Chapter 9

Effects of Alloxan-Diabetes and Subsequent Treatment with Insulin on Kinetic Properties of Na^+, K^+-ATPase and Glucose-6-Phosphatase from Rat Kidney Microsomes

Introduction

As described earlier, alloxan-diabetes and subsequent insulin treatment differentially altered the kinetic properties of Na^+, K^+-ATPase and glucose-6-phosphatase (G6Pase) in liver microsomes. Also, lipid/phospholipid profile in liver microsomes changed significantly under these conditions (Chapter 8 of the Thesis). Hence, studies were carried out to examine early and late effects of alloxan-diabetes and subsequent insulin treatment on kinetic properties of Na^+, K^+-ATPase and glucose-6-phosphatase (G6Pase) from kidney microsomes. The lipid/phospholipid profiles were also examined under these conditions. The results are summarized below.

Materials and Methods

Chemicals

Details of chemicals required, procedure of induction of diabetes, insulin treatment, isolation of microsomes, extraction of lipid/phospholipids, estimation of cholesterol, determination of phospholipid profile and membrane fluidity are as described in Chapter 2 of the Thesis.

Details of ATPase and G6Pase assay are as described in Chapter 8 of the Thesis.

The substrate and temperature kinetics data were analyzed as detailed (Chapter 2 of the Thesis).

Results

Effects on ATPase

In the preliminary studies ATPase activity was determined at room temperature (25 °C) and at the physiological temperature (37 °C). The data are given in Table 1. As can be noted at early stage of diabetes ATPase activity decreased by 27 and 12 % at two temperatures and the effect was more substantial at late stage (66-68 % decrease). Insulin treatment in one week diabetic animals resulted in complete restoration of the activity. In one month group activity at 25 °C completely restored while the activity at 37 °C increased beyond control group after insulin treatment. In one week diabetic group, activity ratio was somewhat high which restored to normal after insulin treatment. The activity ratio was unchanged in the one month groups (Table 1).

In view of these differential changes further experiments were carried out to examine the kinetic behavior of ATPase as a response to change in the substrate i.e. ATP concentration. The data on substrate kinetics were analyzed as detailed (Chapter 2 of the Thesis). The typical substrate saturation and corresponding Eadie-Hofstee plots and Hill plots are shown in Fig. 1 and 2 Panels A-C and D-F respectively. As can be noted, in all experimental conditions the enzyme activity resolved in two kinetically distinguishable components (Fig. 1 and 2 Panels D-F).

Data on Km and Vmax are given in Table 2. Thus, in the control group the Km value of the two components were 0.35-0.40 and 1.72-1.82 mM respectively with corresponding values of Vmax being 8.5-9.1 and 19.7-20.9 units. In one week diabetic group the value

Table 1. Effect of alloxan-diabetes and insulin treatment on Na^+, K^+ ATPase activity in rat kidney microsomes

Group	Treatment	Activity		Activity Ratio
		25 °C	37 °C	
One week	Control	7.51 ± 0.24	15.04 ± 0.21	2.00 ± 0.11
	Diabetic	5.47 ± 0.19[b]	13.19 ± 0.28[b]	2.41 ± 0.13[a]
	Diabetic + Insulin	7.79 ± 0.24[§]	14.91 ± 0.29[§]	1.91 ± 0.11[ψ]
One month	Control	7.91 ± 0.46	15.31 ± 0.59	1.94 ± 0.08
	Diabetic	2.71 ± 0.14[b]	5.59 ± 0.15[b]	2.06 ± 0.12
	Diabetic + Insulin	7.99 ± 0.11[§]	17.61 ± 0.44[a,§]	2.20 ± 0.11

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

Activity ratio was calculated by : activity at 37 °C/activity at 25 °C

The activities are expressed as µmole of Pi liberated / hr / mg protein.

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

Figure 1

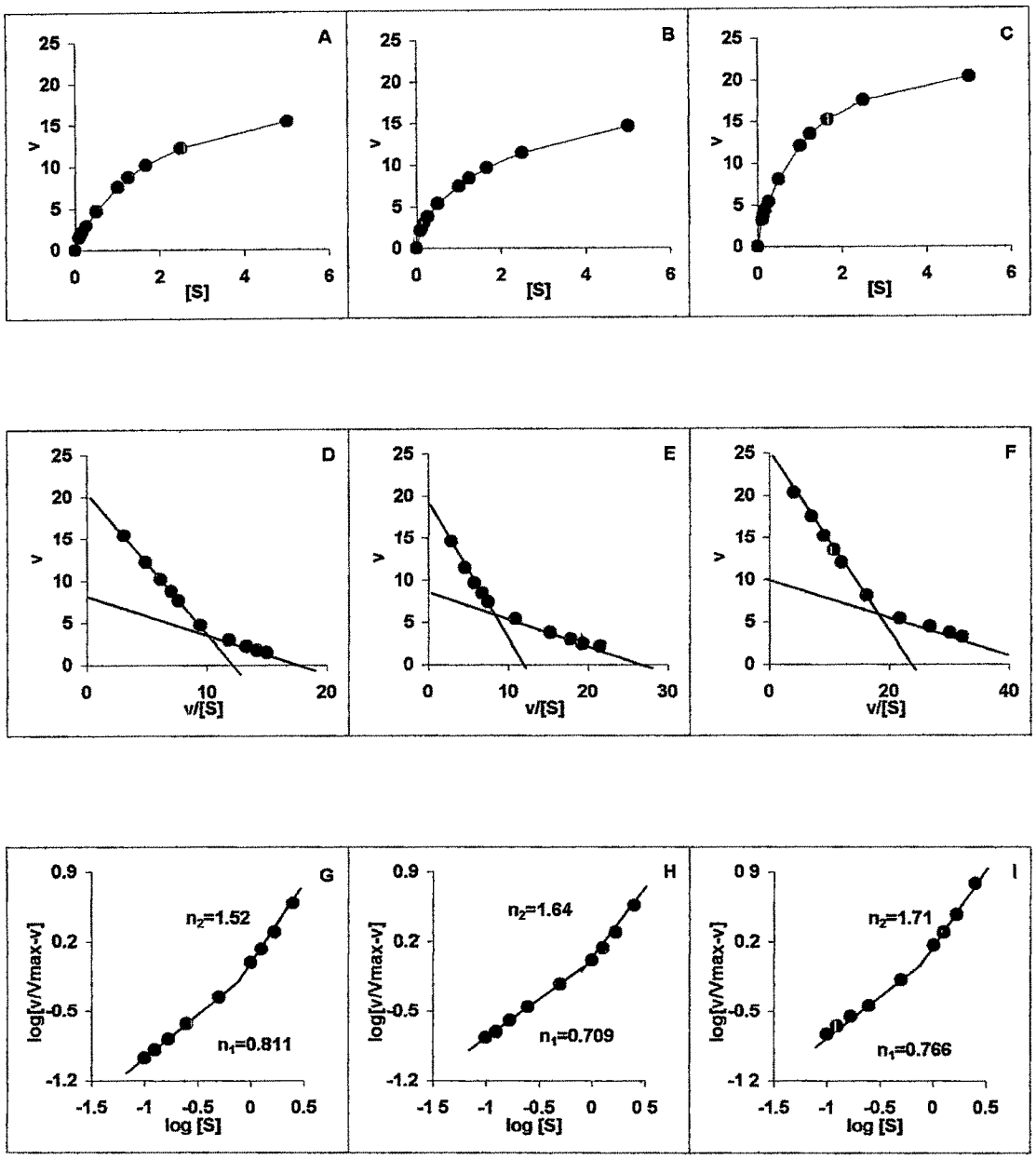
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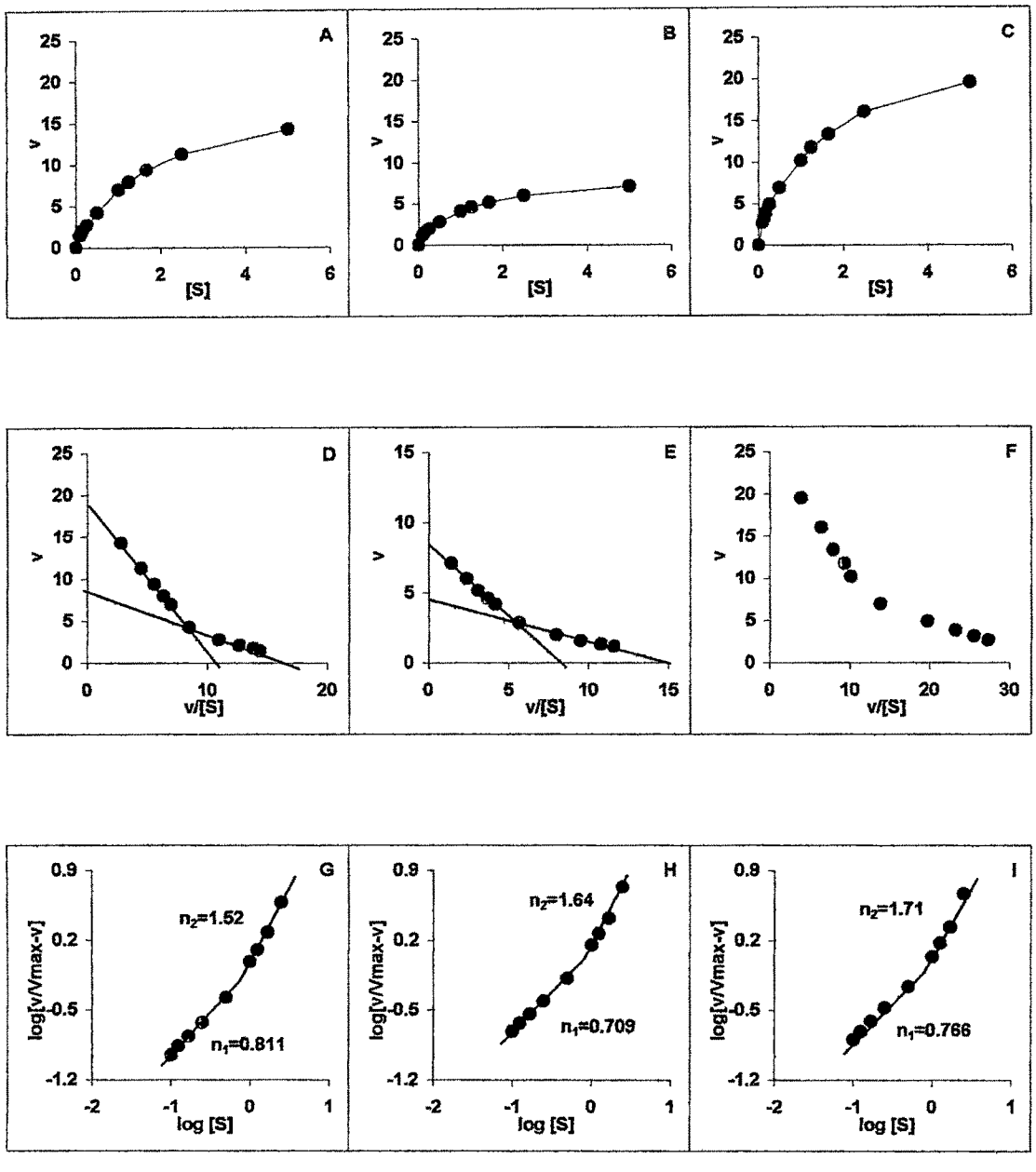
Figure 2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n2=1.52
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n2=1.64
n1=0.709
n2=1.71
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Figure 1. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for Na^+, K^+-ATPase from kidney microsomes 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 Na^+, K^+-ATPase from kidney microsomes 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.

of Km of component I decreased but that of component II was unchanged. In one month diabetic state Km of both the components decreased significantly. At early stage of diabetes, the Vmax of both components was unchanged while at late stage substantial decrease in the Vmax of both the components was noted (50-52 % decreased). Insulin treatment in one week diabetic group was ineffective in restoring the Km value of component I to normality. Under these conditions the Km of component II decreased further. In one month diabetic animals, insulin treatment completely restored the Km of component I whereas partial restoration was noted in Km value of component II. With insulin treatment Vmax value of both the component were comparable to controls (Table 2).

Analysis of the data by Hill plots indicated that up to 0.65 mM ATP concentration one ATP molecule was bound to the enzyme while beyond this concentration two molecules of ATP were bound under all experimental conditions (data not given) Typical Hill plots are shown in Fig. 1 and 2, Panels G-I.

In the next set of experiment the temperature dependence of the enzyme activity was examined. The temperature kinetics data were analyzed as detailed (Chapter 2 of the Thesis). The typical activity versus temperature plots and corresponding Arrhenius plots for early and late effects are given in the Fig. 3 and 4, Panels A-C and D-F respectively. As can be seen, in one week as well as one month groups the activity versus temperature plots in the experimental groups differed considerably from the corresponding controls (Fig. 3 and 4). The optimum temperature in the control group was 41 °C. In one week

Table 2. Effect of alloxan-diabetes and insulin treatment on substrate kinetics properties of Na^+, K^+ ATPase in rat kidney microsomes

Group	Treatment	Component I		Component II	
		Km	Vmax	Km	Vmax
One week	Control	0.40 ± 0.02	9.11 ± 0.54	1.72 ± 0.09	20.88 ± 1.23
	Diabetic	0.31 ± 0.01[b]	8.82 ± 0.43	1.49 ± 0.07	18.82 ± 0.94
	Diabetic + Insulin	0.21 ± 0.01[c,§]	10.01 ± 0.88	1.04 ± 0.06[c,§]	24.41 ± 1.05[ψ]
One month	Control	0.35 ± 0.02	6.47 ± 0.35	1.82 ± 0.08	19.70 ± 0.92
	Diabetic	0.25 ± 0.01[b]	4.24 ± 0.25[c]	1.05 ± 0.04[c]	8.65 ± 0.54[c]
	Diabetic + Insulin	0.31 ± 0.01[‡]	11.18 ± 0.51[b,§]	1.46 ± 0.05[b,§]	25.29 ± 0.96[b,§]

The Km (mM) and Vmax (μmole of Pi liberated / hr / mg protein) values were calculated as described in the text.

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, $p< 0.0$a; b, $p< 0.002$ and c, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.01$; ‡, 0.002 and §, $p<0.001$ compared to the corresponding diabetic.

Figure 3

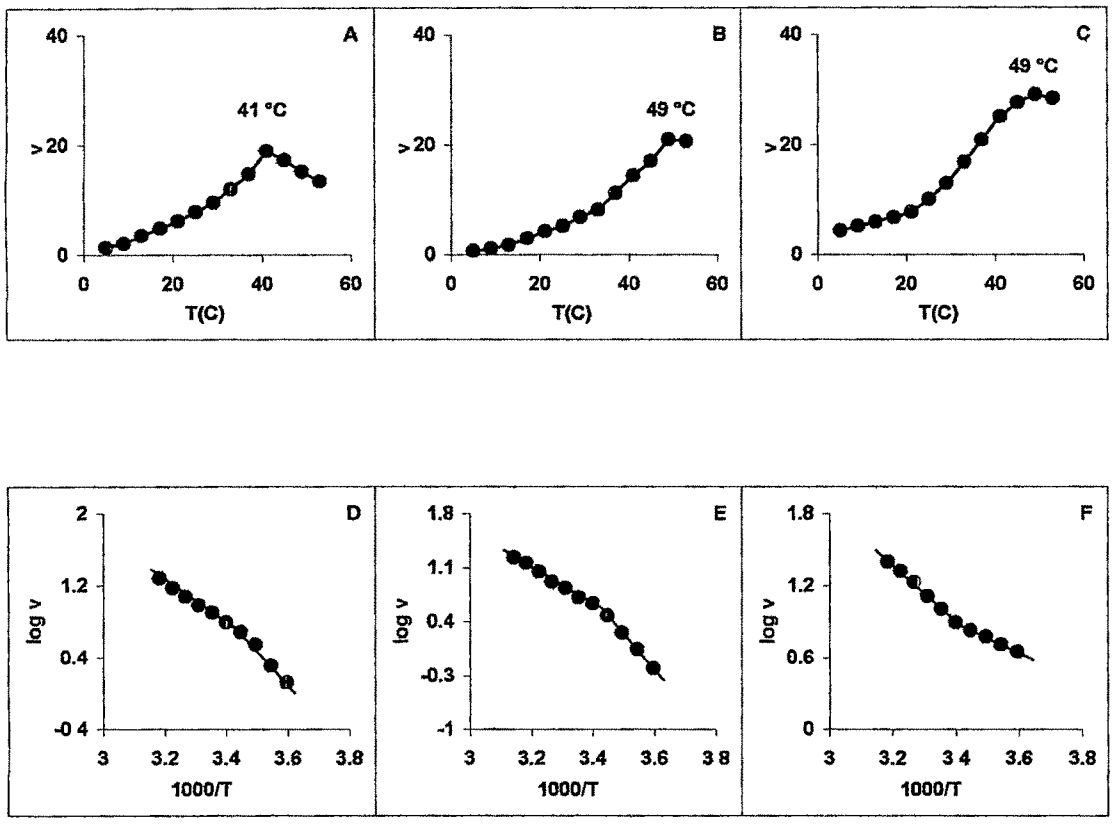
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Figure 4

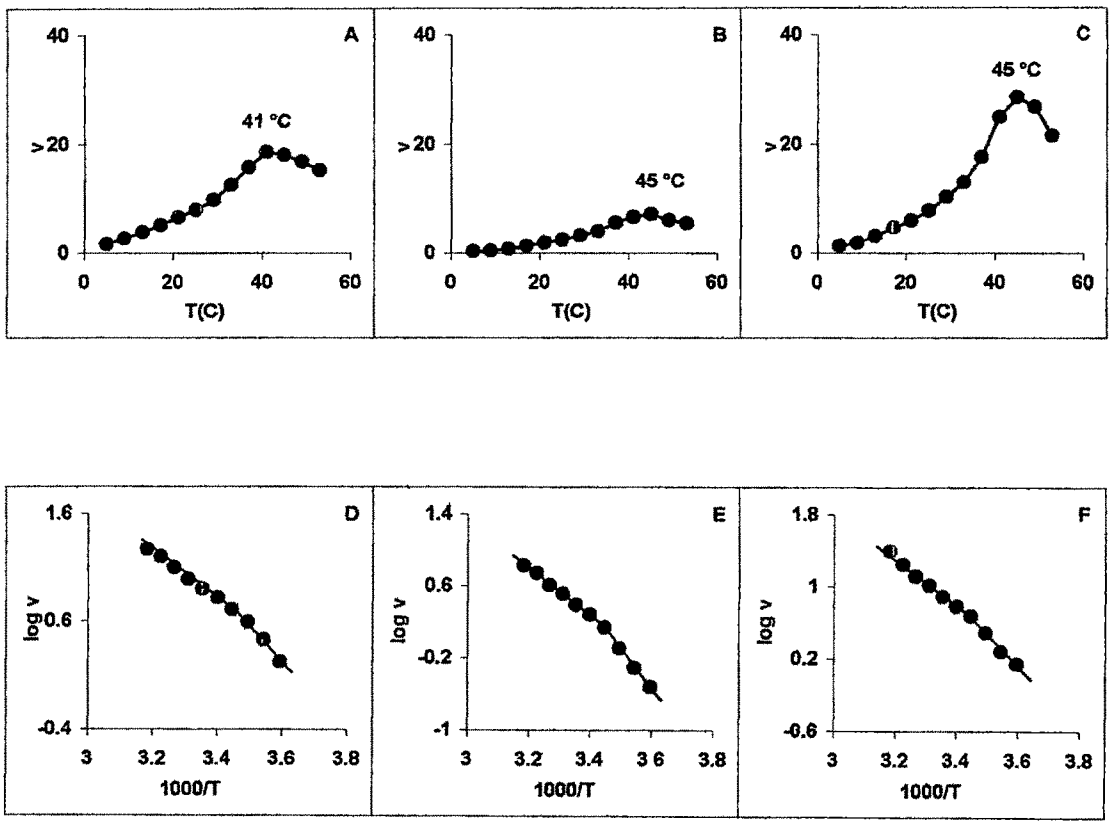
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Figure 3. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for Na^+, K^+-ATPase from kidney microsomes for one week groups. In temperature curves, enzyme activity v on abscissa is plotted verses 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 Na^+, K^+-ATPase from kidney microsomes 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.

Table 3. Effect of alloxan-diabetes and insulin treatment on Arrhenius kinetics properties of Na^+, K^+ ATPase in rat kidney microsomes

		Energy of activation (KJ/mole)		Phase transition temperature Tt (°C)
Group	Treatment	E_H	E_L	Tt
One week	Control	43.32 ± 1.76	75.57 ± 2.87	13.39 ± 0.83
	Diabetic	34.67 ± 1.50[a]	69.55 ± 2.31	14.04 ± 0.49
	Diabetic + Insulin	47.38 ± 1.01[§]	26.95 ± 0.74[b,§]	20.60 ± 1.14[b,§]
One month	Control	44.53 ± 2.39	75.56 ± 4.71	13.93 ± 0.27
	Diabetic	48.95 ± 1.38	85.19 ± 2.79	17.22 ± 0.38[b]
	Diabetic + Insulin	50.02 ± 2.28	69.38 ± 1.90[§]	13.82 ± 0.57[§]

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

a, $p< 0.0a$; b, $p< 0.002$ and c, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.01$; ‡, 0.002 and §, $p<0.001$ compared to the corresponding diabetic.

diabetic group the optimum temperature shifted to 49 °C while in one month group the optimum temperature was 45 °C. Insulin treatment at early and late stages was ineffective in normalizing the optimum temperature (Fig. 3 and 4 Panels A-C).

These differences were also reflected in terms of corresponding Arrhenius plots (Fig. 3 and 4, Panels D-F). In the control group Arrhenius plots depicted typical biphasic pattern i.e. the energy of activation in high temperature ranges (E_H) was lower than that in low temperature ranges (E_L). In diabetic animals the pattern was unchanged (Fig. 3 and 4 Panels E). However, at early stage of diabetes, following insulin treatment, the pattern reversed i.e. energy of activation in high temperature ranges (E_H) was higher than that in low temperature ranges (E_L). (Fig. 3 Panel F). The data on energies of activation and phase transition temperature are given in Table 3. In control group the values of E_H and E_L was about 44 and 76 KJ/mole with phase transition at around 13.5 °C. At early diabetic state the E_H decreased whereas E_L was unchanged. At late stage of diabetes E_H was unchanged whereas marginal increase in E_L was noted which was restored to normality by insulin treatment. At early stage of diabetes phase transition temperature (T_t) was unchanged while at late state it increased. Insulin treatment at early diabetic state resulted in increased in T_t by about 7 °C compared to the control of 13.4 °C whereas at late state T_t was restored to normality.

Effects on G6Pase

Data on effects of alloxan-diabetes and subsequent insulin treatment on G6Pase activity are given in Table 4. Thus, in one week diabetic group, measurements of G6Pase activity

Table 4. Effect of alloxan-diabetes and insulin treatment on glucose-6-phosphatase activity in rat kidney microsomes

Group	Treatment	Activity		Activity Ratio
		25 °C	37 °C	
One week	Control	6.29 ± 0.28	18.61 ± 0.99	2.96 ± 0.12
	Diabetic	8.37 ± 0.41[b]	24.95 ± 0.42	2.98 ± 0.11
	Diabetic + Insulin	7.06 ± 0.29[‡]	20.95 ± 0.88[ψ]	2.97 ± 0.11
One month	Control	7.67 ± 0.28	20.85 ± 1.37	2.72 ± 0.13
	Diabetic	59.26 ± 2.23[c]	156.81 ± 3.19[c]	2.65 ± 0.10
	Diabetic + Insulin	13.92 ± 0.43[c,§]	45.84 ± 1.25[c,§]	3.45 ± 0.16[b,§]

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

Activity ratio was calculated by : activity at 37 °C/activity at 25 °C

The activities are expressed as μmole of Pi liberated / hr / mg protein.

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

at 25 °C revealed 33 % increase in the activity while in one month diabetic group a phenomenal 7.7 fold increase was noted. Almost similar picture was noted when the activity measurements were carried out at 37 °C. In early stage of diabetes the activity increased by 34 % and at the late stage 7.5 fold increase was noted. Insulin treatment completely restored the enzyme activity at early stage while showed partial restoration at late stage of the disease. The activity ratio was higher only in one month diabetic group treated with insulin (Table 4).

In the next set of experiment the kinetic behavior of G6Pase in response to change in substrate i.e. G6P concentration was evaluated. Typical substrate saturation curves and corresponding Eadie-Hofstee plots and Hill plots for are shown in Fig. 5 and 6. The Eadie-Hofstee plots revealed that the enzyme activity resolved in two kinetically distinguishable components in all the groups (Fig. 5 and 6, Panels D- F).

Data on Km and Vmax values of the two components as affected by insulin status are given in Table 5. Thus, in the control group the Km value of the two components were 0.61-0.68 and 4.5-4.7 mM respectively with corresponding values of Vmax of 9.03-10.2 and 33.8-35.2 units. Diabetic state in general increased the Km of both the component and the effect was more pronounced on Km of component II at early stage of diabetes where the Km doubled. Insulin treatment in one week diabetic group completely restored the Km of component I with partial restoration in Km of component II. At late stage insulin treatment completely restored the Km only of component I. In early stage of diabetes Vmax of component I was unchanged whereas Vmax of component II increased

Table 6. Effect of alloxan-diabetes and insulin treatment on substrate kinetics properties of glucose-6-phosphatase in rat kidney microsomes

Group	Treatment	Component I		Component II	
		Km	Vmax	Km	Vmax
One week	Control	0.61 ± 0.023	9.03 ± 0.515	4.70 ± 0.103	33.81± 0.84
	Diabetic	0.78 ± 0.040	9.81 ± 0.314	9.30 ± 0.381	64.6 ± 3.06
	Diabetic + Insulin	0.65 ± 0.042$^{\Psi}$	8.42 ± 0.657	6.16 ± 0.341^{b,§}	44.0 ± 1.43^{c,§}
One month	Control	0.68 ± 0.032	10.2 ± 0.341	4.46 ± 0.246	35.2 ± 0.840
	Diabetic	1.15 ± 0.032^{c}	105.9 ± 2.47^{c}	5.69 ± 0.170^{b}	317.1 ± 6.93^{c}
	Diabetic + Insulin	0.61 ± 0.021§	19.9 ± 0.916^{c,§}	6.28 ± 0.107^{c,¶}	102.5 ± 1.32^{c,§}

The Km (mM) and Vmax (μmole of Pi liberated / hr / mg protein) values were calculated as described in the text.

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

a, $p< 0.01$; b, $p< 0.002$ and c, $p<0.001$ compared to the corresponding control.
Ψ, $p< 0.05$ ¶, 0.02 and §, $p<0.001$ compared to the corresponding diabetic.

Figure 5

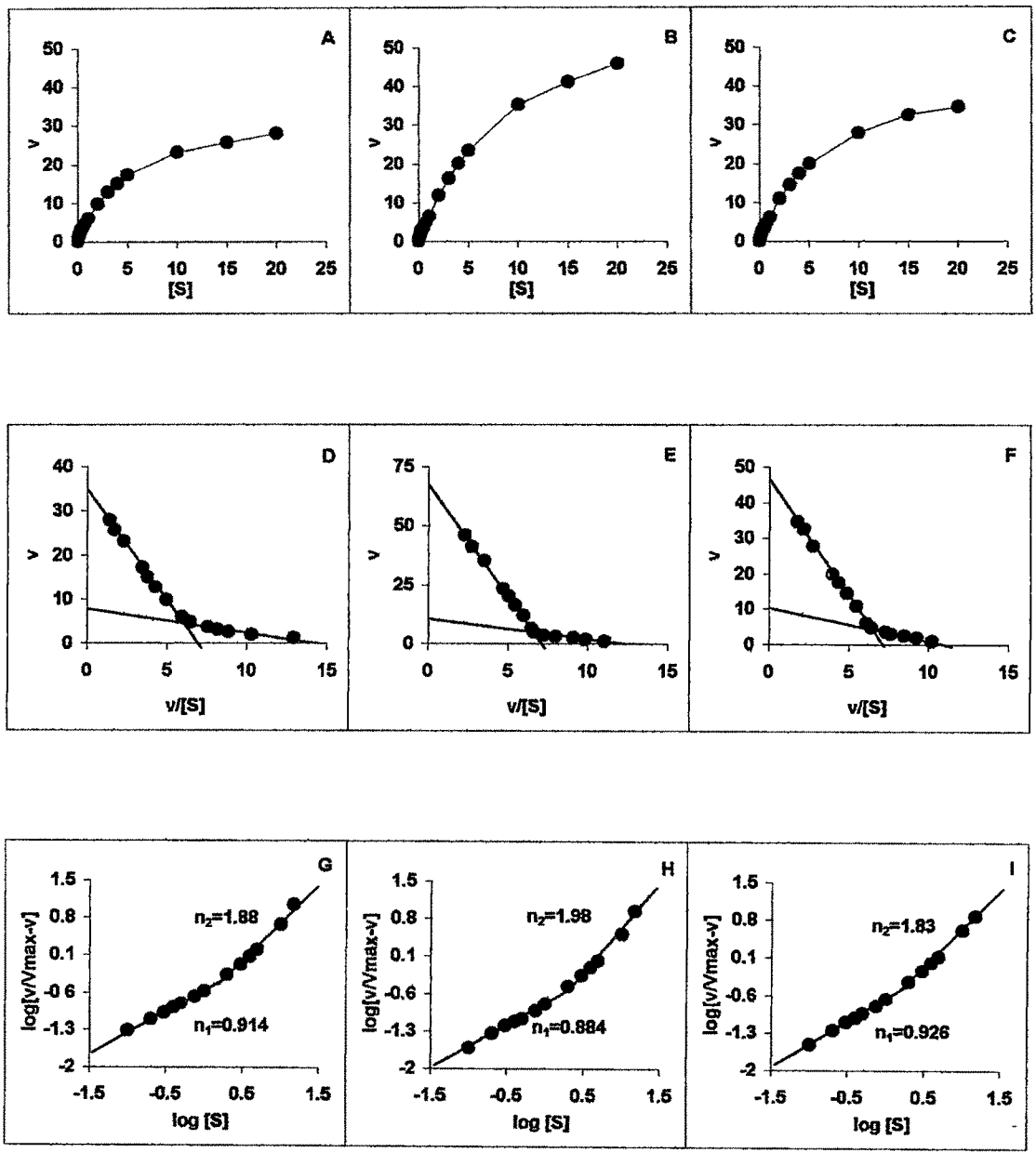
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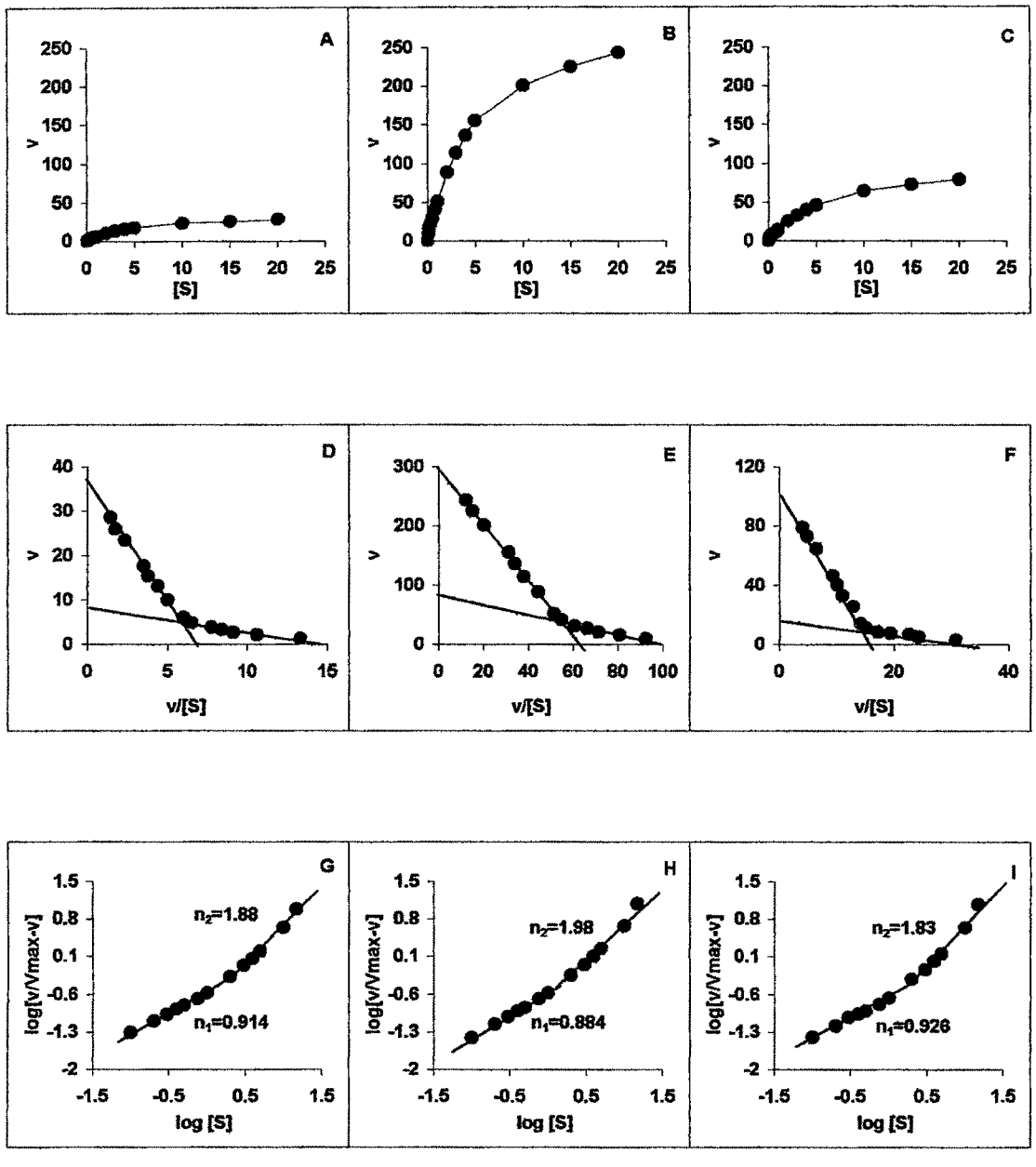
Figure 6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n2=1.88
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n2=1.98
n1=0.884
n2=1.83
n1=0.926

Figure 5. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for G6Pase from kidney microsomes 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 6. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for G6Pase from kidney microsomes 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.

almost by double. At late stage Vmax of both the components showed phenomenal increase (10.4 and 9 fold increase). Insulin treatment showed only partial restorative effect (Table 5).

Analysis of the data by Hill plots indicated that up to 1.6 mM G6P concentration one G6P molecule was bound to the enzyme while beyond this concentration two molecules of G6P were bound under all experimental conditions (data not given) Typical Hill plots are shown in Fig. 5 and 6 Panels G-I.

In the next set of experiments, the temperature dependence of the G6Pase was examined. Typical temperature versus activity and corresponding Arrhenus plots are shown in Fig. 7 and 8, Panels A-C and D-F. In the experimental groups the temperature versus activity curves differed considerably from the control group (Fig. 7 and 8, Panels A-C). The optimum temperature in control group was 45 °C. In diabetes the optimum temperature decreased by 4 °C and shifted to 41 °C. Insulin treatment restored the optimum temperature to normality (Fig. 7 and 8, Panels A-C). The differences in activity versus temperature plots were also reflected in terms of corresponding Arrhenius plots (Fig. 7 and 8 Panels D-F). In control animals typical biphasic pattern was noted for Arrhenius plots. In one week diabetic group the pattern reversed i.e. E_H is higher than E_L; insulin treatment was ineffective (Fig. 7, Panel D-F). In one month diabetic group, the Arrhenius pattern resembled the control group; insulin treatment reversed the pattern (Fig. 8, Panel D-F). The data on energies of activation and phase transition temperature are given in Table 6. In the control group the values of E_H and E_L were 67 and 111 KJ/mole

Figure 7

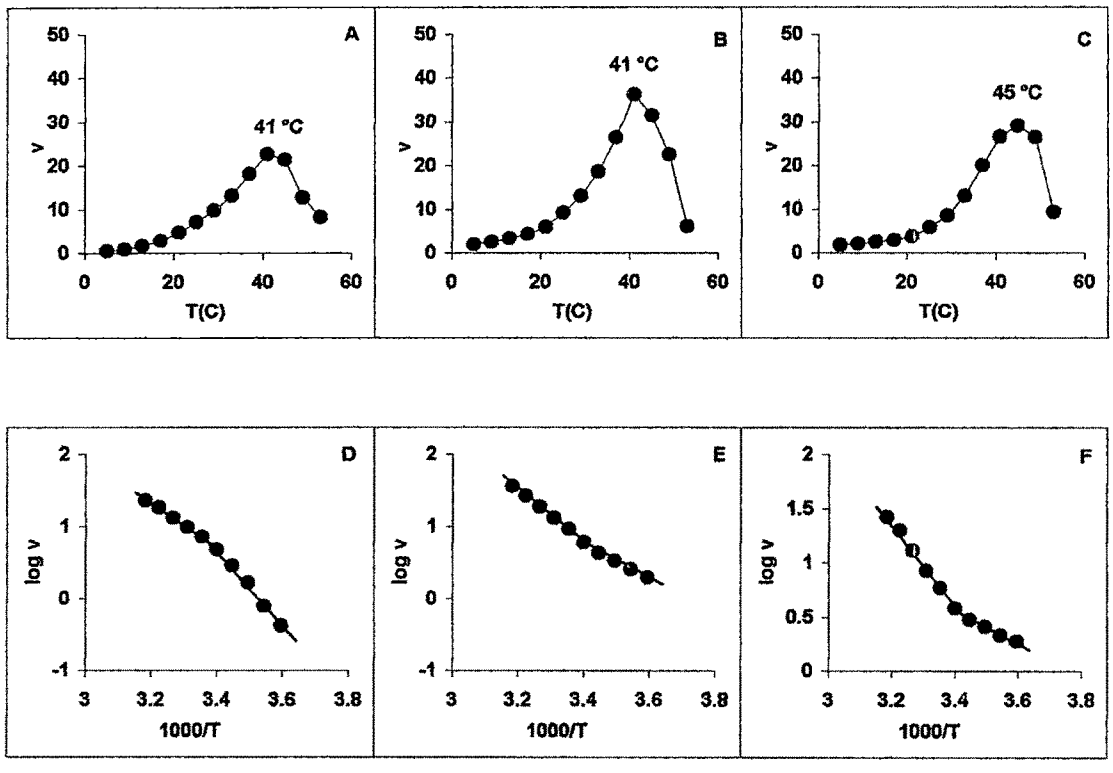
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Figure 8

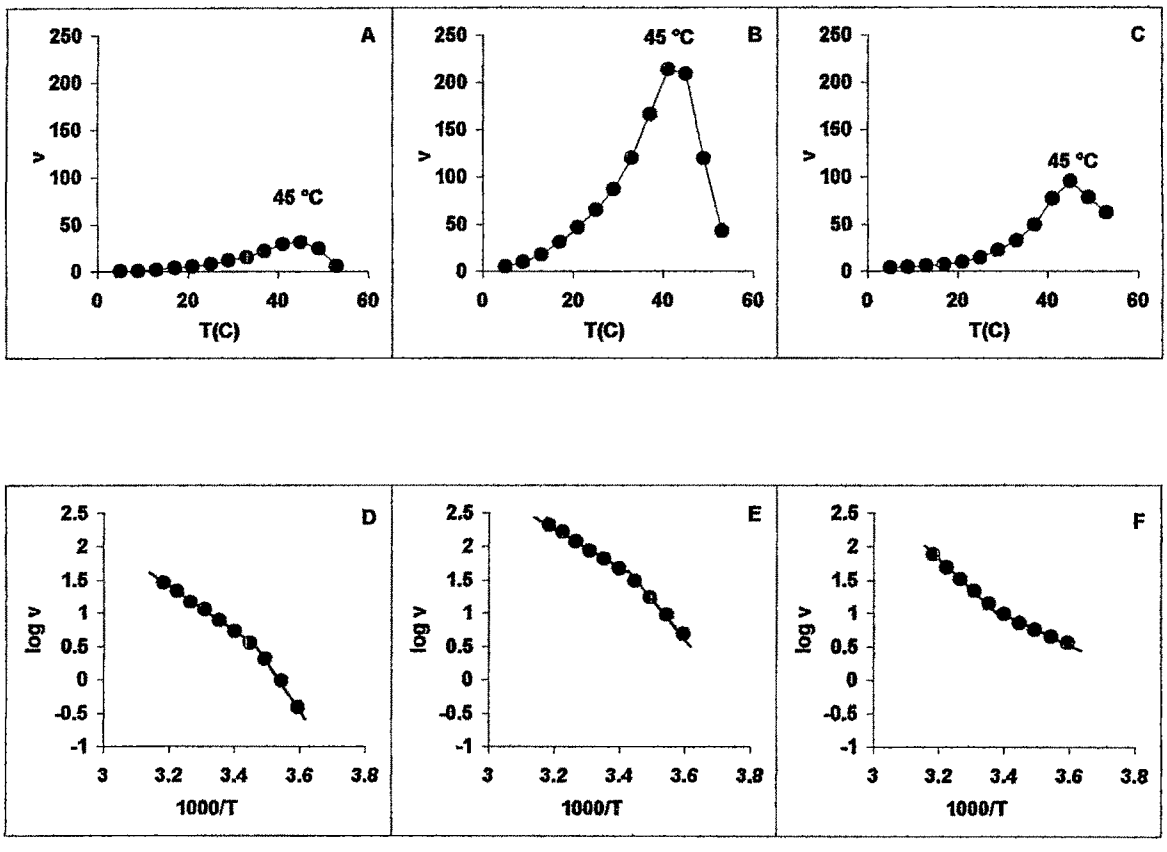
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Figure 7. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for G6Pase from kidney microsomes for one week groups. In temperature curves, enzyme activity v on abscissa is plotted verses temperature (°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 8. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for G6Pase from kidney microsomes 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.

Table 5. Effect of alloxan-diabetes and insulin treatment on Arrhenius kinetics properties of glucose-6-phosphatase in rat kidney microsomes

		Energy of activation (KJ/mole)		Phase transition temperature Tt (°C)
Group	Treatment	E_H	E_L	Tt
One week	Control	70.28 ± 4.52	110.21 ± 1.73	18.36 ± 0.66
	Diabetic	91.51 ± 2.16[a]	61.66 ± 1.14[b]	16.47 ± 0.71[a]
	Diabetic + Insulin	78.86 ± 1.87[§]	39.54 ± 2.34[b,§]	19.15 ± 0.39[ψ]
One month	Control	63.55 ± 3.07	112.89 ± 1.97	19.93 ± 0.69
	Diabetic	42.58 ± 1.78[b]	71.70 ± 1.01[b]	19.14 ± 0.19
	Diabetic + Insulin	70.44 ± 2.98[§]	30.24 ± 1.82[b,§]	18.06 ± 0.40

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

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

respectively. In early diabetic state the E_H was increased (21 KJ increase) while the E_L decreased significantly (49 KJ decrease) and became almost half. At late stage, significant reduction was noted for both E_H and E_L. Insulin treatment restored the E_H in both the diabetic group but E_L registered further decrease. Insulin status had marginal effect on the phase transition temperature (Table 6).

In view of the observed changes in the substrate and temperature kinetics properties of ATPase and G6Pase, the effects of insulin status on lipid/phospholipid profiles of the microsomal membrane were examined. The results are given in Tables 7-9. Early diabetic state resulted in increased TPL and CHL contents (28 and 47 % increase respectively) in the microsomal membranes. Insulin treatment was ineffective in restoring the TPL content while CHL content increased further. The TPL content was unaltered in the late diabetic stage but the CHL content increased by 59 %. Insulin treatment had marginal restorative effect only on CHL content. The TPL/CHL (mole : mole) ratios decreased in the diabetic groups and remained low after insulin treatment (Table 7).

The data on phospholipid composition of microsomal membranes are given in Table 8. Early diabetic state resulted in significant increase in lysophospholipids (Lyso), phosphatidylcholine (PC) and phosphatidylinositol (PI) components whereas sphingomyelin (SPM), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidic acid (PA) components decreased. Insulin treatment had no effect on PC component which remained elevated. PE increased beyond control level after insulin treatment; while there was a further lowering of the other phospholipid classes.

Table 6. Effects of alloxan diabetes on total phospholipids (TPL), cholesterol (CHL) and TPL/CHL ratio in rat kidney microsomes

Groups	Treatment	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)
One week	Control	268.8 ± 7.34	171.6 ± 4.44	0.79 ± 0.03
	Diabetic	344.1 ± 12.0	252.4 ± 3.65[b]	0.68 ± 0.02[a]
	Diabetic + Insulin	352.2 ± 12.3	324.5 ± 7.16[§]	0.54 ± 0.02[§]
One month	Control	272.9 ± 6.98	174.9 ± 3.08	0.78 ± 0.02
	Diabetic	285.9 ± 8.51[b]	279.5 ± 7.39[b]	0.51 ± 0.02[b]
	Diabetic + Insulin	280.6 ± 8.71[b,§]	241.5 ± 4.06[b,§]	0.58 ± 0.02[b,§]

The results are given as mean ± SEM of the number of independent observations indicated in parentheses

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

Table 7. Effects of alloxan diabetes and insulin treatment on phospholipid composition in rat kidney microsomes

Phospholipid Class	Composition (% of total)					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	3.08 ± 0.14	3.82 ± 0.26^{d}	0.50 ± 0.02^{c,§}	3.44 ± 0.18	6.10 ± 0.53^{d}	7.83 ± 0.58^{d,§}
SPM	24.50 ± 0.31	21.80 ± 0.31^{d}	19.90 ± 0.58§	25.10 ± 0.50	27.20 ± 0.91^{d}	23.92 ± 0.55^{d}
PC	35.71 ± 0.45	45.52 ± 0.54^{a}	45.46 ± 0.70^{a}	34.55 ± 0.88	37.27 ± 0.74	34.82 ± 0.44^{a,§}
PI	3.93 ± 0.13	4.69 ± 0.19^{d}	2.15 ± 0.12§	3.29 ± 0.11	2.33 ± 0.21^{d}	3.03 ± 0.15^{d}
PS	5.00 ± 0.12	4.30 ± 0.13^{d}	3.64 ± 0.18^{a,§}	4.91 ± 0.07	2.45 ± 0.14^{d}	3.96 ± 0.13^{d,§}
PE	22.40 ± 0.47	16.11 ± 0.60^{d}	26.58 ± 0.40§	23.01 ± 0.42	20.10 ± 0.92^{d}	23.01 ± 0.43^{b,§}
PA	5.37 ± 0.10	3.73 ± 0.20^{d}	1.77 ± 0.15^{d}	5.43 ± 0.09	4.49 ± 0.37^{d}	3.54 ± 0.20$^{d,\Psi}$

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

Lyso: Lysophospholipid; SPM: sphinghomyelin; PC: phosphatidylcholine; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PA: phosphatidicacid

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 8. Effects of alloxan-diabetes and insulin treatment on phospholipid content in rat kidney microsomes

Phospholipid Class	Content, μg / mg protein					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	8.24 ± 0.36	13.33 ± 1.22[c]	1.77 ± 0.12[e,¶]	7.92 ± 0.44	15.68 ± 1.24[e]	21.81 ± 0.59[e,¶]
SPM	66.14 ± 1.82	74.89 ± 2.58[b]	69.77 ± 2.43	66.89 ± 2.39	76.07 ± 3.31[a]	66.94 ± 2.45[ψ]
PC	95.93 ± 1.83	156.87 ± 6.02[e]	160.20 ± 6.41[e]	95.99 ± 2.43	104.46 ± 4.31	97.61 ± 3.22
PI	10.62 ± 0.48	16.15 ± 0.79[e]	7.60 ± 0.56[d,¶]	11.05 ± 0.86	7.03 ± 0.49[d]	8.54 ± 0.56[a]
PS	13.50 ± 0.52	14.74 ± 0.60	12.88 ± 0.87	13.87 ± 0.66	7.93 ± 0.72[e]	11.13 ± 0.55[c,§]
PE	60.33 ± 1.84	55.21 ± 2.43	93.73 ± 3.77[e,¶]	59.58 ± 2.43	58.39 ± 3.08	64.53 ± 2.46
PA	14.45 ± 0.38	12.91 ± 1.00	6.23 ± 0.55[e,¶]	14.29 ± 0.49	12.83 ± 0.69	10.02 ± 0.80[e,ψ]

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

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

One month diabetic group was characterized by the lowering of PI, PS, PE and PA components while Lyso increased and PC was somewhat elevated. Insulin treatment completely restored PE and PI with partial restoration of PS; Lyso was further elevated while opposite effect was noted for PA component (Table 8). The computed contents of the individual phospholipid classes were generally consistent with the compositional changes (Table 9).

Changes in the levels of cholesterol and altered phospholipid composition and contents could alter the fluidity of the membrane. This was ascertained by measuring the fluidity of the membranes. It can be noted from the data in Table 10 that the membrane fluidity decreased in diabetic animals and treatment with insulin significantly fluidized the membrane in one week diabetic animals whereas opposite effect was seen in the one month diabetic animals (Table 10).

Discussion

It is clear from the data presented that as in the case of liver (Chapter 8 of the Thesis) insulin status affected the two enzyme systems differently, but the effects were opposite. The Km for ATPase decreased in diabetes whereas that for G6Pase increased.

The decrease in Km for ATPase is suggestive of increased efficiency of the enzyme. However, earlier studies from our laboratory showed that streptozotocin-diabetes differentially affects the oxidative capacity of the kidney mitochondria; the oxidation of β hydroxybutyrate and ascorbate + TMPD decreased whereas that of succinate and Table

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

Groups	Treatment	Fluidity Parameters			
		Fluorescence Polarization, p	Fluorescence anisotropy, r	Limited hindered anisotropy, rα	Order parameter, S
One week	Control	0.231 ± 0.009	0.167 ± 0.006	0.122 ± 0.005	0.553 ± 0.019
	Diabetic	0.280 ± 0.004[e]	0.206 ± 0.007[e]	0.175 ± 0.006[e]	0.661 ± 0.016[d]
	Diabetic + Insulin	0.135 ± 0.003[e,§]	0.094 ± 0.004[e,§]	0.026 ± 0.002[e,§]	0.253 ± 0.011[e,§]
One month	Control	0.242 ± 0.007	0.175 ± 0.009	0.134 ± 0.007	0.579 ± 0.018
	Diabetic	0.263 ± 0.004[a]	0.192 ± 0.011	0.156 ± 0.009	0.625 ± 0.022[b]
	Diabetic + Insulin	0.283 ± 0.009[c]	0.208 ± 0.015	0.178 ± 0.007[e]	0.667 ± 0.026[b]

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, $p < 0.02$; c, $p < 0.01$; d, $p < 0.002$ and d, $p < 0.001$ compared to the corresponding control.
§, $p < 0.001$ compared to the corresponding diabetic.

pyruvate + malate increased. However creating imbalance in the oxidative energy potential of the kidney mitochondria (1).

As described earlier (Chapter 8 of the Thesis), the component I could be important in physiological context because of low Km value which is compatible with intracellular concentration of the substrate. Such an assumption is also supported by the fact that up to the concentration of 0.65 mM, 1 ATP molecule is bound to the enzyme (Fig. 1 and 2 Panels G-I) under same condition up to 1.6 mM concentration, 1 G6P molecule is bound to the enzyme G6Pase (Fig. 5 and 6 Panels G-I).

As noted earlier (Chapter 8 of the Thesis), the analysis of the data in terms of apparent efficiency index revealed that at early stage of diabetes the apparent efficiency index for component I of ATPase increased whereas at the late stage it decrease. The component I became highly efficient following insulin treatment and the effect was more pronounced at early stage. A similar picture was noted even for component II (data not shown but can be calculated easily from values of Vmax and Km in Table 2).

Studies on temperature kinetics showed that in diabetes the energies of activation in general were unchanged; however the phase transition temperature increased by 4.2 °C at late stage of the disease (Table 3). This is consistent with the fact that, in diabetes the fatty acid desaturase activity decreased (2, 3).

As described earlier, regulation of Na^+, K^+-ATPase is a complex process controlled by

several factors which include the subunit composition, stoichiometry of the subunits, interaction of the enzyme with membrane proteins and also by the acidic phospholipids (Chapter 8 of the thesis). Data of the present study on phospholipid composition show that at early stages of diabetes, SPM and PC were affected reciprocally. The acidic phospholipid PI and PS were also affected reciprocally at early stage of diabetes whereas at late stage both the phospholipid classes decreased. The PE component decrease of at both the stages of diabetes. Insulin treatment had also differential effects on the phospholipid classes. Regression analysis across the groups correlating kinetics parameters and lipid/phospholipid classes revealed that, the acidic phospholipid PI and PS seem to be positive regulator for Km of both the components. The CHL and SPM seem to be negative regulator of Km and Vmax respectively. Temperature kinetics parameters were regulated differentially by different lipid/phospholipid components (Table 11).

As pointed out earlier, G6Pase activity significantly increased in diabetes (Chapter 8 of the Thesis). Results of the present studies are consistent with the reported observations (4). As in the case of liver, the kidney G6Pase activity also resolved in two kinetically distinguishable components. However, Km of component I decreased in diabetes (Table 5) which similar to that noted earlier for liver (Chapter 8 of the Thesis) is suggestive of increased efficiency of the enzyme G6Pase. Energy imbalance in diabetic kidney referred to above (1) could affect the rate of phosphorylation of glucose and thereby a net intracellular G6P concentration. If one analyzes the data in terms of apparent efficiency, at early stage of diabetes, the apparent efficiency index of component I of G6Pase was

Table 11. Correlation of kinetic parameters of Na^+, K^+-ATPase and glucose-6-phosphatase with membrane lipid/phospholipid composition

Parameter	Correlation with Phospholipid Class			
	Na^+, K^+-ATPase		Glucose-6-phosphatase	
	Positive	Negative	Positive	Negative
Substrate kinetics				
Km_1	PS (+ 0.801) PI (+ 0.694)	CHL (- 0.964) TPL (- 0.650)	SPM (+ 0.577)	PS (- 0.790)
Km_2	PI (+ 0.678) PS (+ 0.901)	CHL (- 0.946)	PC (+ 0.733) TPL (+ 0.725)	CHL (- 0.925)
$Vmax_1$	—	SPM (- 0.708)	SPM (+ 0.683)	PS (- 0.842)
$Vmax_2$	PC (+ 0.745)	SPM (- 0.689)	SPM (+ 0.637)	PS (- 0.875)
Temperature Kinetics				
E_H	PI (+ 0.885) PE (+ 0.681)	— —	PC (+ 0.637) TPL (+ 0.643) PS (+ 0.523)	SPM (- 0.855)
E_L	SPM (+ 0.877)	PC (- 0.657) PE (- 0.610) TPL (- 0.729) CHL (-0.619)	PS (+ 0.528)	CHL (- 0.794) TPL (- 0.561)
Tt	CHL (+ 0.851) TPL (+ 0.598)	PI (- 0.757) PS (- 0.625)	PE(+ 0.698)	PI (- 0.720)

The experimental details are given in the text.

Values given in the parentheses indicate r, regression coefficient which is based on 6-8 independent experiments in each group.

comparable to the control whereas at late stage it increased almost by 6 fold; insulin treatment partially restored the apparent index but it was still significantly higher than that seen in the control groups. It is also known that insulin treatment suppresses the activity of G6Pase by decreasing the amount of messenger ribonucleic acid (m RNA) of the catalytic subunit (5, 6). The results would suggest that insulin treatment suppressed but could not bring the G6Pase activity to normal level.

The regression analysis of kinetic parameters of G6Pase with phospholipid classes across the groups revealed that SPM seems to be the positive and PS a negative regulator for the substrate kinetics parameters. These effects are opposite to that seen for ATPase. PS seems to be the positive regulator of energies of activation in both high and low temperature ranges (Table 11). The regulation of kinetic properties of kidney microsomal G6Pase by specific lipids has been reported for the first time in this study.

Summary

Effects on ATPase

Na^+, K^+-ATPase activity increased by at the early stage whereas decreased by late stage of diabetes. Insulin treatment caused hyper-stimulation of the Na^+, K^+-ATPase activity.

In all the experimental groups the kidney microsomal Na^+, K^+-ATPase resolved in two kinetic components. Diabetic state, in general, lowered the Km and Vmax values and the effects were more pronounced at late stage. Insulin treatment only marginally corrected the Km values in one month group; Vmax in general increased beyond control values upon insulin treatment.

The data from Hill plot analysis showed that up to 0.85 mM ATP concentration one ATP molecule was bound while beyond this concentration two ATP molecules were bound to the enzyme under all the experimental conditions.

In one week diabetic animals E_H decreased and upon insulin treatment it was restored. Insulin treatment in one week diabetic animals significantly lowered the E_L value. T_t increased at late stage of diabetes and also in one week diabetic group treated with insulin.

Effects on G6Pase

The G6Pase activity increased in diabetes with the magnitude being greater in the one month group. Insulin treatment was somewhat effective in restoring the activity to normality.

As in the case of liver, the kidney enzyme displayed two kinetic components.
The diabetic state increased the Km as well as Vmax values. Insulin treatment restored the Km of component I in both the diabetic groups whereas marginally restored the Km of component II only in one week diabetic group. Vmax values in general were partially restored upon insulin treatment.

Hill plot analysis suggested that up to 2.2 mM G6P concentration one G6P molecule was bound while beyond this concentration two G6P molecules were bound to the enzyme under all the experimental conditions.

The temperature kinetic analysis revealed that the early diabetic state as well as insulin treatment in early and late state reversed the Arrhenius pattern. The value for E_H increased in early diabetic state but decreased at the late stage. E_L values decreased in both the diabetic groups. Insulin treatment restored the E_H but not the E_L values. No change was observed in T_t under any of the experimental conditions.

Early diabetic state resulted in increased TPL and CHL contents. The TPL content was unchanged in the late diabetic stage but the CHL content increased. Insulin treatment was effective only in restoring the CHL value at late stage.

The membrane fluidity decreased in diabetic animals and treatment with insulin significantly fluidized the membrane in one week diabetic animals; opposite effect was seen in the one month diabetic animals.

In one week diabetic group, Lyso, PC and PI increased whereas SPM, PE, PS and PA decreased; insulin treatment had no effect on PC component which remained elevated. PE increased beyond control level after insulin treatment while there was a further lowering of the other phospholipid classes. In one month diabetic animals Lyso and PC increased whereas PI, PS, PE and PA decreased; insulin treatment completely restored PE and PI with partial restoration of PS; Lyso was further elevated while opposite effect was noted for PA component.

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