Bacterial Phosphotransferase System: Regulation of the Glucose and Mannose Enzymes II by Sulfhydryl Oxidation†

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ABSTRACT: We have investigated the effect of oxidizing agents on methyl α-glucoside phosphorylation by the *Escherichia coli* phosphotransferase system (PTS). Oxidizing agents inhibited methyl α-glucoside phosphorylation at low methyl α-glucoside concentrations, and the degree of inhibition was shown to decrease with increasing concentrations of methyl α-glucoside. Results of studies with mutant bacteria and substrate analogues of the glucose and mannose enzymes II showed that contrary to the interpretation of Robillard and Konings [Robillard, G. T., & Konings, W. N. (1981) *Biochemistry 20*, 5025–5032] the apparent change in the K_m value for methyl α-glucoside phosphorylation induced by sulfhydryl oxidation is not due to the formation of a low-affinity, oxidized form of the glucose enzyme II. Rather, the results are explained by the presence of two phosphotransferase systems that phosphorylate methyl α-glucoside with different affinities and that are differentially sensitive to oxidizing agents. The low K_m system corresponds to the glucose enzyme II, which is strongly inhibited by potassium ferricyanide, phenazine methosulfate, and plumbagin. The high K_m system corresponds to the mannose enzyme II, which is less sensitive to inhibition by these oxidizing agents. This differential sensitivity to inhibition by oxidizing agents can account for the apparent K_m change for methyl α-glucoside phosphorylation reported by Robillard and Konings. The physiological significance of sulfhydryl oxidation in the enzymes II of the PTS has yet to be ascertained.

The bacterial phosphotransferase system (PTS)[1] catalyzes the translocation and phosphorylation of a variety of sugars (Dills et al., 1980). While several different proteins comprise the system, it is the integral-membrane, sugar-specific enzymes II that directly transport and phosphorylate the sugar substrates. Accordingly, considerable research has been directed toward understanding the regulatory mechanisms that control the activities of the enzymes II.

One regulatory mechanism controlling enzyme II activities in vivo involves responses to the proton electrochemical gradient. A number of investigators have shown that the proton electrochemical gradient inhibits methyl α-glucoside uptake in vivo and in membrane vesicles (Hernandez-Asensio et al., 1975; del Campo et al., 1975; Reider et al., 1979). Recently, data and a hypothesis were presented that offered an explanation for this type of inhibition (Robillard & Konings, 1981; Robillard, 1982). Enzymes II were envisioned to exist as either low-affinity oxidized forms or high-affinity reduced forms with nearly identical V_{max} values. Dithiols in the enzymes II were presumed to be oxidized to disulfides. The protonmotive force was proposed to interconvert the two forms in vivo, and oxidizing agents such as potassium ferricyanide were suggested to mimic the effect of the protonmotive force in vitro. These conclusions were based primarily on studies conducted with enzyme II^{Glc}.

In a recent report we showed that enzyme II^{Mtl} is oxidized to an inactive state rather than a low-affinity form (Grenier et al., 1985). This observation indicated that the model proposed by Robillard & Konings (1981) explaining the regulation of enzyme II^{Glc} was not applicable to all enzymes II. In the present investigation, we show that this model is incorrect for enzyme II^{Glc}. Sulfhydryl oxidation inactivates this enzyme in a fashion analogous to that reported for enzyme II^{Mtl}. It has yet to be determined if the in vitro enzyme II oxidation is relevant to the in vivo response of the enzyme to the proton electrochemical gradient.

Materials and Methods

Chemicals. Methyl α-D-[U-^{14}C]glucopyranoside (279 mCi/mmol) was obtained from Amersham, and 2-deoxy-D-[U-^{14}C]glucose (282 mCi/mmol) was obtained from New England Nuclear. Phenazine methosulfate and plumbagin were from Sigma. All other reagents were of reagent grade or better.

Preparation of Vesicles. Isogenic wild-type and *manA* (enzyme II^{Man} negative) mutant strains of *Escherichia coli* (ML308 and LJ246) and *Salmonella typhimurium* (LT-2 and LJ218) were grown aerobically to the late logarithmic phase in Luria broth containing 0.5% glucose. The cells were harvested, and inverted vesicles were prepared as described by Reenstra et al. (1980). Membrane preparations were kept at −20 °C until used.

Enzyme II Assays. Phosphoenolpyruvate-dependent ^{14}C-labeled sugar phosphorylation reactions for both enzyme II^{Glc} and enzyme II^{Man} were carried out in 0.1-mL volumes containing 50 mM potassium phosphate, pH 7.5, 10 mM $MgSO_4$, 10 mM KF, and 5 mM phosphoenolpyruvate (1× reaction mix). Nonradioactive methyl α-glucoside used in these assays was purified free of glucose and recrystallized as described by Roseman et al. (1952). The specific activities of methyl α-

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[1] Abbreviations: PTS, phosphotransferase system; HPr, heat-stable phosphocarrier protein of the PTS; enzyme II^{Glc}, glucose enzyme II; enzyme II^{Man}, mannose enzyme II; PEP, phosphoenolpyruvate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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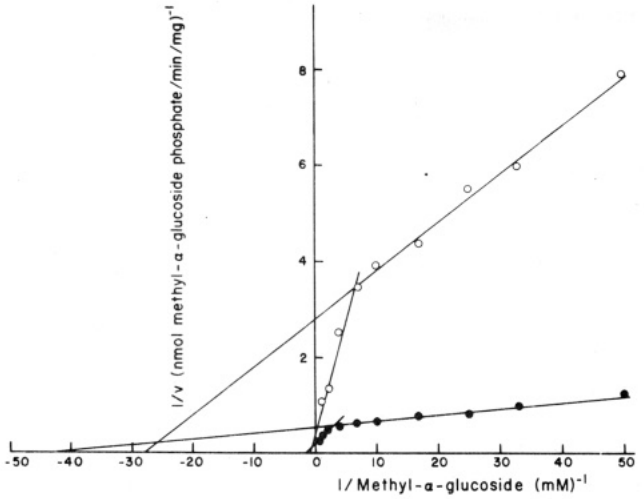

FIGURE 1: Effect of potassium ferricyanide and potassium ferrocyanide on methyl α-glucoside phosphorylation catalyzed by *E. coli* membranes. Ferricyanide (3 mM) or ferrocyanide (3 mM) was added to a 50-μL reaction mixture containing 4 μM enzyme I, 60 μM HPr, 40 μM enzyme III^{Glc}, and 35 μg of membranes from *E. coli* strain ML308. After 20 min at 37 °C, the reaction was initiated by addition of 50 μL of a 2-fold concentrated assay mixture, and reaction was allowed to proceed at 37 °C as described under Materials and Methods. The assay mixture was prepared with different concentrations of substrate. Enzyme I activity is not inhibited by ferricyanide under these conditions (Grenier et al., 1984). Enzyme III^{Glc} lacks cysteine (Meadow & Roseman, 1982; Nelson et al., 1984). Open circles, plus ferricyanide; closed circles, plus ferrocyanide.

[^{14}C]glucoside and 2-deoxy[^{14}C]glucose varied in these experiments but were as high as 250 mCi/mmol. When possible, much lower specific activities were used in order to conserve the radioactive label. The concentrations of enzyme I, HPr, and enzyme III^{Glc} varied and are given in the figure legends. In all cases, enzyme II was the rate-limiting component. Incubations were at 37 °C for 20–30 min and were stopped by the addition of ice-cold water (3 mL). The ^{14}C-labeled sugar phosphate was separated from free sugar on Dowex (AG 1-X2) columns and quantitated as described (Kundig & Roseman, 1971).

Purification of Enzymes. Enzyme I and HPr were purified as described by Waygood & Steeves (1980). Enzyme III^{Glc} was purified essentially as described by Meadow & Roseman (1982) with the following modification. As in the original procedure, preparative gel electrophoresis remained the final purification step. The enzyme III^{Glc} region of the gel was excised from the gel, and the gel was pulverized in a small volume of 0.01 M Tris-HCl, pH 7.5. Following a 48-h incubation period at 4 °C, the gel particles were removed by centrifugation. Protein determinations were performed as described by Lowry et al. (1951).

RESULTS

Inhibition of Methyl α-Glucoside Phosphorylation by Potassium Ferricyanide. Figure 1 shows a double-reciprocal plot of the initial rate of methyl α-glucoside phosphorylation vs. methyl α-glucoside concentration. In this experiment, methyl α-glucoside phosphorylation catalyzed by washed *E. coli* membranes was assayed after treatment of the membranes with either potassium ferricyanide or potassium ferrocyanide. Ferricyanide clearly inhibited methyl α-glucoside phosphorylation, and the percent of inhibition decreased as the methyl α-glucoside concentration increased. The interpretation of this result is not obvious, however, because both the ferrocyanide- and ferricyanide-treated membranes exhibited nonlinear kinetics. As will be discussed below, these nonlinear kinetics

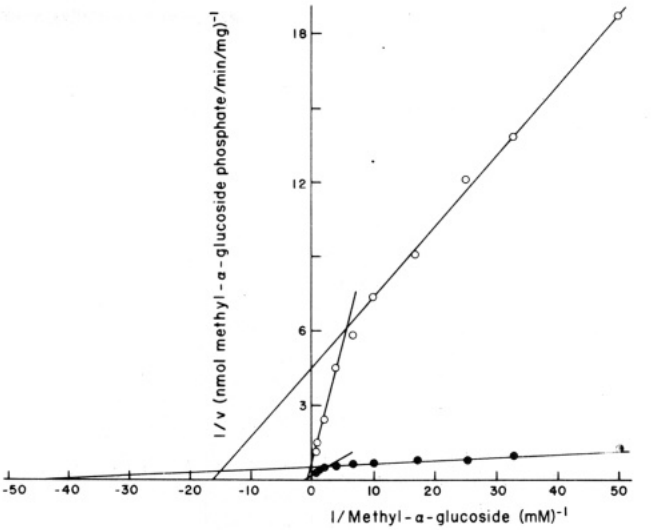

FIGURE 2: Requirement of enzyme III^{Glc} for optimal enzyme II^{Glc} activity. The concentrations of membranes (ML308), HPr, enzyme I, enzyme III^{Glc}, and substrate were as in Figure 1. There was no preincubation period, and enzyme III^{Glc} was omitted from one series of assays. Closed circles, plus enzyme III^{Glc}; open circles, minus enzyme III^{Glc}.

are due to the presence of two different enzymes II in the membranes, both of which phosphorylate methyl α-glucoside. The low K_m system had a K_m^{app} for methyl α-glucoside of 23 μM when treated with ferrocyanide and 36 μM when treated with ferricyanide. The high K_m system had K_m^{app} values of approximately 700 μM and 1100 μM, respectively, under these same conditions. Ferricyanide treatment did not eliminate either system but decreased the V_{max} of the low K_m system by 80% and the V_{max} of the high K_m system by 40%. One obvious interpretation of these results is that the low and high K_m systems are differentially sensitive to ferricyanide.

The presence of two enzymes II in *E. coli* and *S. typhimurium* membranes that phosphorylate methyl α-glucoside has been reported previously (Curtis & Epstein, 1975; Saier et al., 1976, 1977; Rephaeli & Saier, 1980; Stock et al., 1982). Enzyme II^{Glc} has a high affinity for methyl α-glucoside (the low micromolar range), while enzyme II^{Man} has a low affinity for methyl α-glucoside (in the millimolar range). It should be noted that nonlinear double-reciprocal plots of the type shown in Figure 1 typically overestimate the low K_m value and underestimate the high K_m value (Segel, 1975). The specific activities of enzyme II^{Glc} and enzyme II^{Man} in our experiments were higher than those reported by Robillard & Konings (1981) and lower than those reported by Stock et al. (1982). In general, the specific activities reported in the literature vary considerably.

Identification and Characterization of the Low- and High-Affinity Systems. Enzyme II^{Glc} functions in conjunction with an enzyme III^{Glc} for the phosphorylation of methyl α-glucoside. Washed *E. coli* membranes generally contain enzyme III^{Glc} in amounts that are far less than required for saturation of enzyme II. The addition of exogenous enzyme III^{Glc} is therefore required for expression of maximal activity, and any stimulatory effect of enzyme III^{Glc} can be attributed to the enzyme II^{Glc}–enzyme III^{Glc} pair. The experiment shown in Figure 2 indicates that, as expected, one of the two systems catalyzing methyl α-glucoside phosphorylation is the enzyme II^{Glc}. Omitting enzyme III^{Glc} from the assay decreased the V_{max} of the high-affinity system by 90%. The requirement for enzyme III^{Glc} as well as the high affinity of this system for methyl α-glucoside is consistent with the conclusion that this activity is attributable to enzyme II^{Glc}.

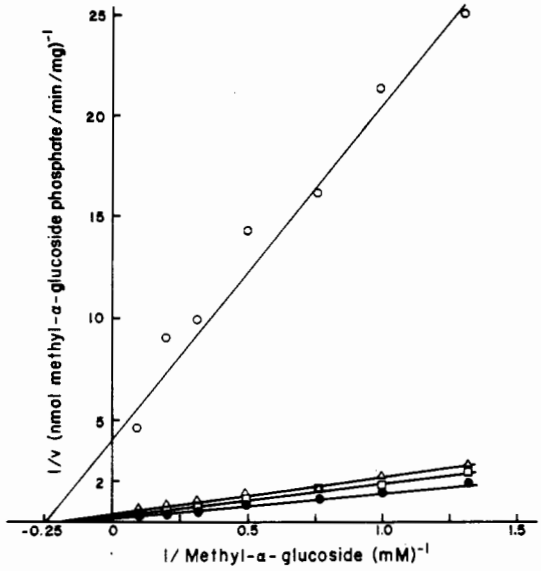

FIGURE 3: Enzyme II^{Man} activity at high concentrations of methyl α-glucoside. Concentrations of HPr and enzyme I were as described in the legend to Figure 1. Enzyme III^{Glc} was omitted from all assays. Samples were preincubated for 20 min at 37 °C with or without ferrocyanide (3 mM) or ferricyanide (3 mM). Membranes derived from *E. coli* strain ML308 or LJ246 (*manA*) were included at a final concentration of 2 mg/mL. Open circles, LJ246 membranes; closed circles, ML308 membranes without ferricyanide or ferrocyanide; triangles, ML308 membranes plus ferrocyanide; squares, ML308 membranes plus ferricyanide.

The high K_m activity observed in Figure 1 was shown to be enzyme II^{Man} by several criteria. Figure 3 shows a double-reciprocal plot in which methyl α-glucoside phosphorylation was measured at high methyl α-glucoside concentrations (0.75–10 mM) in the absence of enzyme III^{Glc}. As shown in Figure 2, these conditions minimize the contribution of enzyme II^{Glc} to the total activity. The K_m^{app} for methyl α-glucoside determined under these conditions ranged from 5 to 10 mM, which approximated previously published values (Stock et al., 1982). An isogenic enzyme II^{Man} mutant, strain LJ246, showed only about 5% of the wild-type activity under these conditions (Figure 3). Ferricyanide caused little inhibition of methyl α-glucoside phosphorylation under these conditions.

The enzyme II^{Man} was also assayed with its alternate substrate, 2-deoxyglucose. The K_m value of both enzyme II^{Glc} and enzyme II^{Man} for 2-deoxyglucose has been reported to be approximately 200 μM (Stock et al., 1982). The V_{max} for the phosphorylation of this sugar by enzyme II^{Man}, however, is nearly 10 times that of enzyme II^{Glc}. Consequently, 2-deoxyglucose phosphorylation in the absence of enzyme III^{Glc} provided a highly specific assay for enzyme II^{Man}. If, as we expected, high K_m methyl α-glucoside phosphorylation was attributable to enzyme II^{Man}, then methyl α-glucoside should be a competitive inhibitor of 2-deoxyglucose phosphorylation. Moreover, if the enzyme follows Michaelis–Menten kinetics, with $k_2 \ll k_{-1}$ (Segel, 1975), the K_i for methyl α-glucoside should approximate the K_m for methyl α-glucoside. As shown in Figure 4A, both of these predictions were verified. Methyl α-glucoside was a competitive inhibitor of 2-deoxyglucose phosphorylation with a K_i of 9 mM. The K_m for 2-deoxyglucose was approximately 100 μM, close to the previously published value of 180 μM (Stock et al., 1982). The *E. coli manA* mutant studied had only about 5% of the wild-type activity as determined by this assay (data not shown).

All of these results provide support for the conclusion that the high K_m methyl α-glucoside phosphorylating system is enzyme II^{Man}. That ferricyanide oxidation does not appreciably inhibit enzyme II^{Man} is shown in Figure 4B. In this experiment, 2-deoxyglucose phosphorylation was inhibited 18% and 28% by ferricyanide and ferrocyanide, respectively. Because ferrocyanide inhibited as well as or better than ferricyanide, the observed inhibition is apparently nonspecific and not related to sulfhydryl oxidation. Two other oxidizing agents, phenazine methosulfate and plumbagin, both at a concentration of 200 μM, caused less than 10% inhibition of 2-deoxyglucose phosphorylation under similar conditions.

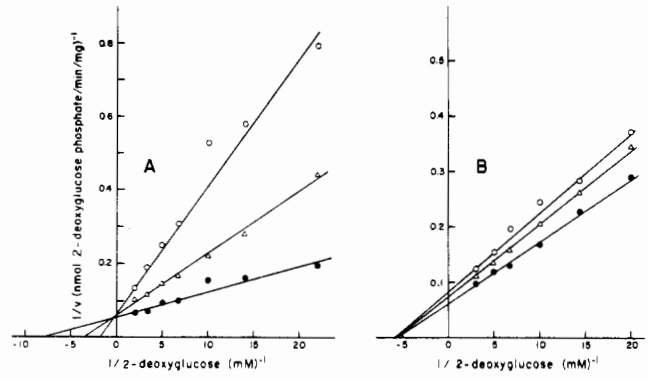

FIGURE 4: Phosphorylation of 2-deoxyglucose by enzyme II^{Man}. In both (A) and (B), HPr and enzyme I concentrations were as described in the legend to Figure 1, and enzyme III^{Glc} was omitted from all samples. Membranes from *E. coli* strain ML308 were included at a final concentration of 80 μg/mL. (A) Effect of methyl α-glucoside on 2-deoxyglucose phosphorylation. Closed circles, triangles, and open circles represent data obtained in the absence of methyl α-glucoside, in the presence of 7.5 mM methyl α-glucoside, and in the presence of 25 mM methyl α-glucoside, respectively. (B) Effects of ferricyanide and ferrocyanide on 2-deoxyglucose phosphorylation. All samples were preincubated for 20 min at 37 °C prior to initiation of the reaction. Closed circles, triangles, and open circles depict the rates of 2-deoxyglucose phosphorylation in the absence of an oxidizing agent, in the presence of ferricyanide (3 mM), or in the presence of ferrocyanide (3 mM), respectively.

The data presented thus far are consistent with the conclusions that the high K_m activity reported in Figure 1 is correctly attributable to enzyme II^{Man} and that oxidizing agents are not appreciably inhibitory under the conditions employed. Enzyme II^{Glc}, however, is inhibited by oxidizing agents under these conditions, and the oxidized enzyme retains less than 15% of its original activity. In order to confirm this conclusion, enzyme II^{Glc} activity was measured at high methyl α-glucoside concentrations in the presence and absence of ferricyanide under conditions where enzyme II^{Man} activity was essentially absent. For this purpose, an *E. coli manA* mutant was employed. The results of this experiment are shown in Figure 5. Ferricyanide inhibited methyl α-glucoside phosphorylation approximately 85% as determined by the differences in V_{max} values. Under these same conditions, the enzyme II^{Man} was not significantly inhibited (Figure 3).

Several of the critical observations made with the *E. coli* enzymes were confirmed with *S. typhimurium* enzymes. A wild-type strain (LT-2) possessed both enzyme II^{Glc} and enzyme II^{Man} activities. While enzyme II^{Man} activity was not inhibited by treatment with oxidizing agents, greater than 85% inhibition of enzyme II^{Glc} activity was observed in a *manA* mutant (LJ218) at a methyl α-glucoside concentration of 10 mM, the highest concentration tested.

DISCUSSION

In 1981, Robillard and Konings presented a model that provided an explanation for the known regulation of *Escherichia coli* phosphotransferase systems by the protonmotive force. Their proposal consisted of two distinct postulates. First, changes in the proton electrochemical gradient (which

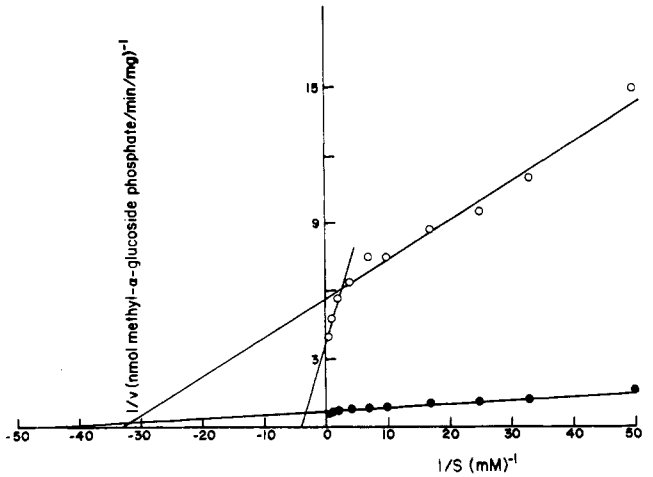

FIGURE 5: Effect of potassium ferricyanide and potassium ferrocyanide treatments on methyl α-glucoside phosphorylation catalyzed by membranes derived from an *E. coli* enzyme II^{Man} mutant. Concentrations of HPr, enzyme I, and enzyme III^{Glc} and the duration of exposure to ferricyanide or ferrocyanide were as described in the legend to Figure 1. Membranes from *E. coli* strain LJ246 were included in the assay solution at a final concentration of 2.4 mg/mL. Open circles, plus ferricyanide; closed circles, plus ferrocyanide.

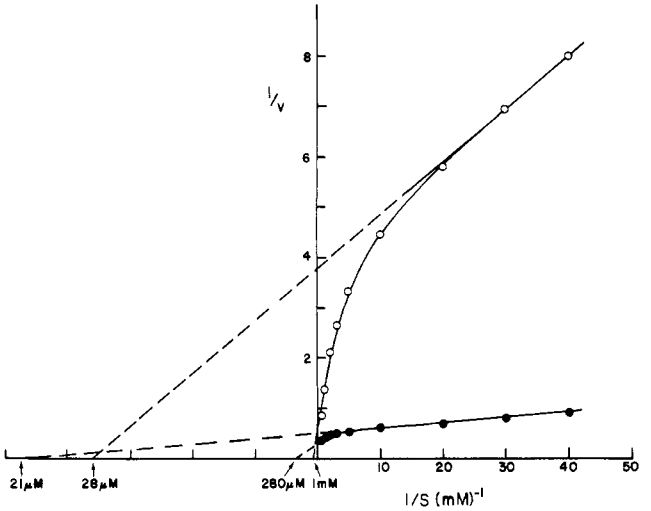

FIGURE 6: Theoretical Lineweaver–Burk plots. K_m values for the high- and low-affinity systems are 20 μM and 10 mM, respectively. Closed circles represent the control condition with V_{max} values of 2 units and 6 units for the high- and low-affinity systems, respectively. Open circles show the theoretical effect observed when only the high-affinity system is inhibited to 10% of its initial value. A marked alteration in the apparent K_M value for the low-affinity system is observed.

could be simulated by oxidizing agents in vitro) caused the conversion of certain dithiols in the enzymes II to disulfides. Second, these modified enzymes II possessed activities that were characterized by unaltered V_{max} values but high K_m values. This model was based primarily on studies of methyl α-glucoside phosphorylation catalyzed by washed *E. coli* membranes supplemented only with enzyme I and HPr.

Our recent studies with purified enzyme II^{Mtl} (Grenier et al., 1984) demonstrated that the second postulate of this model was incorrect for enzyme II^{Mtl}. In agreement with the work of Roossien & Robillard (1984), we found that the oxidizing agent, potassium ferricyanide, inhibited mannitol phosphorylation and that oxidation converted dithiols in enzyme II^{Mtl} into disulfides. In disagreement with their model, however, was the observation that the oxidized enzyme II^{Mtl} was inactive at all mannitol concentrations tested (1 μM to 25 mM).

Membranes prepared from either *E. coli* or *S. typhimurium* contain elevated levels of both enzyme II^{Glc} and enzyme II^{Man} after growth in the presence of glucose (Saier et al., 1976, 1977; Rephaeli & Saier, 1980; Stock et al., 1982). Both of these enzymes phosphorylate methyl α-glucoside with similar V_{max} values but different K_m values. The enzyme II^{Glc} has a K_m for methyl α-glucoside of approximately 10 μM while the enzyme II^{Man} has a K_m for methyl α-glucoside of approximately 10 mM. The results reported in this paper show that enzyme II^{Man} and enzyme II^{Glc} are differentially sensitive to oxidizing agents. Oxidation inhibited enzyme II^{Glc} at all methyl α-glucoside concentrations tested (20 μM to 10 mM), and in all cases, the V_{max} value declined. Inhibition in excess of 85% was documented. In no instance, however, was 100% inhibition of enzyme II^{Glc} achieved. The residual activity may have either reflected inherent activity of the oxidized enzyme II^{Glc}, or unoxidized enzyme may have been present in the preparation. The data presented do not allow us to distinguish between these possibilities. It should be noted that, under conditions that resulted in the extensive oxidation of enzyme II^{Glc}, enzyme II^{Man} activity was not significantly inhibited.

When two enzymes act upon the same substrate with differing affinities, nonlinear Lineweaver–Burk plots are observed. The apparent K_m values for the two enzymes will, in general, lie between the actual K_m values (Segel, 1975). Precisely this behavior was observed when we measured enzyme II^{Man} and enzyme II^{Glc} activities simultaneously. The apparent K_m values depend on the actual K_m and V_{max} values of both systems, and the K_m and V_{max} values of one system more closely approximate the true values only when the contribution of the second system is minimized. Figure 6 exemplifies this behavior with theoretical curves. As can be seen, changing the contribution of one system (in this case the high-affinity system) changes the apparent K_m values of both the high- and low-affinity systems. Robillard & Konings measured methyl α-glucoside phosphorylation with washed *E. coli* membranes in the presence of enzyme I and HPr but in the absence of added enzyme III^{Glc}. It is likely, therefore, that the enzyme II^{Glc} activity was greatly underestimated in their experiments. Their assay conditions would be expected to amplify the contribution made by enzyme II^{Man} to the rate of methyl α-glucoside phosphorylation. Under oxidizing conditions, the contribution of enzyme II^{Glc} would be even further diminished, so that even at low methyl α-glucoside concentrations the contribution of enzyme II^{Glc} to the total activity of the system would be minimal. Over a narrow range of methyl α-glucoside concentrations a straight line could be approximated in a double-reciprocal plot, which would extrapolate to a high apparent K_m. The nearly identical V_{max} values noted by Robillard & Konings (1981) under reducing and oxidizing conditions were coincidental.

In conclusion, this and our previous study (Grenier et al., 1985) indicate that (1) enzymes II are differentially sensitive to oxidation by agents such as potassium ferricyanide, phenazine methosulfate, and plumbagin and (2) the oxidized forms of enzyme II^{Mtl} and enzyme II^{Glc} are essentially inactive. Further studies will be required to determine the physiological significance of these observations. The relationship between sulfhydryl oxidation and the control of enzyme II activity by the proton electrochemical gradient is unknown.

Registry No. PTS, 56941-29-8; PTS enzyme II, 37278-09-4; methyl α-glucoside, 97-30-3; 2-deoxyglucose, 154-17-6; potassium ferricyanide, 13746-66-2; potassium ferrocyanide, 13943-58-3.

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Coupling of Ca^{2+} Transport to ATP Hydrolysis by the Ca^{2+}-ATPase of Sarcoplasmic Reticulum: Potential Role of the 53-Kilodalton Glycoprotein†

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ABSTRACT: An essential feature of the function of the Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) is the close coupling between the hydrolysis of ATP and the active transport of Ca^{2+}. The purpose of this study is to investigate the role of other components of the SR membrane in regulating the coupling of Ca^{2+}-ATPase in SR isolated from rabbit skeletal muscle, reconstituted SR, and purified Ca^{2+}-ATPase/phospholipid complexes. Our results suggest that (1) it is possible to systematically alter the degree of coupling obtained in reconstituted SR preparations by varying the [KCl] present during cholate solubilization, (2) the variation in coupling is not due to differences in the permeability of the reconstituted SR vesicles to Ca^{2+}, and (3) vesicles reconstituted with purified Ca^{2+}-ATPase are extensively uncoupled under our experimental conditions regardless of the lipid/protein ratio or phospholipid composition. In reconstituted SR preparations prepared by varying the [KCl] present during cholate treatment, we find a direct correlation between the relative degree of coupling between ATP hydrolysis and Ca^{2+} transport and the level of the 53-kilodalton (53-kDa) glycoprotein of the SR membrane. These results suggest that the 53-kDa glycoprotein may be involved in regulating the coupling between ATP hydrolysis and Ca^{2+} transport in the SR.

A major goal of membrane biology is to understand how interactions between membrane components (both lipids and proteins) may be involved in regulating the function of membrane transport proteins. The Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum (SR),[1] which mediates the active uptake of Ca^{2+} into the SR at the expense of ATP, has proven to be a useful model system for examining such membrane interactions. From these studies, a wealth of detailed information has become available on Ca^{2+} binding and the ATPase hydrolysis reaction cycle (Yamada & Ikemoto, 1978; Ikemoto, 1982) and on lipid composition (Swoboda et al., 1979; Bennett et al., 1978, 1980; Knowles et al., 1976), membrane fluidity (Warren et al., 1974; Hesketh et al., 1976; Hidalgo et al., 1976; Hidalgo, 1985), and membrane thickness effects (Caffrey & Feigenson, 1981; Johannson et al., 1981) on ATPase activity. However, very little is known about the mechanism by which

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[1] Abbreviations: SR, sarcoplasmic reticulum; R-SR, reconstituted sarcoplasmic reticulum; GP-53, 53-kDa glycoprotein; kDa, kilodalton(s); ATP, adenosine 5′-triphosphate; NADH, nicotinamide adenine dinucleotide, reduced form; MOPS, 3-(*N*-morpholino)propanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; TFP, trifluoperazine.

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