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Diversion of the Metabolic Flux from Pyruvate Dehydrogenase to Pyruvate Oxidase Decreases Oxidative Stress during Glucose Metabolism in Nongrowing *Escherichia coli* Cells Incubated under Aerobic, Phosphate Starvation Conditions†

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Ongoing aerobic metabolism in nongrowing cells may generate oxidative stress. It is shown here that the levels of thiobarbituric acid-reactive substances (TBARSs), which measure fragmentation products of oxidized molecules, increased strongly at the onset of starvation for phosphate (P_i). This increase in TBARS levels required the activity of the histone-like nucleoid-structuring (H-NS) protein. TBARS levels weakly increased further in Δ*ahpCF* mutants deficient in alkyl hydroperoxide reductase (AHP) activity during prolonged metabolism of glucose to acetate. Inactivation of pyruvate oxidase (PoxB) activity decreased the production of acetate by half and significantly increased the production of TBARS. Overall, these data suggest that during incubation under aerobic, P_i starvation conditions, metabolic flux is diverted from the pyruvate dehydrogenase (PDH) complex (NAD dependent) to PoxB (NAD independent). This shift may decrease the production of NADH and in turn the adventitious production of H_2O_2 by NADH dehydrogenase in the respiratory chain. The residual low levels of H_2O_2 produced during prolonged incubation can be scavenged efficiently by AHP. However, high levels of H_2O_2 may be reached transiently at the onset of stationary phase, primarily because H-NS may delay the metabolic shift from PDH to PoxB.

Escherichia coli possesses numerous mechanisms aimed at protecting and repairing cell constituents exposed to toxic agents. Defense mechanisms in growing cells are generally transient responses that allow rapid adaptation to stress. However, in nature, bacteria may alternate between growing and nongrowing states because of nutritional starvation. Survival of starved cells poses a specific problem because of the low capacity of stationary-phase cells to synthesize proteins. Starved cells can at least in part resolve this problem by accumulating RpoS (σ^s) at the entry into stationary phase. The induction of the numerous genes of the RpoS regulon helps to protect nongrowing cells against various stresses (14, 16).

Moreover, it has been suggested that metabolic fluxes may be redistributed in starved cells, which may decrease the endogenous production of reactive oxygen species (ROS). For instance, cells incubated under aerobic, glucose starvation conditions shift from aerobic to fermentative metabolism in an ArcA-dependent manner (e.g., pyruvate dehydrogenase synthesis is decreased, whereas pyruvate formate-lyase synthesis is increased) (24). Such a shift may decrease the production of NADH and the respiratory chain-mediated generation of ROS (24, 28). In the aerobic respiratory chain, NADH dehydrogenase II produces superoxide and hydrogen peroxide (H_2O_2) by autooxidation of its reduced flavin adenine dinucleotide cofactor (20). H_2O_2 is primarily detoxified by the alkyl hydroperoxide reductase (AHP) complex (AhpCF), which efficiently scavenges low levels of H_2O_2 but is inefficient against high levels of H_2O_2 (27).

In contrast to cells incubated under aerobic, glucose starvation conditions which shift from aerobic to anaerobic metabolism, cells incubated under aerobic, P_i starvation conditions may continue to metabolize glucose through aerobic metabolism, which may generate ROS (11). The cellular levels of ROS, which are not diluted by growth any more, may hence steadily increase in P_i-starved cells. This idea is supported by the finding that the colony-forming ability of *ahp* mutants decreases after 2 days of incubation under aerobic, P_i starvation conditions (22). However, these data might indicate that P_i-starved cells are primarily exposed to ROS at the exit of rather than during stationary phase (5). It has been shown that *ahp* mutants accumulate DNA damage and fragmentation products of oxidized molecules, measured as thiobarbituric acid-reactive substances (TBARSs), when they exit stationary phase and enter the lag phase in fresh Luria broth (LB) medium (12). Therefore, TBARS measurements were used in this study to determine whether oxidative stress occurs in cells when they are incubated under aerobic, P_i starvation conditions.

MATERIALS AND METHODS

Bacterial strains. The *Escherichia coli* K-12 strains are described in Table 1. Mutations were introduced into strains by P1 transduction (21). For strains carrying the *leuB* and *argE3*(Oc) mutations, cultures were routinely checked for the absence of Leu^+ contaminants and Arg^+ suppressors, which can grow in old cultures by consuming nutrients excreted into the medium (11).

Media and culture conditions. The minimal medium used for liquid cultures was essentially the morpholinepropanesulfonic acid (MOPS) medium described by Neidhardt et al. (23), which notably contained 40 mM MOPS, 86 mM NaCl, 9.5 mM NH_4Cl, 5 mM K_2HPO_4 and 20 mM glucose, supplemented with five vitamins (0.02 mM thiamine, 0.02 mM calcium pantothenate, 0.02 mM *p*-aminobenzoic acid, 0.02 mM *p*-hydroxybenzoic acid, and 0.02 mM 2,3-dihydroxy-

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† This paper is dedicated to the memory of Benjamin Moreau (1977-2000).

TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
ENZ1203	F$^-$ *thr-1*(Am) *leuB6* Δ(*argF-lac*)*U169 glnV44 sulA211 hisG4*(*Oc*) *rpsL31 argE3*(Oc) *thi-1 rpoS*$^+$	22
ENZ1257	ENZ1203 *sulA*::*lacZ*	22
ENZ1399	ENZ1257 Δ*ahpCF*::kan	22
ENZ1643	ENZ1257 *poxB176*::*lacZ* Cm^r	P1(YYC912) × ENZ1257
ENZ1645	ENZ1203 *poxB176*::*lacZ* Cm^r	P1(YYC912) × ENZ1203
ENZ1646	ENZ1203 Δ*ahpCF*::kan	P1(ENZ1399) × ENZ1203
ENZ1667	ENZ1203 *hns*::neo	P1(MC4100*hns*) × ENZ1203
MC4100*hns*	*hns*::neo	30
YYC912	*poxB176*::*lacZ* Cm^r	7

benzoic acid) and six amino acids (0.8 mM leucine, 0.4 mM threonine, 0.2 mM histidine, 0.4 mM arginine, 0.4 mM isoleucine, and 0.6 mM valine) (pH 7.2). The P_i- and glucose-limiting media contained 0.1 mM K_2HPO_4 plus 9.8 mM KCl and 3 mM glucose, respectively (11). Strains were grown in MOPS medium for 24 h, diluted 1:200 into MOPS medium at time zero, and incubated further with aeration in a water bath rotary shaker (Aquatron Infors HT, 150 rpm) at 37°C, unless otherwise indicated. Culture optical density at 600 nm (OD_{600}) was measured with a Jasco V-530 spectrophotometer in cells of 1-cm path length. The pH of the media was determined at ≈25°C.

Levels of glucose and by-products. The concentrations of glucose (glucose HK assay; Sigma), pyruvate (Roche), and glutamate, formate, acetate, and D-and L-lactate (Boehringer Mannheim/R-Biopharm) were assayed with enzymatic tests according to the instructions of the manufacturers.

Determination of TBARS concentration. Cells were centrifuged at 12,000 × *g* for 15 min, suspended in phosphate buffer (pH 7.0) containing 0.1 mM butylated hydroxytoluene (Sigma) as an antioxidant, and disrupted by one passage through a French pressure cell at 10^8 Pa. The extract was clarified by centrifugation at 27,000 × *g* for 20 min, and the supernatant was centrifuged for 90 min at 230,000 × *g* (k = 96, rotor type 70.1Ti; Beckman) at 4°C. The pellet fraction, which contained cytoplasmic membrane vesicles, was suspended in phosphate buffer containing 0.1 mM butylated hydroxytoluene, precipitated with 10% (wt/vol) trichloroacetic acid, incubated with 4.6 mM thiobarbituric acid (Sigma) in 50% (vol/vol) glacial acetic acid plus 0.1 mM butylated hydroxytoluene for 1 h at 95°C, mixed with 1 ml of butanol, and centrifuged at 11,000 × *g* for 3 min. The absorbance of the butanol phase was determined at 532 nm. The concentration of TBARS (ε_{532} = 156 mM^{-1} cm^{-1}) was expressed in nanomoles per milligram of protein (6, 13). Protein content was determined by the Bradford dye-binding assay (Bio-Rad) with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

TBARS levels increase significantly in cells incubated under P_i starvation conditions. Cells incubated for 4 days under glucose and P_i starvation conditions exhibited TBARS levels 2.5- and 8-fold higher than those of exponentially growing cells, respectively (Table 2, Fig. 1). These data suggest that ROS levels may eventually increase more strongly in P_i- than in glucose-starved cells.

TBARS levels depend on the amount of glucose metabolized in *ahp* cells. Inactivation of the *ahpCF* genes increased by ≈1.9-fold the levels of TBARS in cells incubated for 4 days in P_i-limiting medium initially containing 20 mM glucose (from 0.48 nmol/mg in *ahp*$^+$ cells to 0.90 nmol/mg in *ahp* mutants; Tables 2 and 3). These data suggest that one role of AHP is to protect cell constituents against ROS produced during P_i starvation.

TBARS levels measured on day 4 of incubation in *ahp* mutants increased from ≈0.5 to ≈1.0 nmol/mg as a function of the concentration of glucose added in the range from 5 to 30 mM (Table 3). TBARS levels remained steady at ≈0.5 nmol/mg in *ahp*$^+$ cells incubated under the same conditions (data not shown). Therefore, AHP may help scavenge ROS produced during prolonged incubation in P_i-limiting medium in the presence of excess glucose (≈3 mM glucose was consumed at the entry into stationary phase by ≈10 h of incubation; data not shown).

However, when *ahp* mutants were incubated for 4 days in P_i-limiting medium initially containing 30 or 40 mM glucose, similar levels of TBARS were produced and similar amounts of glucose were consumed (≈26 mM) (Table 3). Therefore, the ROS generated during incubation in P_i-limiting medium may primarily be produced endogenously through glucose metabolism rather than by autooxidation of glucose present in the medium (27).

Overall, these data suggest that metabolism of glucose in P_i-starved cells may generate ROS. However, consumption of glucose had little effect on biomass formation (OD_{600}) (Table 3), which suggests that metabolism may primarily be achieved by overflow metabolism, i.e., by excretion of by-products.

AHP prevents a late increase in TBARS levels. TBARS levels were measured in *ahp*$^+$ and *ahp* mutant strains as a function of the time of incubation in P_i-limiting medium containing 20 mM glucose (Fig. 1). TBARS levels increased in roughly two steps. First, a strong increase (≈8-fold) occurred during the transition from exponential growth to stationary phase (day 1 of incubation) in both *ahp*$^+$ and *ahp* mutant cells. Second, a weaker increase (≈2-fold) preferentially occurred between day 1 and day 4 of incubation in *ahp* mutants.

The differential roles of AHP suggest that the levels of H_2O_2

TABLE 2. TBARS levels increase in cells incubated for 4 days under aerobic, P_i starvation conditions[a]

Medium	Growth phase	OD_{600}	Mean TBARS concn, nmol/mg, ± SD (fold increase)
MOPS	Exponential	0.7	0.06 ± 0.01
Glucose limiting	Stationary	0.7	0.15 ± 0.02 (2.5)
P_i limiting	Stationary	0.8	0.48 ± 0.04 (8)

[a] Strain ENZ1257 was inoculated into 100 ml of MOPS (5 mM P_i plus 20 mM glucose), glucose-limiting (5 mM P_i plus 3 mM glucose), or P_i-limiting (0.1 mM P_i plus 20 mM glucose) medium at time zero and incubated further with aeration in 500-ml Erlenmeyer flasks. When cells were either in exponential growth phase or in stationary phase, i.e., on day 4 of incubation under glucose or P_i starvation conditions (starved cells entered stationary phase after ≈10 h of incubation), the OD_{600} of the cultures and TBARS concentrations were determined. TBARS values are the means of two experiments. The values relative to those in exponentially growing cells are indicated in parentheses.

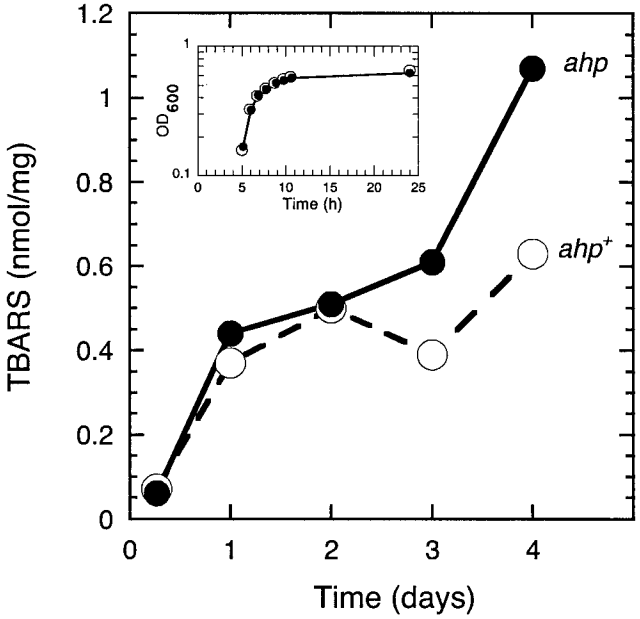

FIG. 1. TBARS levels as a function of time of incubation under aerobic, P_i starvation conditions in *ahp*$^+$ and *ahp* mutant cells. Strains ENZ1257 (*ahp*$^+$) (○) and ENZ1399 (ENZ1257 Δ*ahpCF*) (●) were inoculated into 100 ml of P_i-limiting medium (0.1 mM P_i plus 20 mM glucose) (time zero) and incubated under aerobic conditions in 500-ml Erlenmeyer flasks. TBARS concentrations were determined between days 1 and 4 of incubation ($OD_{600} \approx 0.7$). The values for exponentially growing cells were obtained from cells grown for 6.5 h ($OD_{600} \approx 0.7$) in MOPS medium (5 mM P_i plus 20 mM glucose). The data are from a representative experiment from three separate trials. Variations between experiments were ≤20%. The inset shows the growth curves of *ahp*$^+$ and *ahp* mutant cells in P_i-limiting medium.

reached at the onset of P_i starvation may be too high to be scavenged efficiently by AHP (27). However, between days 1 and 4 of incubation, when the rate of production of H_2O_2 may be reduced, AHP can scavenge the low levels of H_2O_2 accumulated in P_i-starved cells as a result of glucose metabolism.

TBARS levels increase strongly in *poxB* mutants. In P_i-starved cells, glucose was primarily metabolized to acetate, which was excreted into the incubation medium. On day 5 of incubation in P_i-limiting medium initially containing 40 mM glucose, acetate levels reached 29.4 mM and the pH of the medium decreased from ≈7.2 to ≈4.8 (Table 4); the concentrations of pyruvate, lactate, aspartate, glutamate and formate in the incubation medium were lower than 0.4 mM (Table 4 and data not shown).

TABLE 3. TBARS levels increase as a function of the amount of glucose consumed in *ahp* mutants starved of P_i for 4 days[a]

Initial glucose concn (mM)	No. of expt	OD_{600}	Glucose (mM)	Mean TBARS concn (nmol/mg) ± SD
5	2	0.6	≤0.4	0.52 ± 0.04
10	2	0.7	≤0.4	0.66 ± 0.03
20	4	0.7	≤0.4	0.90 ± 0.07
30	4	0.7	4.2 ± 0.1	1.09 ± 0.10
40	2	0.7	14.3 ± 0.1	0.93 ± 0.10

[a] Strain ENZ1399 (ENZ1257 Δ*ahpCF*) was inoculated into 100 ml of P_i-limiting medium (0.1 mM P_i) containing increasing concentrations of glucose, and incubated further under aerobic conditions in 500-ml Erlenmeyer flasks. On day 4 of incubation, the OD_{600} of the cultures, glucose concentrations in the spent medium, and TBARS levels were determined.

TABLE 4. Metabolic pattern in cells starved of P_i for 5 days[a]

Strain	OD_{600}	Final pH	Mean concn (mM) ± SD		
			Glc	Ace	Pyr
ENZ1257	0.7	4.8	11.6 ± 0.2	29.4 ± 1.8	0.31 ± 0.01
ahp	0.7	5.1	13.2 ± 0.6	24.0 ± 0.1	0.08 ± 0.01
poxB	0.8	4.7	14.3 ± 0.6	13.0 ± 0.1	3.9 ± 0.1

[a] Strains ENZ1257, ENZ1399 (ENZ1257 Δ*ahpCF*), and ENZ1643 (ENZ1257 *poxB*) were inoculated into 50 ml of P_i-limiting medium (0.1 mM P_i plus 40 mM glucose) and incubated further under aerobic conditions in 500-ml Erlenmeyer flasks. On day 5 of incubation, the OD_{600} and pH of the cultures and the concentrations in the culture supernatants (adjusted to pH 7) of glucose (Glc), acetate (Ace), and pyruvate (Pyr) were determined. Data are the means of two experiments.

The low concentration of formate in the spent medium indicates that pyruvate formate-lyase played no significant role in the metabolism of pyruvate in P_i-starved cells. Therefore, the metabolism of pyruvate should occur through aerobic enzymes: the pyruvate dehydrogenase (PDH) complex (AceEF Lpd) or pyruvate oxidase (PoxB) or both (10).

The PDH complex is primarily used during aerobic growth to catalyze the oxidative decarboxylation of pyruvate to acetyl coenzyme A (acetyl-CoA) with the concomitant reduction of NAD. Since the rate of synthesis of the AceF subunit of the PDH complex decreases by approximately twofold at the onset of P_i starvation (29), a decrease in PDH activity might account at least in part for the apparent decrease in the production of H_2O_2 during prolonged incubation under P_i starvation conditions.

PoxB can catalyze the oxidative decarboxylation of pyruvate directly to acetate with the concomitant reduction of flavin adenine dinucleotide and ubiquinone, bypassing NADH oxidation by the respiratory chain (10). It has been suggested that PoxB, which is strictly regulated by RpoS, could play a beneficial role at very low growth rates and at the entry into stationary phase under microaerobic conditions, when neither PDH nor pyruvate formate-lyase can function efficiently (1, 7).

Inactivation of *poxB* caused significant changes in the metabolic pattern of cells incubated for 5 days in P_i-limiting medium initially containing 40 mM glucose (Table 4). Whereas the total amount of glucose consumed by *poxB* mutants (25.7 mM) was only slightly lower than that consumed by *poxB*$^+$ cells (28.4 mM), the amount of acetate excreted into the culture medium by *poxB* mutants (13.0 mM) was 2.2-fold lower than that excreted by *poxB*$^+$ cells (29.4 mM) (the concentrations of acetate remained steady between days 4 and 6 in *poxB* mutants, whereas they continued to increase for 6 days up to ≈33 mM in *poxB*$^+$ cells; data not shown). In contrast, the amount of pyruvate excreted by *poxB* mutants was 12.5-fold higher than that excreted by *poxB*$^+$ cells (Table 4).

These data suggest that (i) at least half of the pyruvate that is metabolized to acetate in P_i-starved cells is metabolized through PoxB rather than through the PDH complex-phosphotransacetylase-acetate kinase pathway and (ii) part of the pyru-

TABLE 5. TBARS levels in *poxB* mutants[a]

Strain	Mean TBARS concn (nmol/mg) ± SD		
	Exponential	Day 1	Day 4
ENZ1203	0.06 ± 0.01	0.41 ± 0.01	0.46 ± 0.01
poxB	0.06 ± 0.01	0.45 ± 0.03	1.79 ± 0.13

[a] Strains ENZ1203 and ENZ1645 (ENZ1203 *poxB*) were inoculated into 100 ml of MOPS containing 30 mM glucose and 5 mM P_i (exponential) or 0.1 mM P_i (day 1 and day 4). Data are the means of two experiments.

vate that is not metabolized in *poxB* mutants is excreted directly into the medium.

Inactivation of *poxB* caused a dramatic increase in TBARS levels during prolonged incubation in P_i-limiting medium initially containing 30 mM glucose (Table 5). Whereas *poxB*$^+$ and *poxB* mutant cells exhibited similar levels of TBARS during the exponential growth phase (0.06 nmol/mg) and on day 1 of incubation (≤0.45 nmol/mg), TBARS levels increased sharply to 1.79 nmol/mg on day 4 of incubation in *poxB* mutants, while TBARS levels barely increased in *poxB*$^+$ cells (0.46 nmol/mg) (Table 5). Under the same conditions (on day 4 of incubation in P_i-limiting medium containing 30 mM glucose), TBARS levels increased to 1.09 nmol/mg in *ahp* mutants (Table 3).

Overall, these data suggest that in *poxB* mutants, although part of the pyruvate that is not metabolized through PoxB is excreted, part may be metabolized through the PDH complex in addition to the amount normally metabolized in *poxB*$^+$ cells, which may increase the production of NADH and eventually the production of H_2O_2 by the respiratory chain. Therefore, by diverting part of the metabolic flux from the PDH complex, PoxB may protect P_i-starved cells against oxidative stress.

H-NS modulates the production of TBARS. During the exponential growth phase, synthesis of the PDH complex is decreased, whereas synthesis of PoxB is increased in *hns* mutants compared to the *hns*$^+$ parents (2, 15, 19). Therefore, a role in growing cells of the histone-like nucleoid-structuring protein H-NS may be to increase PDH activity (which gives rise to NADH and acetyl-CoA) and to decrease PoxB activity (which gives rise directly to acetate and eventually to acetyl-CoA through acetate kinase and acetyl-CoA synthetase, both activities requiring ATP) (1, 18). This specific role of H-NS, which may result primarily from its capacity to decrease RpoS levels in exponential phase (4), may help growing cells to sustain an efficient energetic metabolism and a rapid growth rate. In fact, the generation time of *hns* mutants was 3.5-fold higher than that of *hns*$^+$ cells in MOPS medium (4) (Table 6). However, it was rather surprising that TBARS levels were threefold higher in *hns* mutants than in *hns*$^+$ cells during the exponential growth phase (Table 6). This result may be explained at least in part by the recent finding that overexpression of *poxB* in PDH$^-$ mutants, which mimic the behavior of *hns* mutants, increases by ≈2-fold the specific rate of consumption of oxygen during growth (1). This is probably required to compensate for the higher consumption of ATP needed to metabolize further PoxB-derived acetate to acetyl-CoA (1). Higher activity of the tricarboxylic acid cycle enzymes and of the aerobic respiratory chain (8, 10) may eventually increase the production of ROS during growth in *hns* mutants.

Whereas H-NS activity decreased TBARS levels during growth, H-NS activity increased TBARS levels at the onset of P_i starvation. TBARS levels increased strongly at the entry into stationary phase in *hns*$^+$ cells (Table 6), while they barely increased in *hns* mutants (Table 6), although similar amounts of glucose were eventually consumed by both strains. These data may be explained simply by the hypothesis that at the entry into stationary phase, pyruvate metabolism occurs through the PDH complex in *hns*$^+$ cells, whereas part of the metabolism may have already occurred through PoxB in *hns* mutants.

TABLE 6. H-NS modulates the production of TBARS[a]

Strain	P_i (mM)	Growth phase (h of growth)	Glucose consumed (mM)	Mean TBARS concn, nmol/mg, ± SD (fold increase)
ENZ1203	5	Exponential (7)	3.3	0.05 ± 0.01
ENZ1203	0.1	Stationary (24)	8.1	0.41 ± 0.02 (8.2)
hns	5	Exponential (22)	2.1	0.15 ± 0.02 (3)
hns	0.1	Stationary (47)	9.8	0.20 ± 0.03 (4)

[a] Strains ENZ1203 and ENZ1667 (ENZ1203 *hns*::neo) were inoculated (125 μl from overnight cultures in LB medium) into 100 ml of MOPS (5 mM P_i plus 20 mM glucose) or P_i-limiting medium (0.1 mM P_i plus 20 mM glucose) at time zero. Exponentially growing cells in MOPS medium were taken at an OD_{600} of 0.7 (*hns*$^+$; time = 7 h; generation time ≈ 52 min) or at an OD_{600} of 0.4 (*hns* mutants; time = 22 h; generation time ≈ 3 h). TBARS values are the means of two experiments. The values relative to those in *hns*$^+$ cells in the exponential growth phase are indicated in parentheses.

Overall, these data suggest that at the onset of P_i starvation, H-NS delays the shift from PDH to PoxB, which prolongs the production of NADH by the PDH complex and the production of ROS by NADH dehydrogenase II. The ≈4-fold increase in the rate of synthesis of H-NS at the onset of P_i starvation (29) may thus help to increase oxidative stress in P_i-starved cells.

Conclusion. The data presented here provide the outlines to an understanding of how oxidative stress occurs in P_i-starved cells during the metabolism of glucose to acetate (Fig. 2). At the onset of stationary phase, H-NS may help to sustain a high metabolic flux through the PDH complex, which generates NADH and eventually ROS as a result of the activity of

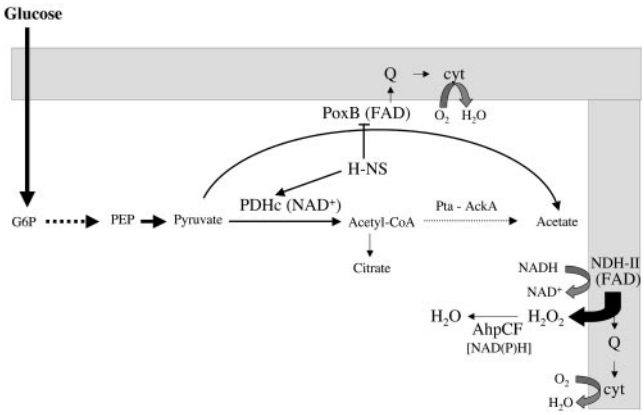

FIG. 2. Diagram showing the proposed pathways for the metabolism of pyruvate in P_i-starved cells. Abbreviations: Q, ubiquinone; G6P, glucose 6-phosphate; cyt, cytochrome quinol oxidase; FAD, flavin adenine dinucleotide; PEP, phosphoenolpyruvate; PDHc, PDH complex; Pta, phosphotransacetylase; AckA, acetate kinase.

NADH dehydrogenase II in the respiratory chain. However, the rate of production of NADH may decrease rapidly, primarily because of diversion of the metabolic flux from PDH to PoxB. The residual low levels of H_2O_2 generated during prolonged metabolism of glucose may be efficiently scavenged by AHP.

There is evidence that the PoxB shunt-AHP antioxidant activity integrated system may be specific to P_i-starved cells. First, even though glucose-starved cells accumulate RpoS and induce the expression of *poxB* (although at levels approximately twice lower than in P_i-starved cells; unpublished data), metabolism of glucose could occur primarily through an aerobic phosphoenolpyruvate-glyoxylate cycle (9), which may generate ROS (Table 2) (22), and eventually fermentative pathways, which may prevent the generation of ROS (17, 24). However, the increase in oxidative protein damage in glucose-starved cells results primarily from the synthesis of aberrant proteins rather than from an increase in ROS levels (3). Second, nitrogen-starved cells accumulate low levels of RpoS and poorly induce *poxB* (unpublished data). However, nitrogen-starved cells could prevent the generation of ROS by diverting glucose metabolism towards products such as glycogen (26). Finally, sulfur-starved cells may be exposed to oxidative stress as a result of the reduction of Fe^{3+} to Fe^{2+} by cystine, which increases the production of hydroxyl radicals through the Fenton reaction (25). Therefore, it appears that each type of metabolic perturbation may generate oxidative stress through different mechanisms, and cells may respond to these perturbations by specific strategies which are just beginning to be revealed.

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REFERENCES

1. **Abdel-Hamid, A. M., M. M. Attwood, and J. R. Guest.** 2001. Pyruvate oxidase contributes to the aerobic growth efficiency of *Escherichia coli*. Microbiology **147:**1483–1498.
2. **Atlung, T., and H. Ingmer.** 1997. H-NS: a modulator of environmentally regulated gene expression. Mol. Microbiol. **24:**7–17.
3. **Ballesteros, M., Å. Fredriksson, J. Henriksson, and T. Nyström.** 2001. Bacterial senescence: protein oxidation in non-proliferating cells is dictated by the accuracy of the ribosomes. EMBO J. **20:**5280–5289.
4. **Barth, M., C. Marschall, A. Muffler, D. Fischer, and R. Hengge-Aronis.** 1995. Role for the histone-like protein H-NS in growth phase-dependent and osmotic regulation of σ^s and many σ^s-dependent genes in *Escherichia coli*. J. Bacteriol. **177:**3455–3464.
5. **Bogosian, G., and E. V. Bourneuf.** 2001. A matter of bacterial life and death. EMBO Rep. **2:**770–774.
6. **Brown, R. K., and F. J. Kelly.** 1996. Peroxides and other products, p. 110–131. *In* N. A. Punchard and F. J. Kelly (ed.), Free radicals: a practical approach. IRL Press, New York, N.Y.
7. **Chang, Y.-Y., A.-Y. Wang, and J. E. Cronan, Jr.** 1994. Expression of *Escherichia coli* pyruvate oxidase (PoxB) depends on the sigma factor encoded by the *rpoS* (*katF*) gene. Mol. Microbiol. **11:**1019–1028.
8. **Cronan, J. E., Jr., and D. LaPorte.** 1996. Tricarboxylic acid cycle and glyoxylate bypass, p. 206–216. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, ASM Press, Washington, D.C.
9. **Fischer, E., and U. Sauer.** 2003. A novel metabolic cycle catalyzes glucose oxidation and anaplerosis in hungry *Escherichia coli*. J. Biol. Chem. **278:**46446–46451.
10. **Gennis, R. B., and V. Stewart.** 1996. Respiration, p. 217–261. *In* F. C. Neidhardt, R. Curtiss, III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
11. **Gérard, F., A.-M. Dri, and P. L. Moreau.** 1999. Role of *Escherichia coli* RpoS, LexA and H-NS global regulators in metabolism and survival under aerobic, phosphate starvation conditions. Microbiology **145:**1547–1562.
12. **González-Flecha, B., and B. Demple.** 1997. Homeostatic regulation of intracellular hydrogen peroxide concentration in aerobically growing *Escherichia coli*. J. Bacteriol. **179:**382–388.
13. **Griffiths, H. R., and J. Lunec.** 1996. Investigating the effects of oxygen free radicals on carbohydrates in biological systems, p. 185–199. *In* N. A. Punchard, and F. J. Kelly (ed.), Free radicals: a practical approach. IRL Press, New York, N.Y.
14. **Hengge-Aronis, R.** 1996. Regulation of gene expression during entry into stationary phase, p. 1497–1512. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular Biology. ASM Press, Washington, D.C.
15. **Hengge-Aronis, R.** 1999. Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli*. Curr. Opin. Microbiol. **2:**148–152.
16. **Huisman, G. W., D. A. Siegele, M. M. Zambrano, and R. Kolter.** 1996. Morphological and physiological changes during stationary phase, p. 1672–1682. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
17. **Koo B.-M., M.-J. Yoon, C.-R. Lee, T.-W. Nam, Y.-J. Choe, H. Jaffe, A. Peterkofsky, and Y.-J. Seok.** 2004. A novel fermentation/respiration switch protein regulated by enzyme IIA^{Glc} in *Escherichia coli*. J. Biol. Chem. **279:**31613–31621.
18. **Kumari, S., E. J. Simel, and A. J. Wolfe.** 2000. σ^{70} is the principal sigma factor responsible for transcription of *acs*, which encoded acetyl coenzyme A synthetase in *Escherichia coli*. J. Bacteriol. **182:**551–554.
19. **Laurent-Winter, C., S. Ngo, A. Danchin, and P. Bertin.** 1997. Role of *Escherichia coli* histone-like nucleoid-structuring protein in bacterial metabolism and stress response. Identification of targets by two-dimensional electrophoresis. Eur. J. Biochem. **244:**767–773.
20. **Messner, K. R., and J. A. Imlay.** 1999. The identification of primary sites of superoxide and hydrogen peroxide formation in the aerobic respiratory chain and sulfite reductase complex of *Escherichia coli*. J. Biol. Chem. **274:**10119–10128.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Moreau, P. L., F. Gérard, N. W. Lutz, and P. Cozzone.** 2001. Non-growing *Escherichia coli* cells starved for glucose or phosphate use different mechanisms to survive oxidative stress. Mol. Microbiol. **39:**1048–1060.
23. **Neidhardt, F. C., P. L. Bloch, and D. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
24. **Nyström, T., C. Larsson, and L. Gustafsson.** 1996. Bacterial defense against aging: role of the *Escherichia coli* ArcA regulator in gene expression, readjusted energy flux and survival during stasis. EMBO J. **15:**3219–3228.
25. **Park, S., and J. A. Imlay.** 2003. High levels of intracellular cysteine promote oxidative DNA damage by driving the Fenton reaction. J. Bacteriol. **185:**1942–1950.
26. **Preiss, J.** 1996. Regulation of glycogen synthesis, p. 1015–1024. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
27. **Seaver, L. C., and J. A. Imlay.** 2001. Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. J. Bacteriol. **183:**7173–7181.
28. **Storz, G., and J. A. Imlay.** 1999. Oxidative stress. Curr. Opin. Microbiol. **2:**188–194.
29. **VanBogelen, R. A., E. Olson, B. L. Wanner, and F. C. Neidhardt.** 1996. Global analysis of proteins synthesized during phosphorus restriction in *Escherichia coli*. J. Bacteriol. **178:**4344–4366.
30. **Yamada, H., T. Yoshida, K. Tanaka, C. Sasakawa, and T. Mizuno.** 1991. Molecular analysis of the *Escherichia coli hns* gene encoding a DNA-binding protein, which preferentially recognizes curved DNA sequences. Mol. Gen. Genet. **230:**332–336.