week groups. In temperature curves, enzyme activity v on abscissa is plotted verses temperature (°C) on ordinate. 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 ° Celcius + 273.2). 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 4. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for one month groups. In temperature curves, enzyme activity v on abscissa is plotted verses temperature (°C) on ordinate. 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 ° Celcius + 273.2). 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.

with insulin proved to be an exception where the pattern was reversed (Fig. 4) i.e. value of E_H was high and that of E_L was substantially reduced. These differences are summarized in Table 6.

Thus, in one week diabetic animals the value of E_L increased by 2 fold. Insulin treatment partially corrected this defect and marginally lowered E_H. In one month diabetic group E_H decreased whereas E_L increased significantly. Insulin treatment had effects opposite to those noted in one week group. Thus E_L increased beyond control whereas and E_H was reduced by a factor of three (Table 6).

The generalized feature in both the groups was decrease in T_t in diabetes which could not be corrected by insulin treatment (Table 6).

The observed changes in the substrate and temperature kinetics prompt us to examine the effect of insulin status on lipid/phospholipids profiles of the mitochondria. The results of which are given in Tables 7-9. In one week diabetic animals the TPL content did not change while the CHL content increased by 16%. Insulin treatment had no effect on TPL content but restored the CHL content. In one month diabetic group the TPL content decreased by 52 % and CHL content increased by 63 %. Insulin treatment partially restored the TPL and CHL contents. The TPL/CHL (mole : mole) ratios decreased in the diabetic groups and insulin treatment was effective in normalizing it only in early stage. The membrane fluidity decreased in diabetic animals and insulin treatment fluidized the membrane in one week diabetic animals (Table 7).

Table 6 Effect of alloxan diabetes and insulin treatment on Arrhenius kinetics properties of $FoF_1ATPase$ in rat kidney mitochondria

Group	Treatment	Energy of activation (KJ/mole) E_H	Energy of activation (KJ/mole) E_L	Phase transition temperature T_t (°C)
One week	Control	52.3 ± 1.93	77.2 ± 1.09	23.78 ± 0.45
	Diabetic	56.8 ± 1.65	151.1 ± 5.03^{c}	17.44 ± 0.28^{c}
	Diabetic + Insulin	47.9 ± 1.11$^{\Psi}$	111.6 ± 3.63^{c,§}	17.76 ± 0.68^{c}
One month	Control	55.3 ± 2.34	76.4 ± 1.28	24.00 ± 0.55
	Diabetic	45.1 ± 1.29^{b}	101.5 ± 3.27^{c}	18.99 ± 0.64^{c}
	Diabetic + Insulin	69.3 ± 2.09$^{c,\Psi}$	23.9 ± 2.52$^{c,\Psi}$	15.20 ± 1.02$^{c,\Psi}$

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

a, $p< 0.02$; b, $p< 0.01$ and c, $p<0.001$ compared to the corresponding control.
§, $p<0.01$ and Ψ, $p<0.001$ compared to the corresponding diabetic.

Table 7. Effects of alloxan diabetes on total phospholipids (TPL), cholesterol (CHL), TPL/CHL ratio and fluorescence polarization in rat kidney mitochondria

Groups	Treatment	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)	Fluorescence polarization p
One week	Control	262.4 ± 4.64	91.2 ± 2.71	1.45 ± 0.05	0.207 ± 0.001
	Diabetic	255.0 ± 2.86	106.2 ± 2.86[b]	1.21 ± 0.04[a]	0.273 ± 0.004[b]
	Diabetic + Insulin	239.0 ± 9.75	82.33 ± 4.19[§]	1.46 ± 0.03[§]	0.124 ± 0.004[b,§]
One month	Control	267.7 ± 2.57	89.7 ± 2.67	1.51 ± 0.05	0.211 ± 0.002
	Diabetic	129.2 ± 7.47[b]	146.5 ± 5.90[b]	0.44 ± 0.02[b]	0.264 ± 0.012[b]
	Diabetic + Insulin	206.7 ± 11.5[b,§]	114.6 ± 3.69[b,§]	0.90 ± 0.03[b,§]	0.239 ± 0.002[b,§]

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

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

The data on phospholipid composition (Table 8) reveled that diabetic state had a generalized effect of increasing lysophospholipids (Lyso), sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS) and a tendency to decrease phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) components. Insulin treatment partially corrected the Lyso and PS composition in both the diabetic groups. Insulin treatment could not correct the PI and PE components in one week group and elevated the latter component in the one month group (Table 8). The computed contents of the individual phospholipid classes were generally consistent with the above data (Table 9).

Change in the altered CHL and phospholipid composition and contents could alter the membrane fluidity and the data for the fluidity parameter are given in Table 10. From the data it is revealed that the diabetic state decreased the membrane fluidity. Treatment with insulin in early diabetic state increased the membrane fluidity whereas at late stage partial restoration was seen (Table 10).

Discussion

The basic observation of the present studies is that diabetic state decreased the enzyme activity and insulin treatment resulted in hyper-stimulation (Table 3). Also, the experimental conditions altered the substrate and temperature kinetics properties of the enzyme in a differential manner. Thus in diabetic state component III was not discernible whereas upon insulin treatment component II was found to be missing (Fig. 1 and 2 Table

8. Effects of alloxan diabetes and insulin treatment on phospholipid composition in rat kidney mitochondria.

Phospholipid Class	Composition (% of total)					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	2.19 ± 0.07	5.99 ± 0.29^{d}	1.59 ± 0.13^{c,§}	2.16 ± 0.07	3.63 ± 0.20^{d}	1.70 ± 0.05^{d,§}
SPM	7.58 ± 0.11	10.30 ± 0.29^{d}	7.25 ± 0.18§	7.55 ± 0.11	10.18 ± 0.40^{d}	11.01 ± 0.36^{d}
PC	38.31 ± 0.63	40.33 ± 0.52^{a}	40.52 ± 0.31^{a}	38.70 ± 0.51	40.25 ± 0.59	36.82 ± 0.54^{a,§}
PI	1.08 ± 0.15	2.90 ± 0.15^{d}	1.10 ± 0.05§	1.09 ± 0.10	2.25 ± 0.10^{d}	2.33 ± 0.11^{d}
PS	1.01 ± 0.06	2.66 ± 0.13^{d}	1.27 ± 0.08^{a,§}	1.01 ± 0.05	4.78 ± 0.17^{d}	5.96 ± 0.22^{d,§}
PE	34.73 ± 0.50	25.27 ± 0.62^{d}	35.68 ± 0.29§	34.61 ± 0.46	27.03 ± 0.88^{d}	32.77 ± 0.33^{b,§}
DPG	15.10 ± 0.33	12.70 ± 0.27^{d}	12.51 ± 0.24^{d}	15.01 ± 0.31	11.82 ± 0.41^{d}	9.41 ± 0.52$^{d,\Psi}$

The results are given as mean ± SEM of the 6-8 independent observations.

Lyso: Lysophospholipid; SPM: sphinghomyelin; PC: phosphatidylcholine; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; DPG: diphosphatidylglycerol.

a, $p< 0.05$; b, $p< 0.02$; c, $p<0.002$ and d, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.02$ and §, $p<0.001$ compared to the corresponding diabetic.

Table 9. Effects of alloxan-diabetes and insulin treatment on phospholipid ccontent in rat kidney mitochondria.

Phospholipid Class	Content, μg / mg mitochondrial protein					
	one week			one month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	5.74 ± 0.23	15.10 ± 0.68^{d}	3.74 ± 0.30^{d,¶}	5.78 ± 0.18	4.77 ± 0.46^{c}	3.50 ± 0.16^{d}
SPM	19.91 ± 0.50	26.03 ± 0.63^{d}	17.19 ± 0.71^{b,¶}	20.21 ± 0.40	13.31 ± 1.11^{d}	22.51 ± 0.78^{a,¶}
PC	100.30 ± 1.39	100.17 ± 1.61	96.90 ± 4.21	103.40 ± 1.58	52.17 ± 3.17^{d}	76.00 ± 2.80^{d,¶}
PI	2.85 ± 0.39	7.36 ± 0.42^{d}	2.62 ± 0.17¶	2.43 ± 0.16	2.94 ± 0.25	4.93 ± 0.36^{d,¶}
PS	2.65 ± 0.19	6.74 ± 0.36^{d}	3.01 ± 0.20¶	2.70 ± 0.17	6.16 ± 0.42^{d}	12.71 ± 0.88^{d,¶}
PE	91.18 ± 2.27	64.21 ± 1.80^{d}	85.61 ± 3.47¶	92.48 ± 1.48	80.80 ± 2.22^{c}	68.20 ± 2.93^{d,ψ}
DPG	39.69 ± 1.50	32.08 ± 0.91^{c}	29.93 ± 1.49^{d}	40.10 ± 0.98	29.02 ± 0.88^{d}	20.53 ± 1.85^{d,ψ}

The results are given as mean ± SEM of the 6-8 independent observations.

a, $p< 0.05$; b, $p< 0.02$; c, $p<0.002$ and d, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.02$ and §, $p<0.001$ compared to the corresponding diabetic.

Table 10. Effects of alloxan-diabetes and insulin treatment on fluidity parameters of kidney mitochondria.

Groups	Treatment	Fluidity Parameters			
		Fluorescence Polarization, p	Fluorescence anisotropy, r	Limited hindered anisotropy, rα	Order parameter, S
One week	Control	0.207 ± 0.001	0.151 ± 0.001	0.101 ± 0.002	0.503 ± 0.005
	Diabetic	0.273 ± 0.004[a]	0.201 ± 0.003[a]	0.167 ± 0.004[a]	0.646 ± 0.009[a]
	Diabetic + Insulin	0.124 ± 0.004[a,§]	0.086 ± 0.003[a,§]	0.015 ± 0.004[a,§]	0.176 ± 0.020[a,§]
One month	Control	0.211 ± 0.002	0.156 ± 0.007	0.051 ± 0.007	0.513 ± 0.011
	Diabetic	0.264 ± 0.012[a]	0.194 ± 0.010[a]	0.158 ± 0.013[a]	0.624 ± 0.024[a]
	Diabetic + Insulin	0.239 ± 0.002[a,¶]	0.173 ± 0.001[a,ψ]	0.131 ± 0.002[a,ψ]	0.571 ± 0.004[a,ψ]

The results are given as mean ± SEM of the 6-8 independent observations.

a, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.05$; ¶, $p< 0.01$ and §, $p<0.001$ compared to the corresponding diabetic.

Panels D, E and F and Table 4). Early diabetic state caused increase in the Km of component I; an opposite effect i.e. decrease was noted at the late state. Insulin treatment elevated the Km of component I in both the diabetic groups (Table 4). The Km of component II increased in late state. The effects of insulin treatment on Km of component III were of opposite nature; decrease in one week and increase in one month groups. The Vmax of all the components were high under all experimental groups and insulin treatment accentuated the effect. The data on ^{App}Kcat/Km would suggest that the observed increase in the diabetic groups may be related to the increased catalytic efficacy of the enzyme. This may possibly represent a compensatory mechanism. Progressive increase in the ^{App}Kcat/Km with progression of the diabetic state (Table 5) would also support this assumption. By contrast, the observed increase in the activity in the insulin treated diabetic animals may be attributable to increased enzyme units rather than to increased catalytic efficacy (Tables 3-5). It has been suggested that increased AGEs formed in diabetes react with peptide and protein and can modulates or influence their biological reactivity (5-7). However, this possibility seems to be unlikely in the present situation. Firstly, in the diabetic groups where the blood sugar levels are significantly high, (e.g. see Results Section) the activity actually decreased (Table 3). By contrast, when the blood sugar levels were almost normalized following insulin treatment, there was hyper-stimulation of the activity in a progressive manner (Table 3). It may hence be suggested that insulin action may be at the level of gene regulation. Since the subunits of the FoF_1 ATPase are coded by the nuclear as well as mitochondrial DNA (16), it would

be of interest to find out how insulin activates the subunit synthesis by the nuclear and mitochondrial DNAs

The increased energies of activation in the low temperature range (E_L) in the diabetic animals are consistent with the lowered activity of the enzyme (Table 3). It is possible that to overcome this barrier, the catalytic efficacy of the enzyme increased in the diabetic state (Table 5). However, the underlying mechanism for the improved catalytic efficacy remains unclear at this stage. Insulin treatment had diverse effects on E_L; in one week group there was partial correction while in the one month group insulin treatment overcorrected the E_2 value while reversing the pattern of Arrhenius plot (Table 6, Fig. 3 and 4 Panels D, E and F). The latter could to be another compensatory mechanism. With respect to the phase transition temperature T_t, the generalized feature was decrease in T_t in diabetic groups and insulin treatment accentuated the effect further in the one month group.

It has been reported that in diabetes the desaturases activities decrease thereby decreasing the unsaturation index (17, 18). One would therefore anticipate that the phase transition temperature should have increased. In related studies we have observed that indeed this is the case for the ATPase activity in liver mitochondria (Chapter 2). The present observations on the kidney enzyme therefore seem to be paradoxical. However, it is possible that glycation of the membrane proteins and/or changes in lipid/phospholipid components may play a role in modulating the T_t. Data on effect of insulin status on lipid/phospholipid composition (Table 8) and regration analysis suggest that

phospotidylserine may have a strong negative correlation with the phase transition temperature, T_t

The overall results point out that the diabetic state and subsequent insulin treatments substantially modulate the kinetic properties of kidney mitochondrial ATPase in a differential manner. The results also point out that the kinetics properties are not restored to the control status. These results are consistent with the well acknowledged fact that insulin treatment corrects the blood sugar levels but cannot correct all the maladies of diabetes (19-21). It has been reported that pathies associated with diabetes are corrected by treatment with C-peptide (20, 21). One therefore wonders if combined treatment with insulin and C-peptide would restore the kinetics properties of kidney mitochondrial FoF_1 ATPase. Role of C-peptide in modulating the Na^+, K^+ ATPase activity from proximal and tubular segment of the rat nephron has been demonstrated (22).

In previous chapter 2 we noted that in rat liver mitochondria from control, diabetic as well as insulin treated diabetic animals three kinetically distinguishable components were evident. These observations differ considerably from those of the present studies on kidney mitochondria. The results thus suggest that the insulin action is also tissue-specific.

Summary

Diabetic state resulted in significant decrease in the activity while insulin treatment caused hyper-stimulation. In control animals the enzyme activity resolved in three kinetic components. In diabetic condition only component I and II were present. With insulin treatment component III was restored but component II was abolished. Diabetic state and insulin treatment had varied effects on Km values of the three components whereas the Vmax values were generally on the higher side.

Evaluation of the ^{App}Kcat / Km values revealed that diabetic state resulted in increased catalytic efficiency; insulin treatment brought back these values to normality.

Temperature kinetics studies indicated that the phase transition temperature decreased significantly in the diabetic and insulin treated diabetic animals. The energy of activation in low temperature range increased in the diabetic animals. Insulin treatment corrected the Arrhenus pattern at early stage of diabetes; at late stage the pattern was reversed. The results are suggestive of subtle insulin-status-dependent alterations in membrane structure-function relationships.

Early stage of diabetes marginally increased CHL content which was restored by insulin treatment. Long term diabetes lowered the TPL and the CHL content elevated. Insulin treatment partially restored the TPL and CHL content.

Diabetic state decreased the proportion of PE and diphosphatidylglycerol (DPG) components while increased in the Lyso, SPM, PC, PI and PS components in the mitochondria. Insulin treatment had partial restorative effect.

The membrane fluidity decreased in general in diabetic condition and was not corrected by insulin treatment at late stage. However, at early stage, insulin treatment fluidized both the membranes.

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