^{31}P NMR Saturation-Transfer Measurements in *Saccharomyces cerevisiae*: Characterization of Phosphate Exchange Reactions by Iodoacetate and Antimycin A Inhibition†

S. L. Campbell-Burk,* K. A. Jones,‡ and R. G. Shulman

Department of Molecular Biophysics and Biochemistry and Department of Chemistry, Yale University, New Haven, Connecticut 06511

Received October 15, 1986; Revised Manuscript Received May 18, 1987

ABSTRACT: ^{31}P nuclear magnetic resonance (NMR) saturation-transfer (ST) techniques have been used to measure steady-state flows through phosphate–adenosine 5′-triphosphate (ATP) exchange reactions in glucose-grown derepressed yeast. Our results have revealed that the reactions catalyzed by glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase (GAPDH/PGK) and by the mitochondrial ATPase contribute to the observed ST. Contributions from these reactions were evaluated by performing ST studies under various metabolic conditions in the presence and absence of either iodoacetate, a specific inhibitor of GAPDH, or the respiratory chain inhibitor antimycin A. Intracellular phosphate (P_i) longitudinal relaxation times were determined by performing inversion recovery experiments during steady-state ATP_γ saturation and were used in combination with ST data to determine P_i consumption rates. ^{13}C NMR and O_2 electrode measurements were also conducted to monitor changes in rates of glucose consumption and O_2 consumption, respectively, under the various metabolic conditions examined. Our results suggest that GAPDH/PGK-catalyzed P_i–ATP exchange is responsible for antimycin-resistant saturation transfer observed in anaerobic and aerobic glucose-fed yeast. Kinetics through GAPDH/PGK were found to depend on metabolic conditions. The coupled system appears to operate in a unidirectional manner during anaerobic glucose metabolism and bidirectionally when the cells are respiring on exogenously supplied ethanol. Additionally, mitochondrial ATPase activity appears to be responsible for the transfer observed in iodoacetate-treated aerobic cells supplied with either glucose or ethanol, with synthesis of ATP occurring unidirectionally.

^{31}P NMR[1] magnetization-transfer techniques have been used to measure steady-state kinetics of enzyme-catalyzed reactions in a variety of living systems [for reviews, see Alger and Shulman (1984) and Koretsky and Weiner (1984)]. In particular, saturation-transfer experiments performed by selectively saturating the γ-phosphate of ATP and monitoring the change in the intracellular phosphate intensity (P_i^{in}) have measured phosphate–ATP exchange reactions in vivo. In yeast (Alger et al., 1982; Campbell et al., 1985; Brindle & Krikler, 1985), the Langendorf perfused heart (Matthews et al., 1981; Kingsley-Hickman et al., 1986), the perfused and in situ rat kidney (Freeman et al., 1983), and the in situ rat brain (Shoubridge et al., 1982), rates of oxygen consumption were determined and combined with the ST-measured P_i consumption rates to determine P/O ratios. The P/O ratio is defined as the moles of inorganic phosphate incorporated into organic form per mole of oxygen consumed and is an index of oxidative phosphorylation. The P/O ratios for oxidation of NADH and succinate are 3 and 2, respectively. In all but a few cases, the net rate of ATP production calculated from oxygen consumption measurements (assuming P/O = 2–3) was found to be equal to the ST-measured unidirectional rate of ATP synthesis. These calculations are based on the assumption that a two-site exchange model of $P_i \rightleftarrows$ ATP ensues and the fractional change in the intracellular P_i intensity during steady-state ATP_γ saturation are interpreted as measuring the unidirectional rate of ATP synthesis. Results from these studies indicate that mitochondrial ATPase catalyzed ATP production occurs irreversibly. However, a P/O value greater than 3 was obtained in ST studies performed on aerobic yeast suspensions (Alger et al., 1982; Brindle & Krikler, 1985) and perfused heart (Kingsley-Hickman et al., 1986).

In an effort to explain the large yeast P/O values, we recently performed ST studies in the yeast *Saccharomyces cerevisiae* (Campbell et al., 1985). The work was performed on respiratory competent cells, which were grown to mid-log phase on a medium containing acetate as opposed to the original yeast study which was performed on glucose-grown stationary phase cells. A ST effect was observed only under aerobic conditions with P/O values close to 3 obtained in the presence of either glucose or ethanol. These results suggest that the mitochondrial ATPase is responsible for the observed transfer to intracellular P_i^{in} during steady-state saturation of ATP_γ. Moreover, a P/O value close to 3 was obtained in ST studies performed on perfused ethanol-grown yeast suspensions during aerobic glycolysis, when the measured P_i consumption rate was corrected for estimated glycolytic contributions (Brindle & Krikler, 1985). Thus, results obtained from these and previous yeast ST studies suggest a dependence on yeast

† This work was supported by National Institutes of Health Grant AM 27121.

* Address correspondence to this author at the Department of Biochemistry, Brandeis University, Waltham, MA 02254.

‡ Present address: Bureau for Science and Technology, Agency for International Development, Washington, DC 20523.

[1] Abbreviations: NMR, nuclear magnetic resonance; ATP, adenosine 5′-triphosphate; P_i, inorganic phosphate; ST, saturation transfer; NADH, reduced nicotinamide adenine dinucleotide; ADP, adenosine 5′-diphosphate; NOE, nuclear Overhauser effect; PME, phosphomonoester; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate.

0006-2960/87/0426-7483$01.50/0

growth and feeding conditions.

In the present paper, we have investigated the possibility that both glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase (GAPDH/PGK) and mitochondrial ATPase reactions contribute to the ST observed in yeast grown to stationary phase in the presence of glucose. It has been shown that cells grown in the presence of glucose show a 3–100-fold increase in the specific activity of various glycolytic enzymes, with the largest induction observed in GAPDH (Maitra & Lobo, 1971). Since the level of glycolytic enzymes may be much higher in glucose-grown stationary phase cells used in the present studies than in cells grown on acetate, a more important contribution from the GAPDH/PGK coupled system is expected. If this is the case, contributions from the reaction catalyzed by GAPDH/PGK may account for both the large P/O ratios previously observed and the ST-measured P_i consumption activity observed during anaerobic glycolysis (Alger et al., 1982) in glucose-grown derepressed cells.

Possible ST contributions from the GAPDH/PGK can be tested by inhibiting GAPDH. The GAPDH/PGK-catalyzed coupled reaction is

$$GAP + P_i + ADP + NAD^+ \rightleftarrows 3PG + NADH + ATP + H^+ \quad (1)$$

In this expression, GAP represents glyceraldehyde 3-phosphate and 3PG is 3-phosphoglycerate. Iodoacetate inhibits GAPDH; it interferes with the binding of NAD and substrate by carboxymethylation of one or more sulfhydryl group(s) at or near the active site of the enzyme (Webb, 1966). Saturation-transfer studies conducted in the presence and absence of this inhibitor should provide information about possible contributions from GAPDH/PGK-catalyzed P_i–ATP exchange.

We have also conducted ST studies in the presence of antimycin A, a specific inhibitor of electron chain site 2, complex III. It is believed that this inhibitor works by prohibiting electron flow between ubiquinol and ferricytochrome *c*. Dissociation of cytochrome *b* from cytochrome c_1 is also blocked (Erecinska & Wilson, 1981). Treatment of cells with antimycin A should inhibit electron transport derived generation of an electrochemical potential, thereby affecting the coupled production of ATP.

Antimycin A was chosen because it penetrates the yeast cell, its inhibition effect is specific in action, and it does not cause extensive deenergization (thus maintaining steady-state reaction concentrations). Direct ATPase inhibitors such as dicyclohexylcarbodiimide (DCCD) and oligomycin could not be used, as these inhibitors did not satisfy one or more of the above criteria necessary to perform ST experiments on intact cells. In particular, oligomycin had been used in a previous ST investigation on yeast (Alger et al., 1982) and had shown a reduced P_i consumption rate to 15% of the original value upon addition to endogenously respiring yeast. Hence it was concluded that the transfer observed in these cells was from ATPase activity. The mitochondrial ATPase was assumed to be in equilibrium since the P/O value of 87 determined from the ST measurements could be explained by very rapid P_i rates. However, we have found in separate experiments that the reduced P_i consumption rate previously observed in the presence of oligomycin can be explained by the presence of ethanol in which the oligomycin was dissolved. From our measurements of this system it appears that the ethanol increased the respiratory rate to the point where the O_2 tension was reduced below the level needed for normal respiration, giving lower P_i consumption rates. When oligomycin was supplied to endogenously respiring yeast at low ethanol concentrations, no change in either O_2 consumption or ST-determined P_i consumption rates was observed (data not shown). These results suggest that the mitochondrial ATPase was not inhibited by oligomycin in the previous yeast study (Alger et al., 1982), possibly because the inhibitor does not enter the intact cell.

In this paper, we have investigated the effect of either antimycin or iodoacetate on the transfer of magnetization from ATP_γ to P_i^{in} by ^{31}P NMR ST techniques in glucose-grown derepressed yeast under the following metabolic conditions: anaerobic glucose-fed cells, aerobic cells supplied with glucose, and aerobic cells supplied with ethanol. The ^{31}P ST studies were supplemented with both ^{13}C NMR measurements and O_2 electrode derived rates of oxygen consumption to determine glucose consumption and oxygen consumption rates, respectively, and to estimate net glycolytic and mitochondrial ATPase flows.

Experimental Procedures

Cell Preparation. The *Saccharomyces cerevisiae* strain NCYC 239 was grown aerobically for 24 h at 30 °C to stationary phase in a liquid medium containing 2% bactopeptone, 1% yeast extract, and 2% glucose. The cultures were cooled on ice and then harvested by low-speed centrifugation at 4 °C. The cells were washed twice in ice-cold buffer containing 5 mM KH_2PO_4, 0.86 mM K_2HPO_4, 2 mM $MgSO_4$, 1.7 mM NaCl, and 50 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) adjusted to pH 6.0 with NaOH. In ^{13}C NMR experiments, 50 mM $Na_2H_2P_2O_7$ was substituted for MES. Cell suspensions were prepared and examined by NMR at densities of 40% wet weight with the harvesting buffer.

NMR Experimental Conditions. Glucose (2 g) was added to 15 mL of a 40% cell suspension in a 20-mm NMR tube. A double-bubbler apparatus consisting of an upper bubbler positioned above the radio frequency (rf) coil and lower bubbler positioned at the bottom of the NMR tube was then inserted into the cell suspension and bubbled with either oxygen or nitrogen. Gas delivery rates corresponding to 1300 mL/min (upper bubbler) and 50 mL/min (lower bubbler) with 95% N_2/5% CO_2 were used for anaerobic experiments, and rates of 1700 mL/min (upper bubbler) and 170 mL/min (lower bubbler) with 95% O_2/5% CO_2 were used for aerobic experiments. For experiments using exogenously added ethanol, 15 mL of the cell suspension was pelleted and resuspended to the original volume in the buffer containing 3.3% ethanol. Gas delivery rates, which ensured O_2 saturation of the cell suspension during aerobic NMR experiments, were determined by monitoring the dissolved oxygen concentration using a Yellow Springs oxygen electrode in an apparatus similar in dimension to the NMR sample tube.

Iodoacetate and Antimycin A NMR Experiments. Iodoacetate was obtained from Sigma Chemical Co. Stock solutions (100 mM) were made up in the harvesting medium and titrated to pH 6.0 with NaOH. In ST NMR experiments with iodoacetate, 52.5 μL of the 100 mM stock solution was added to the cell suspension, a carbon source was added, and the suspension was then bubbled at the appropriate rates with 95% O_2/5% CO_2. ST experiments were initiated after a 30–45-min wait in the presence of iodoacetate to ensure adequate time for inhibition to occur.

Stock solutions (200 mM) of antimycin were made up in acetone. In NMR experiments with antimycin A, 7.5 μL of the antimycin stock solution was added to the NMR sample to obtain a final concentration of 100 μM, and NMR experiments were conducted as described above.

^{31}P ST and T_1 Experiments. ^{31}P NMR spectra were obtained at 145.78 MHz with a Bruker WH 360 NMR spec-

trometer. A frequency synthesizer driving an auxillary amplifier generated the rf field used for saturating the NTP_γ resonance in both T_1 and ST experiments. A 90° pulse and a 2.7-s relaxation delay was used for the ST experiments. Under these pulsing conditions, the intracellular phosphate resonance is fully relaxed. The rf field was positioned alternately between the NTP_γ resonance (M) and a point equidistant from the P_i^{in} resonance on its low-field side every 67 s (24 scans). Data were collected in files of 144 scans. Alteration of the rf field between control and ATP_γ positions helped to reduce errors in the spectra resulting from small variations in metabolite levels in time and from direct saturation of the P_i^{in} resonance. The saturating field was turned off during acquisition. T_1 apparent values were determined from the slope of recovery curves obtained from inversion recovery experiments performed in the presence of a saturating field at NTP_γ by using a 180°–t–90° pulse sequence and varying the t between 0.05 and 5 s. Slopes obtained from these recovery curves provide relaxation times in the presence of exchange (Mann, 1977).

A sealed capillary containing methyl phosphonate was attached to the center of the bubbling apparatus and was used as an external concentration standard. The methyl phosphonate signal was calibrated by comparison with the resonance areas of (1–20 mM) Na_2HPO_4 solutions. $GdCl_3$ (150 μM) was added to the methyl phosphonate solution to enhance relaxation so that the methyl phosphonate resonance was completely relaxed under the pulsing conditions used. The P_i^{in} concentration was calculated by cutting out and weighing both the methyl phosphonate and P_i^{in} peaks in the spectrum obtained with the irradiating field off and comparing the area of the P_i^{in} peak to the fully relaxed calibrated methyl phosphonate peak area. The apparent relaxation rate ($1/T_{1i,app}$) was determined from the slope of an inversion recovery experiment performed during steady-state saturation of NTP_γ. To convert relative to absolute concentrations it was assumed that 1.67 g of wet yeast contains 1 mL of cell water (Gancedo & Gancedo, 1973). Rates of P_i consumption were determined by multiplying the fractional change in P_i^{in} (resulting from ATP_γ saturation) by the measured apparent T_{1i} relaxation rate and the intracellular phosphate concentration.

^{31}P Acid Extracts. The cell suspension was prepared to mimic NMR conditions corresponding to the physiological state of interest. Fifteen milliliters of a 40% yeast suspension was bubbled in the presence of a carbon source for 30–45 min at oxygen-saturated bubbling rates. For inhibitor studies, either iodoacetate or antimycin was added to the cells prior to oxygenation. All 15 mL of the cells was immediately acid extracted according to den Hollander et al. (1986b). Three milliliters of the acid extract was placed in a 10-mm NMR tube, and the fully relaxed ^{31}P spectra were acquired with a pulse interval of 7.5 s and a flip angle of 45°. A decoupling power of 2 W was used only during acquisition to decouple protons and to avoid Overhauser enhancement. A 100 mM methyl phosphonate solution (150 μL) was added to the cell suspension before extraction and its phosphorus resonance used as an internal concentration standard in the ^{31}P spectra.

^{13}C NMR Glucose Consumption Measurements. ^{13}C NMR spectra were acquired with a Bruker WH 360 WB spectrometer operating at 90.55 MHz. Experiments were performed by supplying 2.0 g of glucose to 15 mL of a 40% cell suspension, bubbling with either oxygen or nitrogen, and then monitoring the natural abundance glucose signal as a function of time. Experiments were also performed by adding [1-^{13}C]glucose to 15 mL of a 10% cell suspension and then monitoring end products of glucose catabolism by ^{13}C NMR. Spectra were obtained with a 45° pulse and a 2-s repetition rate using the MLEV pulse sequence (Levitt et al., 1983) to decouple protons. Gated decoupling was employed to avoid NOE enhancement using a decoupling power of 2 W. Glucose utilization rates were determined from the slope of a graph of combined $C_{1\alpha}$ and $C_{1\beta}$ glucose signals versus time. Glucose concentrations were determined by extrapolation of the slope to zero time. Rates of glycerol production were determined by monitoring the glycerol signal as a function of time.

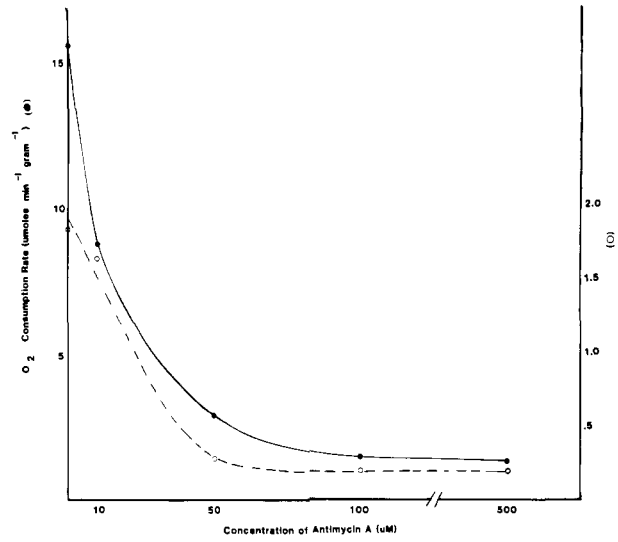

FIGURE 1: Dose–response curve: effect of antimycin on oxygen comsumption rates. Oxygen consumption rates were measured for glucose-grown derepressed stationary phase yeast respiring on glucose (●) or ethanol (○) in the presence of increasing concentrations of antimycin as described under Experimental Procedures.

Oxygen Consumption Measurements. Oxygen consumption rates were measured by using a Yellow Spring oxygen electrode contained in a Model 53 Yellow Spring bath stirrer assembly unit at 25 °C. Aliquots were taken from NMR samples throughout the NMR experiment, diluted to 1% in oxygenated buffer, and oxygenated further for 10 min before the measurement was conducted. Rates were determined from the slope of a plot of O_2 concentration versus time. Measurements of oxygen consumption were also performed on the bench under conditions that simulated NMR experiments.

RESULTS

Antimycin Inhibition Studies. The effect of increasing antimycin A concentration on the rate at which the cells consume oxygen is shown in Figure 1. In aerobic cells supplied with either glucose or ethanol, maximal inhibition in O_2 consumption to 10% of normal occurs at antimycin concentrations of 100 μM or higher.

Due to the insolubility of antimycin A in aqueous solutions, it was necessary to make up antimycin stock solutions in acetone. The presence of acetone in the cell suspension showed no significant effect on oxygen consumption rates or ST results. In addition, plating studies have shown that cell viability was unaffected by either acetone or 100 μM antimycin (data not shown).

Antimycin Saturation-Transfer Experiments. Figure 2 presents saturation-transfer spectra of yeast treated with acetone or antimycin during aerobic metabolism of ethanol. The M^0 spectra (left panel) were obtained with the saturating frequency at the control position (indicated by the arrow). The spectral assignments are similar to those described by Alger et al. (1982) as identified previously (Salhany et al., 1975;

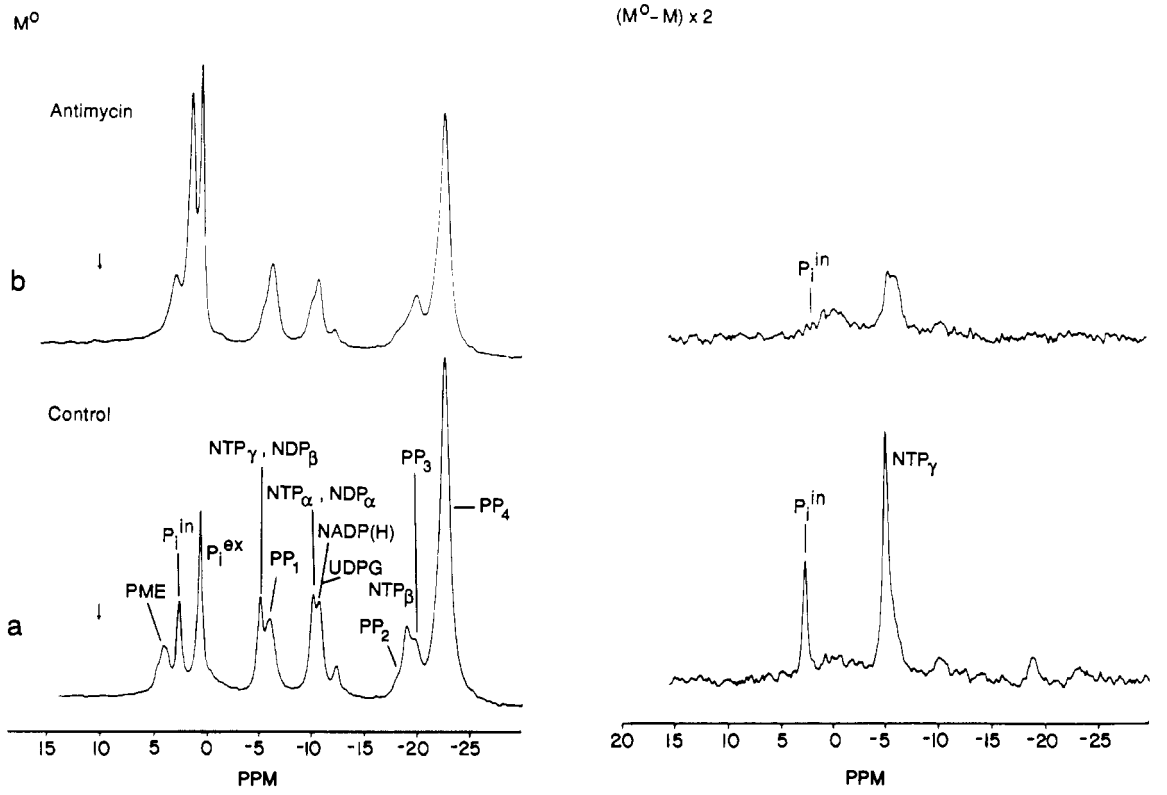

FIGURE 2: Effect of antimycin on aerobic ethanol-fed cells. Cells were grown in the presence of glucose and harvested at stationary phase. The lower trace shows control M^0 and ΔM spectra, whereas the upper trace shows spectra obtained from antimycin-treated cells. Spectral abbreviations are as follows: PME, phosphomonoester; P_i^{in}, intracellular phosphate; P_i^{other}, predominantely inorganic orthophosphate located in vacuole and extracellular medium; NDP_α and NTP_α, α-phosphates of nucleoside di- and triphosphates; NDP_β and NTP_β, β-phosphates of nucleoside di- and triphosphates; PP_1, terminal phosphate of polyphosphate; NTP_γ, γ-phosphate of nucleoside triphosphate; NADP(H), oxidized and reduced form of nicotinamide adenine dinucleotide; UDPG, uridine diphosphoglucose; PP_2 and PP_3, penultimate phosphates of polyphosphate. The NMR data acquisition parameters and sample conditions are described in the text.

Table I: Antimycin Inhibition: Summary of Results

condition	P_i^{in} concn (mM)	$\Delta M/M^{0a}$	T_{1iapp} (s)	P_i consumption rate[b] [μmol s^{-1} (g of wet cells)$^{-1}$]	pH
anaerobic glucose	3.9	0.16 ± 0.02	0.70 ± 0.05	0.53 ± 0.13	7.13
anaerobic glucose (+antimycin)	3.9	0.16 ± 0.02	0.70 ± 0.05	0.53 ± 0.13	7.13
aerobic glucose	1.7	0.52 ± 0.03	0.30 ± 0.04	1.8 ± 0.5	7.17
aerobic glucose (+antimycin)	4.0	0.16 ± 0.02	0.70 ± 0.05	0.55 ± 0.14	7.17
aerobic ethanol	4.7	0.53 ± 0.03	0.50 ± 0.05	3.0 ± 0.7	7.19
aerobic ethanol (+antimycin)	15.0	c	0.70 ± 0.05		6.56

[a] Standard deviation from the mean (n = 3, 4). [b] Error determined according to Alger and Shulman (1984). [c] $\Delta M/M^0$ unquantitable.

Navon et al., 1979; den Hollander et al., 1981). The effects of antimycin were observed immediately upon its addition to the cell suspension, and a new steady state was reached within 20 min, at which time the ST measurements were conducted.

As shown in Figure 2, addition of antimycin to yeast respiring on ethanol dramatically affects the cells. There is a large increase in the intensity as well as an upfield shift (more acidic) in the intracellular P_i peak and a drop in both polyphosphate and ATP levels relative to the control spectrum. The $M-$ spectrum (ATP_γ saturated, not shown) was taken during steady-state saturation of the NTP_γ (predominantly ATP_γ) resonance and was subtracted from the M^0 spectrum to obtain the difference spectrum $(M^0 - M) = \Delta M$. Comparison of M^0 and difference spectra of the control cells in Figure 2 shows a large transfer to P_i^{in} with the P_i consumption rate (V_{ST}) equal to 3.0 ± 0.7 μmol s^{-1} (g of wet cells)$^{-1}$. The P_i consumption rate was determined as described under Experimental Procedures. However, in the presence of antimycin, no detectable transfer occurs. ST data are listed in Table I.

The PP_1 peak also experiences direct saturation, as it is clearly resonant with the saturating rf field. Polyphosphates are localized in the yeast vacuole (Durr et al., 1979). We would not expect partial saturation of the PP_1 resonance to transfer magnetization to intracellular phosphate (resulting from polyphosphate synthesis), since the time constant for P_i transport between vacuolar and cytosolic compartments is much slower than the P_i spin–lattice relaxation time (Gillies et al., 1981; den Hollander et al., 1981). To test this, ST experiments were performed in which the rf frequency was varied [similar to experiments conducted by Koretsky et al. (1984)] from −5 to −7 ppm. The $(\Delta M/M^0)_i$ was reproduced only when the rf field was centered on the ATP_γ resonance at 5.0 ppm and not on the PP_1 peak at 6.0 ppm. A much smaller magnetization-transfer effect was observed when the saturating rf field was positioned predominately on the PP_1 resonance. Results from these experiments indicate that P_i–ATP and not P_i–PP_1 exchange reactions are responsible for the P_i^{in} difference peak.

The difference peaks at the NTP_β and NTP_α regions have been described previously (Alger et al., 1982). The excursion at the PME resonance is a result of glucose 6-phosphate and fructose 6-phosphate exchange reactions with ATP and has

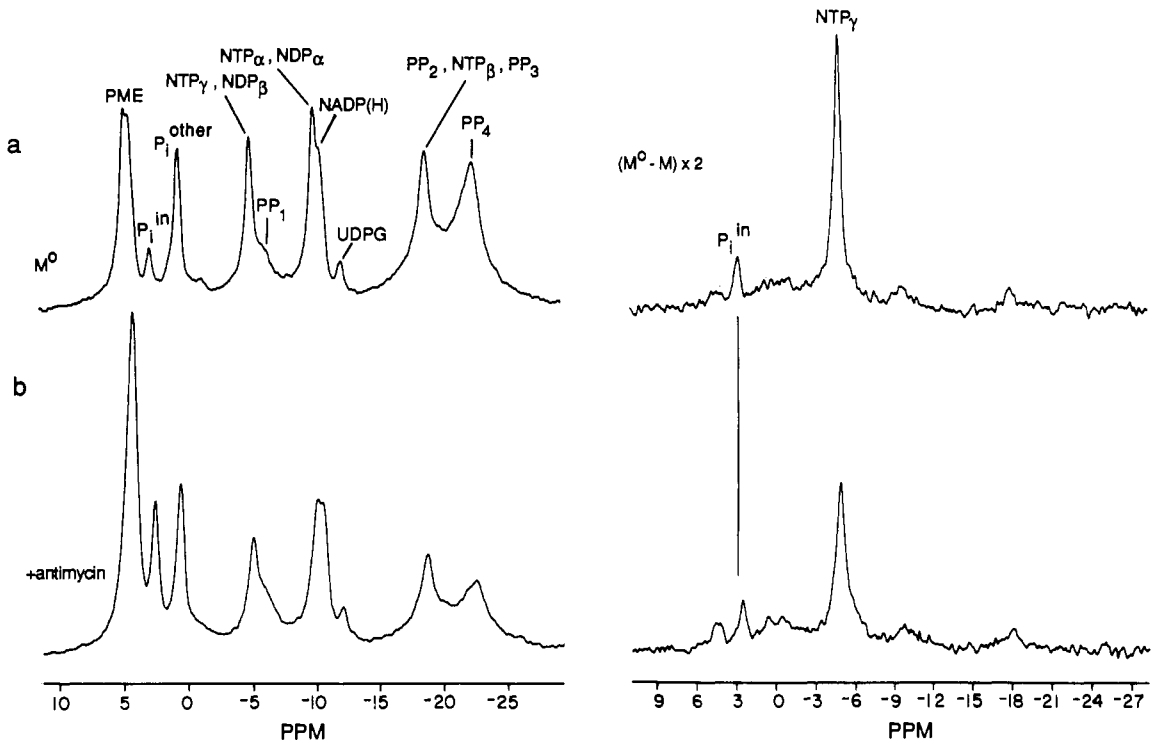

FIGURE 3: Effect of antimycin on aerobic glucose-fed cells. Only the M^0 and ΔM spectra are shown. The control data are presented in the bottom spectra. Top spectra show data obtained from a separate sample to which 100 μM antimycin was added.

Table II: Effect of Antimycin on Glucose Consumption Rate

condition	slope	R value	rate [μmol min^{-1} (g of wet cells)$^{-1}$]
aerobic (0.05% acetone)	-0.072 ± 0.008	0.97	8.1
aerobic (100 μM antimycin)	-0.090 ± 0.011	0.97	12.8

been observed only in glucose-grown stationary phase cells during anaerobic and aerobic glucose metabolism (Campbell-Burk et al., 1987). A difference signal is also observed at ~0 ppm and may result from direct saturation of the P_i^{other} peak due to its proximity to the NTP^γ resonance. It is also possible that magnetization transfer to the P_i^{other} peak occurs upon saturation of ATP_γ since the P_i^{other} peak not only is composed of extracellular and vacuolar P_i but also contains glycerophosphorylcholine and phosphoethanolamine.

In parts a and b of Figure 3, the saturation-transfer spectra of aerobic glucose-fed cells in the presence and absence of antimycin are shown. As is apparent from the M^0 spectrum of antimycin-treated cells, there is an increase in the intensity of the PME and the P_i^{in} peaks and a drop in the ATP resonances relative to the control. The changes in ^{31}P spectra upon treatment with antimycin are similar to those observed when yeast cells are switched from aerobic to anaerobic conditions (den Hollander et al., 1981, 1986a). Moreover, the glucose consumption rate measured in the presence of antimycin A was found to be 1.6 times greater than the rate determined in the absence of the inhibitor (Table II). The P_i consumption activity observed in ST experiments conducted on aerobic cells supplied with glucose has been quantitated and a P_i consumption rate of 1.8 ± 0.5 μmol s^{-1} (g of wet cells)$^{-1}$ determined. In contrast to the results obtained in aerobic cells supplied with ethanol, a transfer to P_i^{in} was observed in aerobic glucose-fed cells treated with antimycin, as shown by the difference spectrum in Figure 3b. ST data obtained from antimycin-treated aerobic-fed cells was quantitated to give a P_i consumption rate of 0.55 ± 0.14 μmol s^{-1} (g of wet cells)$^{-1}$, which is one-third the control rate.

Anaerobic glucose-fed cells do not appear to be affected by this inhibitor. Neither the ST data (Table I) nor the ^{31}P NMR spectrum of antimycin-treated anaerobic glucose-fed cells differs from the control.

Iodoacetate Inhibition Studies. Iodoacetate has been shown to be a specific inhibitor of GAPDH at concentrations below 1 mM in several intact systems (Webb, 1966). The concentration of iodoacetate that produces a significant yet selective inhibition of GAPDH was determined by conducting ^{31}P NMR and O_2 consumption measurements on cell suspensions treated with iodoacetate at concentrations ranging from 0.05 to 1 mM.

Our initial studies were conducted on anaerobic cells supplied with glucose. A block at GAPDH under these conditions will significantly affect glycolytic energy production, and this perturbation should be evident in the levels of phosphate metabolites. In these experiments, a marked change in the ^{31}P NMR spectrum, consistent with that expected from GAPDH inhibition (Webb, 1966), was observed within 15 min after the addition of 0.25 mM iodoacetate to anaerobic glucose-fed cells. The cells did not survive incubation periods of greater than 40 min. Consistent with these results, at 0.25 mM iodoacetate, Stickland (1956a) observed glycolytic inhibition corresponding to 88% in anaerobic glucose-fed yeast suspensions.

Succinate dehydrogenase and alcohol dehydrogenase appear to be susceptible to iodoacetate at higher concentrations than required for GAPDH inhibition (Webb, 1966). Both of these enzymes are necessary for efficient oxidation of ethanol. In aerobic ethanol-fed cells, no significant change in the O_2 consumption rate was observed at 0.35 mM iodoacetate. However, at iodoacetate concentrations greater than 0.5 mM, the rate of oxygen consumption decreases significantly, indicating that iodoacetate no longer appears to be selective for GAPDH. We have chosen to use 0.35 mM iodoacetate in our ST experiments because this concentration appears to both selectively and significantly inhibit GAPDH.

Iodoacetate Saturation-Transfer Studies. ST spectra of

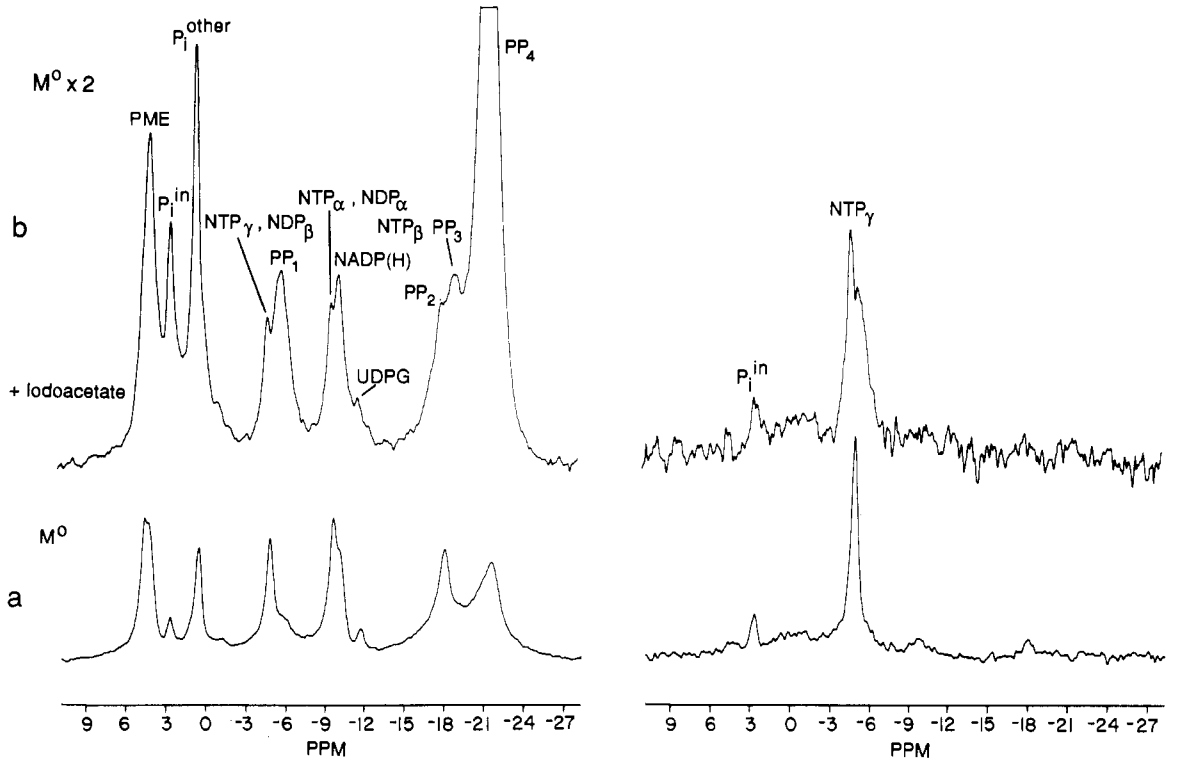

FIGURE 4: Effect of iodoacetate upon saturation-transfer spectra of aerobic glucose-fed cells. Only the M^0 and ΔM spectra are shown. Control data are shown in (a), and (b) shows the effect of 0.35 mM iodoacetate. M^0 and ΔM spectra were acquired by using a 90° pulse and a pulse interval of 2.4 s. Control spectra were collected alternately in eight blocks of 144 FIDs. Spectra of iodoacetate-treated cells were collected in four blocks of 144 FIDs as an incubation period of 45 min was allowed before ST experiments were initiated. Note that the gain in M^0 and ΔM spectra of iodoacetate-treated cells is 2-fold greater than in control cells. Sample conditions are described under Experimental Procedures.

Table III: Phosphomonoester Metabolite Concentrations (mM)

metabolite	aerobic glucose control	aerobic glucose + iodoacetate
fructose 1,6-bisphosphate	3.6	13
fructose 6-phosphate[a]	0.6	2.4
glucose 6-phosphate	1.8	7.2
α-glycerol phosphate	1.0	8.5

[a] Derived from glucose 6-phosphate concentration by assuming glucose-6-phosphate isomerase is in equilibrium (Johnson, 1960).

control and iodoacetate-treated aerobic cells supplied with glucose are shown in Figure 4, with the M^0 spectra depicted in the left panel. The M^0 spectrum taken in the presence of the inhibitor reveals a large increase in the intensity of the PME, P_i^{in}, and polyphosphate peaks (PP_{1-4}) and a decrease in the NTP_γ resonance relative to the control spectrum. We have identified and quantitated the metabolites that comprise the PME peak in acid extract spectra. As shown in Table III, the concentrations of fructose 1,6-bisphosphate, α-glycerol phosphate, and glucose 6-phosphate increase by 4–8-fold in the presence of iodoacetate. Moreover, the rate of oxygen consumption in these iodoacetate-treated cells is approximately half the rate measured in the absence of the inhibitor. The difference spectra $[(M^0 - M) \times 2]$ are shown in the right panel of Figure 4. As shown in Table IV, the P_i^{in} difference peak in the control spectrum yields a $\Delta M/M^0$ of 0.52 ± 0.03 (m = 4) and a P_i consumption rate of 1.8 ± 0.5 μmol s^{-1} (g of wet cells)$^{-1}$. Addition of iodoacetate to these cells significantly reduces the fractional change in the P_i^{in} peak (Figure 4b, right panel). The value of ΔM is about the same while the M^0 is much larger so that the $(\Delta M/M^0)_i$ value is approximately one-fifth the control value. The rate of P_i consumption in the presence of iodoacetate has been determined (Table IV) and used in combination with the rate of oxygen consumption to obtain a P/O value of 2.7 ± 0.7. This value is not significantly different from the value of 3.5 ± 1.0 determined in the absence of the inhibitor.

Table IV: Iodoacetate Inhibition: Summary of Results

condition	P_i^{in} concn (mM)	$\Delta M/M^0$	T_{1app} (s)	P_i consumption rate [μmol s^{-1} (g of wet cells)$^{-1}$]	O_2 consumption rate [μmol s^{-1} (g of wet cells)$^{-1}$]	P/O	V_2 [μmol s^{-1} (g of wet cells)$^{-1}$]
anaerobic glucose	3.9	0.16 ± 0.02	0.70 ± 0.05	0.53 ± 0.13			0.43 ± 0.02
aerobic glucose	1.7	0.52 ± 0.02	0.30 ± 0.03	1.8 ± 0.5	0.26	3.5 ± 1.0	
aerobic glucose (+iodoacetate)	5.4	0.10 ± 0.02	0.50 ± 0.02	0.65 ± 0.16	0.12	2.7 ± 0.7	
aerobic ethanol	4.7	0.53 ± 0.03	0.50 ± 0.08	3.0 ± 0.7	0.14	11 ± 2.6	2.2 ± 0.7
aerobic ethanol (+iodoacetate)	5.5	0.10 ± 0.02	0.50 ± 0.02	0.66 ± 0.16	0.13	2.5 ± 0.6	

[a] Standard deviation from the mean (n = 3, 4). [b] Error estimated as described in the text. [c] $\Delta M/M^0{}_i$ unquantitatable.

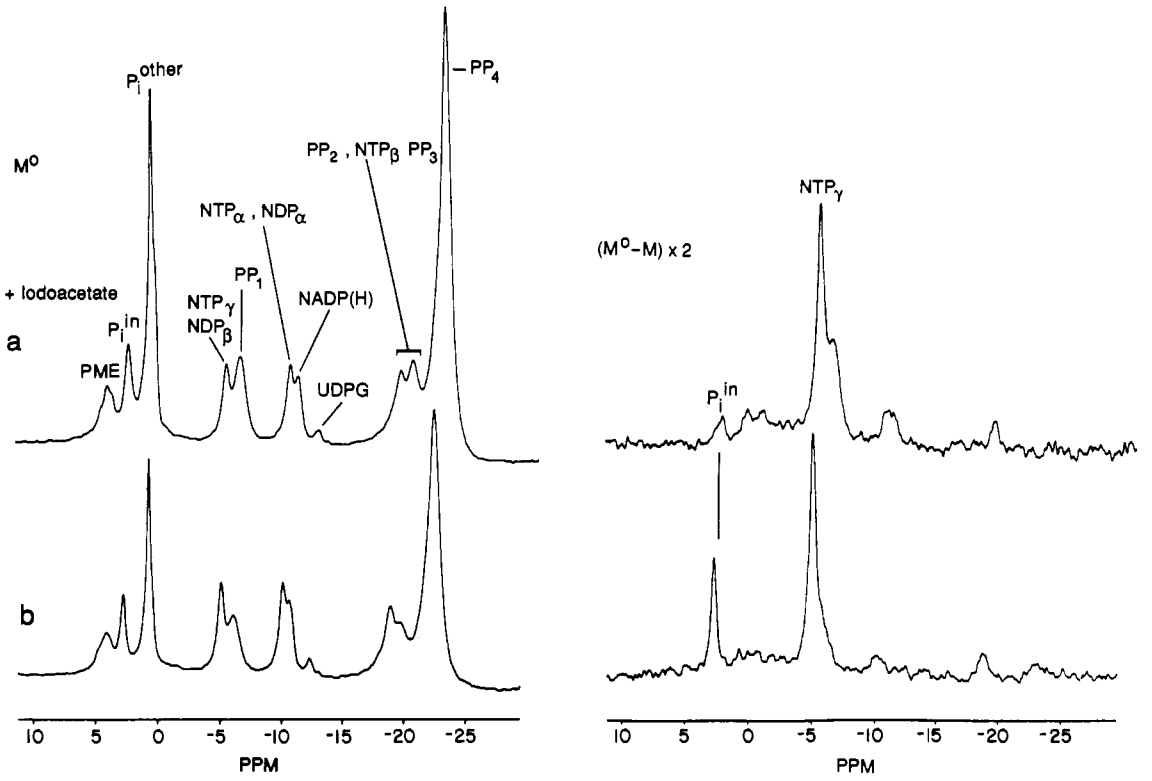

FIGURE 5: Effect of iodoacetate upon saturation-transfer spectra of aerobic ethanol-fed cells. The M^0 and difference spectra ($M_i^0 - M_i$) of control cells (a) and iodoacetate-treated cells (b) are shown.

ST studies with and without iodoacetate were also conducted in aerobic cells supplied with ethanol as shown in Figure 5. The M^0 spectrum of iodoacetate-treated cells in Figure 5a (left panel) contains higher levels of polyphosphates and P_i^{other} relative to the control spectrum but approximately the same levels of ATP and P_i^{in}. The rate of oxygen consumption in iodoacetate-treated cells does not differ appreciably from the control rates (see Table IV), indicating that respiration of ethanol does not appear to be affected by the inhibitor. Moreover, neither the ST-measured P_i consumption activity nor the rate at which the cells consume oxygen varied during NMR experiments performed on aerobic ethanol-fed cell suspensions treated with 0.35 mM iodoacetate, indicating that the extent of inhibition was not changing over the time frame in which the NMR experiments were conducted. The difference spectra are shown in the right panel of Figure 5. The large transfer to P_i observed in the control difference spectrum has been quantitated to give a P_i consumption rate of 3.0 ± 0.7 μmol s^{-1} (g of wet cells)$^{-1}$ and a P/O ratio of 11 ± 2.6 with the data listed in Table IV. Comparison of difference spectra obtained in the presence and absence of iodoacetate shows a significant fraction ($^4/_5$) of the ST appears to be sensitive to iodoacetate. The reduction in $\Delta M/M^0_i$ gives a P_i consumption rate and a P/O value that is approximately 5-fold lower than the control.

Discussion

Results obtained from our ^{31}P and ^{13}C NMR measurements of anaerobic glucose-fed cells suggest that cell metabolism is crippled by the presence of the glycolytic inhibitor iodoacetate but appears to be unaffected by the presence of antimycin. These results are not unexpected since anaerobic glucose-fed cells rely mainly on the glycolytic pathway to generate ATP necessary for cellular energy needs. This pathway is blocked by iodoacetate but appears to be unaffected by antimycin. A magnetization-transfer effect was previously observed in anaerobic glucose-fed cells (Alger et al., 1982). The investigators suggested that glyceraldehyde-3-phosphate dehydrogenase activity may be responsible for the observed magnetization transfer, since the ST-determined P_i consumption rate was similar to the estimated triose phosphate glycolytic flux. Consistent with this notion, our studies have shown that the ST-determined P_i consumption rate measured in anaerobic glucose-fed cells is insensitive to antimycin.

The two major energy-generating routes available to aerobic glucose-fed cells are the glycolytic pathway and respiration via the tricarboxylic acid cycle and electron-transport chain. Thus, when electron transport is inhibited with antimycin A, these cells possess an alternative ATP-generating pathway, i.e., the glycolytic pathway. Results obtained from studies conducted on antimycin-treated aerobic glucose-fed cells indicate an increased glycolytic rate relative to control cells, with changes in phosphorus metabolite levels and the rate of glucose consumption similar to that obtained when the cells are switched from aerobic to anaerobic conditions (Navon et al., 1981). In addition, a decrease in the ST-measured P_i consumption rate was observed in antimycin-treated aerobic glucose-fed cells with the rate equal to that observed under anaerobic conditions. Therefore, it seems likely that GAPDH/PGK and not the mitochondrial ATPase is responsible for the antimycin-resistant P_i consumption activity. ^{31}P NMR spectra of iodoacetate-treated aerobic glucose-fed cells showed significant perturbation in the levels of several phosphorus-containing metabolites and decreased respiration rates. In particular, a significant increase in the levels of glycolytic intermediates located above the block at GAPDH (i.e., F6P, G6P, Fru-1,6P_2) occurred. The accumulation of these metabolites was observed previously in iodoacetate-treated yeast suspensions (Webb, 1966) and conforms to the pattern expected if GAPDH is inhibited. Assuming no transfer occurs as a result of incomplete inhibition of GAPDH, residual respiration during glycolytic inhibition most likely occurs via the phosphogluconate pathway or respiration of endogenous substrates. The lower rates of P_i consumption and O_2 con-

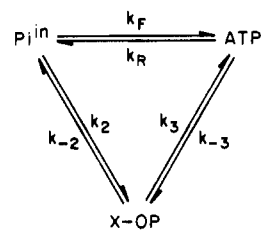

FIGURE 6: Kinetic scheme for the analysis of ST data.

sumption give a P/O value of 3.5 ± 1.0. Our measured P/O value of ~3 indicates that the mitochondrial ATPase is most likely responsible for the residual transfer observed in aerobic glucose-fed cells when glycolytic contributions have been eliminated.

Antimycin A perturbs metabolism and phosphate kinetics in glucose-grown derepressed cells respiring on ethanol. The increase in the intensity of the P_i^{in} resonance and drop in ATP levels are indicative of a reduced phosphorylation potential. Intracellular acidification also occurs as evidenced by an upfield shift in the P_i^{in} resonance. In addition, a ST effect was not observed in antimycin-treated aerobic glucose-fed cells in contrast to the large transfer (0.53 ± 0.03) observed in the absence of antimycin. On the other hand, ^{31}P spectra of iodoacetate-treated aerobic ethanol-fed cells differ only in polyphosphate and P_i^{other} levels with rates of O_2 consumption approximately equal to control values. However, the amount of transfer in ST experiments decreases appreciably, giving rise to a corresponding decrease in the P/O value from 11 ± 2.6 to 2.5 ± 0.6. Again, as in the case with aerobic glucose-fed cells, P/O ratios of ~3 were obtained from aerobic ethanol-fed cells when contributions from GAPDH/PGK-catalyzed P_i–ATP exchange are eliminated with iodoacetate. These results suggest that the mitochondrial ATPase is responsible for the residual iodoacetate-resistant P_i consumption activity.

Our measured P_i consumption rate (V_{ST}) appears to be a weighted average of both mitochondrial ATPase and GAPDH/PGK contributions. We have used the kinetic scheme shown in Figure 6, similar to those described by Alger et al. (1982) and Brindle and Krikler (1985) for ST data analysis, in order to evaluate the relative fluxes associated with these exchange reactions: $V_F = k_F$ [P_i], unidirectional rate of ATP synthesis from P_i^{in} catalyzed by ATPase reactions; $V_R = k_R$ [ATP], unidirectional rate of ATP hydrolysis to P_i^{in} catalyzed by ATPase reactions; $V_2 = k_2$ [P_i], unidirectional rate of P_i^{in} consumption by reactions other than ATPase; $V_{-2} = k_{-2}$ [X–OP], unidirectional rate of appearance of P_i^{in} from phosphorylated compounds other than ATP; $V_3 = k_3$ [X–OP], unidirectional rate of ATP synthesis from phosphorylated compounds; $V_{-3} = k_{-3}$ [ATP], unidirectional rate of ATP hydrolysis by reactions other than ATPase.

Our results indicate that V_2 and V_{-2} correspond to forward and reverse GAPDH-catalyzed flows since a significant fraction of the saturation transfer was sensitive to iodoacetate in these studies. Coupled production of ATP from P_i by GAPDH/PGK occurs via 1,3-diphosphoglycerate (X–OP) by V_2 and V_3 routes. In addition to PGK, reactions such as pyruvate kinase may also contribute to the V_3 term, whereas reversal of the PGK-catalyzed reaction and other kinases such as hexokinase and phosphofructokinase contribute to V_{-3}. We have not considered contributions from phosphatase reactions such as fructose 1,6-bisphosphatase to V_{-2} in these cells because no ST to other phosphate compounds was observed in experiments with P_i saturated, in agreement with earlier observations by Alger et al. (1982). The rate of P_i production by fructose 1,6-bisphosphatase measured in respiratory competent aerobic cells supplied with either ethanol or glucose (den Hollander et al., 1986a,b; Maitra & Lobo, 1978) is too slow relative to the P_i^{in} relaxation rate to contribute significantly to the ST. The mitochondrial ATPase also appears to contribute to the observed rate and is designated V_F in the kinetic scheme of Figure 6. Our measurements of the P_i consumption rate when glycolytic contributions have been eliminated in cells supplied with either ethanol or glucose indicate that synthesis of ATP via the mitochondrial ATPase is irreversible (P/O values = 2–3). Recent results obtained from ST studies performed on acetate-grown cells are consistent with this notion (Campbell et al., 1985). Direct hydrolysis of ATP to P_i (V_R in the kinetic scheme) most likely occurs via the plasma membrane ATPase. The plasma membrane ATPase is a proton pump that closely resembles the Na–K-ATPase found in mammalian systems (Pena, 1976). This proton pump is believed to be responsible for maintenance of cytoplasmic pH and facilitation of transport processes in the cell (Willsky, 1979; Poole, 1978). It has been speculated that transport via the plasma membrane ATPase is the major ATP-utilizing system in yeast, since growing yeast produce more ATP than needed for biosynthetic purposes (Serrano, 1978).

The Bloch equation corresponding to the spin magnetization of inorganic phosphate (M_i) due to the exchange processes shown in the kinetic scheme is

$$dM_i/dt = M_i^0 - M_i/T_{1i} - k_2M_i + k_{-2}M_x - k_FM_i + k_RM_y \quad (2)$$

In this equation, M_y and M_x correspond to the spin magnetizations of ATP and X–OP, respectively, $1/T_{1i}$ is the spin–lattice relaxation rate of M_i in the absence of exchange, and M_i^0 is the equilibrium magnetization of P_i. The steady-state saturation-transfer experiment is conducted by selectively saturating the ATP_γ resonance with a rf field. Assuming both mitochondrial and cytosolic pools of ATP are saturated in our experiments, $k_RM_y = 0$. The reduction in M_i is then measured after steady state is reached. Equation 2 was solved for the fractional reduction in the magnetization of P_i^{in}, $(M_i^0 - M_i)/M_i^0 = \Delta M_i/M_i^0$.

$$\Delta M_i/M_i^0 = [(k_F + k_2)/(1/T_{1i} + k_F + k_2)] - [k_{-2}M_x/(1/T_{1i} + k_F + k_2)(M_i^0)] \quad (3)$$

Rearrangement of this expression gives

$$\Delta M_i/M_i^0(1/T_{1i} + k_F + k_2)(M_i^0) = (k_F + k_2)(M_i^0) - k_{-2}M_x \quad (4)$$

P_i consumption rates (V_{ST}) were determined as described under Experimental Procedures. In these calculations, we have initially assumed that the flow through GAPDH/PGK is unidirectional (i.e., $k_{-2} = 0$), thereby simplifying the above expression to

$$V_{ST} = \Delta M_i/M_i^0(1/T_{1iapp})(M_i^0) = (k_F + k_2)(M_i^0) \quad (5)$$

where the measured apparent longitudinal relaxation rate ($1/T_{1iapp}$) has been substituted for ($1/T_1 + k_F + k_2$). The $\Delta M/M_i^0$ values and P_i^{in} concentration were calculated by using the total observable magnetization, since P_i transport across the mitochondrial membrane is believed to be faster than ATPase-catalyzed production of ATP so that P_i contained in the cytosol and mitochondrial P_i constitute one exchangeable pool (Alger et al., 1982; Brindle & Krikler, 1985). Rearranging and using the definitions $V_F = k_FM_i^0$ and $V_2 = k_2M_i^0$, we have

$$V_{ST} = V_F + V_2 \quad (6)$$

The net rate of mitochondrial ATP synthesis was estimated by measuring rates of oxygen consumption (Table III) and assuming a value of 3 for the P/O ratio. We have substituted these values for V_F in eq 6. The forward unidirectional flow through GAPDH/PGK (V_2) was then determined by subtracting V_F from V_{ST}. We have evaluated these fluxes (V_2) for aerobic ethanol and glucose-fed cells. The values are listed in Table IV.

We have also estimated the net anaerobic glycolytic flow through the trioses in order to compare these rates to our anaerobic ST-derived GAPDH/PGK flow. This rate was estimated by subtracting the fraction of glucose consumed in nonglycolytic pathways from the overall glucose utilization rate (den Hollander et al., 1986). These nonglycolytic flows include trehalose and glycogen biosynthetic pathways, formation of glycerol, and the pentose phosphate shunt. The overall anaerobic glucose utilization rate was measured by monitoring the time course of glucose disappearance by ^{13}C NMR and determined to be 0.27 ± 0.01 μmol s^{-1} (g of wet cells)$^{-1}$. In addition, the fraction of glucose used for the synthesis of trehalose and glycogen in glucose-grown stationary phase cells has been measured previously in this laboratory to be 10.5% (den Hollander et al., 1986) in anaerobic glucose-fed cells. Our ^{13}C NMR measurements have also provided rates of glycerol production, which accounts for approximately 8% of the total amount of glucose consumed under anaerobic conditions. The fraction of glucose consumed by the phosphogluconate pathway under anaerobic conditions has been estimated previously by den Hollander et al. (1986a) and found to be approximately 8% in glucose repressed cells and 29% in acetate-grown derepressed cells. For glucose-grown derepressed cells, we would expect that the fraction of glucose catabolized by the phosphogluconate pathway is between 8% and 29% (den Hollander et al., 1981). However, its overall contribution is limited (with a lower limit of 2.7% and an upper limit of 9.7%) because the pentose phosphates are expected to be predominately channeled back to the Embden–Meyerhof–Parnas pathway under the conditions examined in this study (Lagunas & Gancedo, 1973; den Hollander et al., 1986a). So, for every three hexose phosphate molecules entering the phosphogluconate pathway, two molecules will be resynthesized from pentose phosphates. The fact that ethanol and glycerol fermentative end products account for virtually all of the label initially present in glucose lends support for these assumptions. After the glucose utilization rate was corrected for glucose consumption by nonglycolytic paths, the net glycolytic flow was determined to have a lower limit of 0.20 ± 0.02 μmol s^{-1} (g of wet cells^{-1} and an upper limit of 0.22 ± 0.02 μmol s^{-1} (g of wet cells)$^{-1}$. The net anaerobic glycolytic flow through the trioses is equal to twice this rate. As shown in Table IV, the unidirectional flow through GAPDH/PGK (V_2) was determined to be 0.53 ± 0.13 μmol s^{-1} (g of wet cells)$^{-1}$ for anaerobically fermenting cells and is similar to our estimated anaerobic net triose phosphate glycolytic flux. Thus, the results are consistent with unidirectional operation of GAPDH/PGK during anaerobic glycolysis. Estimates of GAPDH/PGK reactant and product concentrations in anaerobically fermenting yeast suggest that at pH 7 the reaction strongly favors glyceraldehyde 3-phosphate and provides further support for our interpretation of ST results (Johnson, 1960).

The estimated triose phosphate glycolytic flow under aerobic conditions is approximately one-eighth of V_F (following paper). Thus, if the flow through GAPDH/PGK is unidirectional, any contribution from glycolytic exchange reactions is small relative to V_F in aerobic glucose-fed cells. Consistent with this notion, the estimated rate of P_i consumption via the mitochondrial ATPase in aerobic glucose-fed cells appears to account for the ST-measured P_i consumption rate ($V_F \sim V_{ST}$), indicating that contributions from V_2 are small, if present, and cannot be assessed from these measurements.

In the case of aerobic cells supplied with ethanol, V_2 has been determined to be 2.2 ± 0.7 μmol s^{-1} (g of wet cells)$^{-1}$ which is ~4-fold higher than the value determined during anaerobic glucose metabolism. During the oxidation of ethanol, acetyl-CoA is formed. Further oxidation generally occurs by either the tricarboxylic acid cycle (TCA) or the glyoxylate cycle (Strathern et al., 1982). However, a high rate of glycolytic reversal from endogenous phosphoenolpyruvate to hexose monophosphates was measured directly by Maitra and Lobo (1978) in suspensions of respiratory competent *S. cerevisiae* supplied with ethanol under aerobic conditions. Studies conducted with mutants blocked at a number of the glycolytic steps have shown that glycolytic reversal takes place by bypassing pyruvate kinase and phosphofructokinase. The investigators concluded that ethanol promotes glycolytic reversal by providing NADH to the GAPDH reaction, since a large change in the redox potential was observed and the rate of glycolytic reversal was much lower when the cells were supplied with a more oxidized carbon source such as acetaldehyde and acetate. The flux from phosphoenolpyruvate to fructose 1,6-bisphosphate was estimated to be 0.67 μmol of three-carbon unit s^{-1} (g of wet cells)$^{-1}$ (Maitra & Lobo, 1978). Since our ST-determined value of V_2 indicates that a large flux occurs in the presence of ethanol under aerobic conditions and a reverse flow has been observed under similar conditions, it appears that the GAPDH/PGK reaction is bidirectional in these aerobic ethanol-fed cells.

Calculation of V_2 assuming glycolytic reversal occurs, i.e., k_{-2} is nonzero, is not as straightforward. Relaxation of P_i with ATP_γ saturated will in general be a double exponential with time constant dependent on $1/T_{1i}$, $1/T_{1x}$, k_{-2}, k_F, and k_2. A simple two-site analysis leading to a single-exponential fit could either underestimate or overestimate T_{1iapp}. In view of this, V_2 was calculated by estimating the longitudinal relaxation time in the absence of exchange, T_{1i}, from measurements of O_2 consumption and T_{1app} in iodoacetate-treated ethanol-fed cells. With glycolytic contributions eliminated, $1/T_{1app} = 1/T_{1i} + k_F$. Subtracting k_F from our measured T_{1app} gives $1/T_{1i}$. Upon substituting our ST-determined values of $\Delta M/M^0$, $1/T_{1i}$, M_i^0, and V_F into eq 3, we obtain $V_2 = 1.2 + 2.1k_{-2}M_x$. A true estimate of V_2, the forward unidirectional flow through GAPDH, will be obtained if k_{-3} is much greater than $1/T_{1x}$, so that the saturation introduced into ATP_γ will in turn be transferred to X–OP ($M_x = 0$). Under these conditions, there should be no return of z magnetization via the $k_{-2}M_x$ term. However, if k_{-3} is less than $1/T_{1x}$, the $k_{-2}M_x$ term will reduce the magnetization-transfer effect due to V_2 alone. In any case, the fact that a large flux ($V_2 - 2.1k_{-2}M_x = 1.2$) was measured indicates that extensive cancellation between forward and reverse flows does not occur, and at most V_2 will be underestimated.

In antimycin studies of aerobic ethanol-fed cells, the large transfer ($\Delta M/M_i^0 = 0.53$) measured by ST experiments in aerobic ethanol-fed cells was attributed to both mitochondrial ATPase and GAPDH/PGK exchange. However, in the presence of antimycin, no detectable transfer occurs. The measured apparent T_1 (T_{1app}) increases to 0.60 ± 0.06 s and is similar to the estimated T_{1i}, indicating that longitudinal relaxation does not appear to be greatly influenced by exchange. Thus, the large decrease in $\Delta M/M_i^0$ and increase in

T_{1app} more than compensate for the 3-fold increase in P_i^{in} concentration, leading to an overall decrease in flux.

A P/O value of 2–3 was obtained in aerobic ethanol-fed cells when glycolytic contributions were inhibited with iodoacetate, suggesting that mitochondrial ATPase-catalyzed ATP production occurs irreversibly. However, in the presence of antimycin, it is conceivable that a protonmotive force could be produced by hydrolyzing the residual ATP via reversal of the ATPase reaction, with the protonmotive force eventually coming to equilibrium with the phosphate potential. However, our results indicate that this does not occur. If rapid turnover of the mitochondrial ATPase takes place, we would expect to see an appreciable transfer since our experiments measure unidirectional ATP synthesis rates and not net synthesis of ATP. Instead, as stated above, our results indicate a reduced ATPase flux in antimycin-treated cells.

The effect of antimycin on GAPDH/PGK exchange is difficult to assess from our measurements. ST measurements in antimycin-treated ethanol-fed cells indicate a decreased P_i consumption rate. A decreased flux can result from a decrease in the ATP synthetase flux alone or decreased contributions from both GAPDH/PGK and mitochondrial ATPase exchange reactions. Either a decrease in the forward flow through GAPDH/PGK or an increase in the reverse flow could lead to a reduction in V_{ST}.

The results obtained from these studies indicate that unidirectional flows through both GAPDH/PGK and the mitochondrial ATPase can be monitored by saturation-transfer NMR in intact yeast. The relative contributions of these exchange reactions depend on both growth conditions and metabolic state (i.e., carbon source and oxygenation state). The glycolytic pathway plays an important role in these cells. The contribution from GAPDH/PGK complicates ST results and leads to incorrectly high P/O values. Recent ST studies of *Escherichia coli* suggest that the GAPDH/PGK reaction also contributes to ST-measured P_i consumption rates (I. Campbell, personal communication). In the previous study performed on yeast cells grown to mid-log phase in the presence of acetate (Campbell et al., 1985), GAPDH/PGK exchange did not appear to contribute to the ST results. This is consistent with the very low GAPDH activity that has been measured in cultures of *Pseudomonas putida* grown on succinate, lactate, or acetate (Ruiz-Amil et al., 1969). In systems containing lower glycolytic enzyme levels (particularly GAPDH), the mitochondrial ATPase appears to be solely responsible for the ST effect, and P/O values of 2–3 are obtained. Consequently, ST studies performed in any mammalian systems that possess high glycolytic capabilities may have large contributions from GAPDH/PGK exchange.

ACKNOWLEDGMENTS

We thank Dr. J. A. Alger for many helpful discussions and comments during preparation of the manuscript. We also thank Betsy Hodgson for excellent technical assistance.

Registry No. ATP, 56-65-5; PO_4^{3-}, 14265-44-2.

REFERENCES

Alger, J. R., & Shulman, R. G. (1984) *Q. Rev. Biophys. 17*, 83–124.

Alger, J. R., den Hollander, J. A., & Shulman, R. G. (1982) *Biochemistry 21*, 2957–2963.

Brindle, K., & Krikler, S. (1985) *Biochim. Biophys. Acta 847*, 285–292.

Campbell, S. L., Jones, K. A., & Shulman, R. G. (1985) *FEBS Lett. 193*, 189–193.

Campbell-Burk, S. L., den Hollander, J. A., Alger, J. R., & Shulman, R. G. (1987) *Biochemistry* (following paper in this issue).

Den Hollander, J. A., Ugurbil, K., Brown, T. R., & Shulman, R. G. (1981) *Biochemistry 20*, 5871–5880.

Den Hollander, J. A., Ugurbil, K., Brown, T. R., Bednar, M., Redfield, C., & Shulman, R. G. (1986a) *Biochemistry 25*, 203–211.

Den Hollander, J. A., Ugurbil, K., & Shulman, R. G. (1986b) *Biochemistry 25*, 212–219.

Durr, M., Urech, K., Boller, Th., Wiemken, A., Schwencke, J., & Nagy, M. (1979) *Arch. Microbiol. 121*, 169–175.

Erecinska, M., & Wilson, D. F. (1981) in *International Encyclopedia of Pharmacology and Therapeutics*, Sect. 107, Inhibitors of Mitochondrial Function, Pergamon, New York.

Freeman, D., Bartlett, S., Radda, G., & Ross, B. (1983) *Biochim. Biophys. Acta 762*, 325–336.

Gancedo, J. M., & Gancedo, C. (1973) *Biochimie 55*, 205–211.

Gillies, R. J., Ugurbil, K., den Hollander, J. A., & Shulman, R. G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2125–2129.

Johnson, M. J. (1960) *Enzymes, 2nd Ed. 3*, 407–441.

Kingsley-Hickman, P., Sako, E. Y., Andreone, P. A., Cyr, J. A., Michurski, S., Foker, J. E., From, A. H. L., Petein, M., & Ugurbil, K. (1986) *FEBS Lett. 198*, 159–163.

Koretsky, A. P., & Weiner, M. W. (1984) in *Biomedical Magnetic Resonance* (James, T. L., & Margules, A. R., Eds.) Radiology Research and Education Foundation, San Francisco.

Koretsky, A. P., Basus, V. J., James, T. L., Klein, M. P., & Weiner, M. W. (1984) *Biophys. J. 45*, 32a.

Lagunas, R., & Gancedo, J. M. (1973) *Eur. J. Biochem. 37*, 90–94.

Levitt, M. J., Freeman, R., & Frenkiel, T. (1983) in *Advances in Magnetic Resonance* (Waugh, J. S., Ed.) Vol. 2, pp 47–110, Academic, New York.

Maitra, P. K., & Lobo, Z. (1971) *J. Biol. Chem. 246*, 475–488.

Maitra, P. K., & Lobo, A. (1978) *Arch. Biochem. Biophys. 185*, 535–543.

Mann, B. E. (1977) *J. Magn. Reson. 25*, 91–94.

Matthews, P. M., Bland, J. L., Gadian, D. G., & Radda, G. K. (1981) *Biochem. Biophys. Res. Commun. 103*, 1052–1059.

Navon, G., Shulman, R. G., Yamane, T., Eccleshall, T. R., Lam, K. B., Baronfsky, J. J., & Marmur, J. (1979) *Biochemistry 18*, 4487–4499.

Pena, A. (1976) in *Mitochondria: Bioenergetics, Biogenesis, and Membrane Structure* (Packer, L., & Gomez-Puyou, Eds.) pp 21–30, Academic, New York.

Poole, R. J. (1978) *Annu. Rev. Plant Physiol. 29*, 437–460.

Ruiz-Amil, M., Aparicio, M. L., & Canovas, J. L. (1969) *FEBS Lett. 3*, 65–67.

Salhany, J. M., Yamane, T., Shulman, R. G., & Ogawa, S. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 498–503.

Serrano, R. (1978) *Mol. Cell. Biochem. 22*, 51–63.

Shoubridge, E. A., Briggs, R. W., & Radda, G. K. (1982) *FEBS Lett. 140*, 288–292.

Strathern, J. N., Jones, E. W., & Broach, J. R. (1982) in *The Molecular Biology of the Yeast Saccharomyces, Metabolism and Gene Expression*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Webb, J. L. (1966) in *Enzyme and Metabolic Inhibitors*, Vol. 3, pp 1–270, Academic, New York.

Willsky, G. R. (1979) *J. Biol. Chem. 254*, 3326–3332.