JOURNAL OF BACTERIOLOGY, Apr. 1983, p. 336–343
0021-9193/83/040336-08$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 154, No. 1

Proteins Induced by Anaerobiosis in *Escherichia coli*

MARTIN W. SMITH AND FREDERICK C. NEIDHARDT*
Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109

Received 27 September 1982/Accepted 10 January 1983

The contribution of protein induction and repression to the adaptation of cells to changes in oxygen supply is only poorly understood. We assessed this contribution by measuring the levels of 170 individual polypeptides produced by *Escherichia coli* K-12 in cells growing aerobically or anaerobically with and without nitrate. Eighteen reached their highest levels during anaerobic growth. These 18 polypeptides include at least 4 glycolytic enzymes and pyruvate formate-lyase (β-subunit). Most of these proteins were found at significant levels during aerobic growth and appeared to undergo metabolic regulation by stimuli other than anaerobiosis. Anaerobic induction ratios ranged from 1.8- to 11-fold, and nitrate antagonized the anaerobic induction of all of the proteins except one. The time course of synthesis of the proteins after shifts in oxygen supply revealed at least three distinct temporal patterns. These results are discussed in light of known physiological alterations associated with changes in oxygen availability.

Escherichia coli is capable of balanced growth in either the presence or absence of molecular oxygen. During aerobic growth, oxygen serves as terminal electron acceptor for a respiratory chain that provides both a hydrogen sink for metabolically derived electrons and the means for oxidative energy production (8). In the absence of oxygen or other respiratory electron acceptors, *E. coli* carries out a mixed-acid fermentation wherein pyruvate undergoes stepwise reduction to a variety of end products and ATP is produced exclusively by substrate-level phosphorylations.

Many years of investigation into the physiological makeup of respiring and fermenting cells have revealed several key features. First, the rate, route, and efficiency of carbon source utilization by the cell vary according to the supply of oxygen (17, 22, 24). Second, the mechanisms by which electron flow and the maintenance of an oxidation-reduction balance are achieved differ between aerobic and anaerobic cells (8). Third, alternative biosynthetic pathways are used if a reaction is coupled with the respiratory chain or is dependent upon or sensitive to molecular oxygen (2, 7).

These alterations in physiological character are achieved by a combination of enzyme activation/inhibition and induction/repression controls (25). At the level of activation/inhibition, the effector molecules and their impact on the catalytic activity of individual enzymes have been well studied. On the other hand, the mechanism by which a metabolic signal associated with the supply of molecular oxygen can affect the induction or repression of a protein is only poorly understood.

With the use of two-dimensional gel electrophoresis it is possible to resolve the bulk of cellular proteins produced under a given growth condition and, combined with double-isotope labeling, to measure the chemical amount of each protein. These techniques afford a feasible approach to questions concerning the effect of an environmental stimulus on the synthesis of individual cellular proteins (21).

The aims of this and the accompanying paper (27) are directed toward an understanding of the physiological commitment required of *E. coli* for successful growth in the presence or absence of oxygen and other respiratory electron acceptors. We report here (i) an assessment of the number of polypeptides produced in increased amounts by *E. coli* K-12 growing anaerobically; (ii) a quantitative analysis of their proportions in relation to total cellular protein; (iii) their steady-state induction ratios; (iv) the time course of their synthesis after abrupt shifts between aerobic and anaerobic conditions; and (v) biochemical identification of some of these proteins.

MATERIALS AND METHODS

Bacterial strain. *E. coli* K-12 strain W3110 (Thi^-) was used throughout this work.

Media and growth conditions. Cells were grown in MOPS minimal medium (18) modified by increasing the concentration of MOPS buffer to 80 mM. Glucose (0.8%, wt/vol) was added as carbon and energy source; vitamin B_1 (0.01 mM) was added to satisfy the growth requirement.

Aerobic cultures were grown on a rotary shaker at 37 ± 0.1°C. Anaerobic cultures were grown in an anaerobic glove chamber (Standard Safety, Palatine, Ill., and Coy Laboratories, Ann Arbor, Mich.) containing 5% CO_2, 10% H_2, and ≤5 ppm O_2 (3), with the balance being N_2. The chamber enclosed double-walled beakers containing fine sand in which standard Erlenmeyer flasks were immersed. Heated water was circulated through the chamber lining the beakers to warm the sand to 37 ± 0.2°C. The beakers were mounted on magnetic stirrers to allow rapid mixing of the culture medium in the flasks. With this apparatus cultures were easily manipulated without disturbance of the anaerobic environment.

Shifts between aerobic and anaerobic conditions were made by transferring the culture into or out of the anaerobic chamber. Shifts to aerobiosis were achieved by taking a culture flask out of the chamber air lock and immediately putting it in a rotary shaker in the laboratory. Transferring materials into the anaerobic chamber normally requires successive decompression and compression of the air lock. To avoid significant changes in atmospheric pressure, shifts to anaerobiosis were accomplished by rapidly squirting the culture fluid through sterile tubing into a prewarmed, stirring flask inside the chamber.

Growth was monitored spectrophotometrically at 420 nm. Growth of radioactive cultures was followed by monitoring parallel cultures of unlabeled cells.

Determination of steady-state polypeptide levels. A steady-state aerobic reference culture was grown to an optical density of 1.0 in minimal medium containing [^{14}C]leucine (356 mCi/mmol; 40 μCi/ml) plus adequate unlabeled leucine (0.04 mM), isoleucine (0.16 mM), and valine (0.24 mM). Test cultures were grown either aerobically, anaerobically, or anaerobically with 0.5% KNO_3 (wt/vol) in the same medium but with [^{3}H]leucine (145 Ci/mmol; 60 μCi/ml) as label and 0.15 mM cold leucine. Cells were harvested, and a portion of the reference culture was mixed with each test culture. Extracts were prepared by boiling in a sodium dodecyl sulfate lysing solution (1, 15), and individual polypeptides were resolved by two-dimensional electrophoresis according to O'Farrell (19, 20). The ^{3}H and ^{14}C contents of the individual polypeptides and of the unresolved extracts were determined as previously described (21). The ^{3}H/^{14}C ratio for a polypeptide sampled from a gel, divided by the ^{3}H/^{14}C ratio in the total extract, is the level of that protein in the test culture relative to the reference culture.

Visual comparisons were made with gels prepared as described above from cells grown in minimal medium containing $H_2{}^{35}SO_4$ (4,220 Ci/mmol; 75 μCi/ml) plus cold K_2SO_4 (28 μM) to an optical density of 1.0.

The fraction of total protein mass of an individual polypeptide (α'_{+O_2}) was determined as described by Pedersen et al. (21).

Determination of relative differential rates of polypeptide synthesis. Steady-state reference cultures were grown, aerobically or anaerobically, to an optical density of 0.5 in medium containing [^{14}C]leucine (356 mCi/mmol; 20 μCi/ml) plus carrier amino acids. A portion (3 ml) of a parallel nonradioactive culture was removed shortly before, and at intervals after, the shift and labeled for 2 min with [^{3}H]leucine (145 Ci/mmol; 900 μCi total). Labeling was terminated by addition of excess nonradioactive leucine, and incubation was continued for 1 min. Samples were mixed with a portion of the appropriate reference culture and processed as described above. The relative differential rate of synthesis of a polypeptide is the ^{3}H/^{14}C ratio in the gel spot, divided by the ^{3}H/^{14}C ratio in the total extract and normalized to the same ratio for the preshift pulse.

RESULTS

Steady-state levels of polypeptides produced during anaerobic growth. The relative levels of 170 polypeptides produced by *E. coli* K-12 were measured in cells grown in glucose minimal medium either aerobically, anaerobically, or anaerobically with nitrate as respiratory electron acceptor. Individual polypeptides were chosen for sampling according to the following strategy. First, gels prepared from cells labeled with $H_2{}^{35}SO_4$ were visually inspected for spots which varied in intensity with oxygen supply. These polypeptide spots were then sampled from a second set of gels prepared from [^{14}C]leucine- and [^{3}H]leucine-labeled cells for quantitative analysis. Second, protein spots previously identified as enzymes of central metabolic pathways, and therefore expected to respond at least to some extent, were sampled. Finally, a large set of other proteins, some identified and some not, were randomly sampled to complete the survey.

Of the polypeptides sampled, 18 had higher steady-state levels during anaerobic growth. These polypeptides are circled and numbered on the two-dimensional gel displayed in Fig. 1. Their steady-state levels and those of nine control proteins are given in Table 1. The control polypeptides are all of known biochemical identity and have diverse metabolic function and regulatory properties.

Induction by anaerobiosis of the polypeptides that responded was rather modest: two to fivefold. The known Embden-Meyerhof enzymes, F35.8, F43.8, G54.7, and I33.5, exhibited particularly small, but reproducible, elevation; their inclusion in this study was based on their uniform transient behavior after shifts in oxygen supply (discussed below). Polypeptides A48, B11.8, G31.5, G70, and H97.3 displayed greater than sixfold induction by anaerobiosis. Pyruvate formate-lyase (G70 and G74) is known to undergo a complex modification cycle in response to changes in oxygen supply (11, 12), and thus its accumulation during anaerobic growth may be the combined result of gene induction and enzyme modification.

That structural modification of G70 plays a major role in its accumulation was verified by treating cells with chloramphenicol before a shift to anaerobiosis. Visual inspection of gels prepared with these cells revealed that G70 accumulated after the shift even in the presence of chloramphenicol. No other anaerobiosis-in-

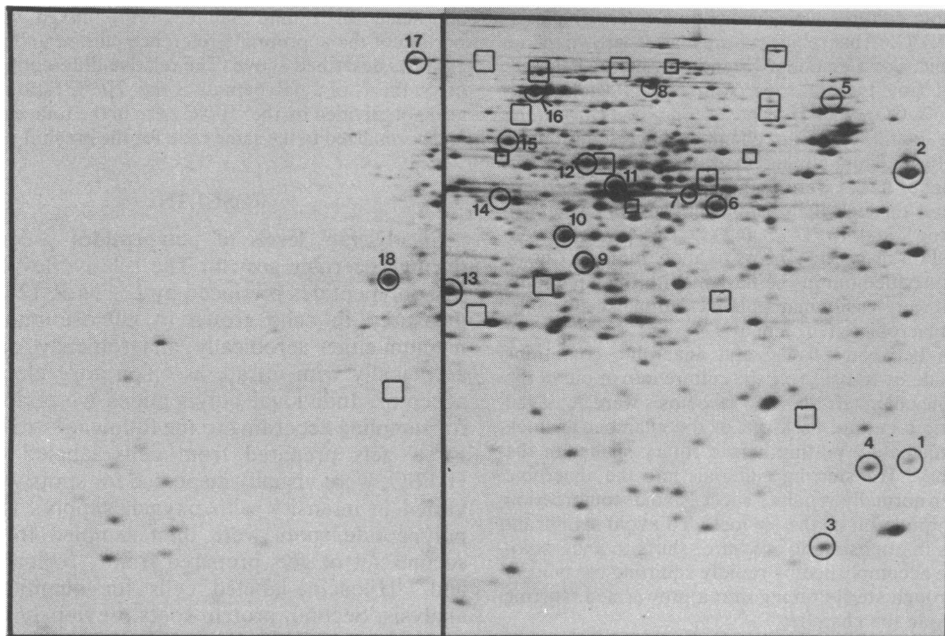

FIG. 1. **Autoradiograms of two-dimensional gels of anaerobically grown *E. coli* K-12 strain W3110 labeled with $H_2{}^{35}SO_4$. This is a composite of two gels prepared with the same extract: right, pH 5 to 7 ampholine equilibrium gel; left, pH 3 to 10 nonequilibrium gel. A 20-μl sample of extract containing ca. 20 μg of protein (4 × 10^6 cpm) was loaded on each gel. Isoelectric focusing in the first dimension was for 7,000 V · h (equilibrium gel) or 1,500 V · h (non-equilibrium gel) through 11.5% sodium dodecyl sulfate-acrylamide. The acid end of each gel is on the right. The second dimension was run at 4 W per gel for 3 to 4 h. The anaerobiosis-induced polypeptides are circled and numbered as in Table 1. For comparison, gels prepared from aerobically grown cells are shown in Fig. 1 of the subsequent paper (27).**

duced polypeptides accumulated under these circumstances (data not shown).

Nitrate as respiratory electron acceptor antagonized the anaerobic induction of most of the 18 polypeptides. For proteins A15.5, A48, B11.8, B76, G70, and I33.5, nitrate completely abolished anaerobic induction; for C40.3, C43.1, E79, F35.8, F38, F43.8, F50.5, G31.5, G41.3, G54.7, and H97.3 it was somewhat less effective. Induction of B15.0 appeared to be insensitive to nitrate.

The control polypeptides generally exhibited little net response to changes in oxygen supply. The changes in level of protein EF-G and ribosomal protein L1 were expected consequences of the decrease in cell growth rate associated with anaerobiosis (68% of the aerobic rate).

Abundance and metabolic regulation of the anaerobiosis-induced polypeptides. Visual inspection of the gels in Fig. 1 reveals that most of the polypeptides had substantial levels during aerobic growth. This impression was verified by measurement of their individual weight fractions of total cellular protein (α'_{+O_2}, Table 1). Most of the polypeptides induced by anaerobiosis appeared at molecular abundances comparable with those of the control proteins, and C40.3 and F43.8 were among the very major polypeptides in aerobically grown cells. Notable exceptions were proteins B11.8, F50.5, and G31.5, which had very low levels during aerobic growth.

The metabolic regulation of the anaerobiosis-induced polypeptides was also assessed, where possible, by measuring their levels during different rates of aerobic growth on a variety of culture media differing (i) in carbon and energy source and (ii) in the presence of biosynthetic building blocks. Individual proteins were classified by their overall regulatory behavior in these media according to the nomenclature established by Pedersen et al. (21). The polypeptides were found to display a very wide variety of regulatory features during aerobic growth (Table 1). Polypeptides A15.5, A48, B15.0, C40.3, C43.1, F35.8, and G70 (class Ib) were found to have aerobic levels that were invariant over the spectrum of conditions tested. Polypeptides F38, G31.5, H97.3, and I33.5 were repressed by the addition of biosynthetic precursors (amino acids, bases, and vitamins) to the medium, and G41.3, G54.7, E79, and I33.5 had very low levels during growth on oxidizable energy sources (ac-

TABLE 1. Steady-state levels of individual polypeptides

Serial no.[a]	Alpha-numeric designation[b]	Identification[c]	Metabolic regulation class[d]	α'_{+O_2} (μg/mg)[e]	Level relative to aerobic	
					Anaerobic	Nitrate
1	A15.5		Ib	2.2	3.37	0.63
2	A48		Ib	9.8	9.62	0.23
3	B11.8			0.4	6.32	0.89
4	B15.0		Ib	1.1	2.99	2.91
5	B76			1.8	3.06	0.65
6	C40.3[f]		Ib	15.5	2.22	1.23
7	C43.1		Ib	1.4	2.18	1.16
8	E79		IIb2	1.2	2.58	1.06
9	F35.8	F P-kinase, I	Ib	1.3	1.85	1.18
10	F38		IIa3	9.8	2.08	1.31
11	F43.8	Enolase	IIa1	20.3	2.16	1.21
12	F50.5			0.1	5.23	2.93
13	G31.5		Ia3	0.3	6.54	4.40
14	G41.3		Ic	2.6	3.06	1.98
15	G54.7	Pyr kinase, I	Ic	7.0	1.84	1.26
16	G70	Pyr formate-lyase, β	Ib	1.3	10.61	2.82
17	H97.3		Ia3	1.4	9.06	0.38
18	I33.5	Glyceral P-dehydro	IIa2	3.6	1.88	1.21
	B46.7	ATPase, β	Ia4	6.2	1.37	0.78
	B58.3	PTS, EI	IIa3	4.3	1.04	0.70
	D84	EF-G	Ic	16.6	0.62	0.76
	F39.7	Asp aminotrans	IIIa	2.2	0.64	0.92
	F46.6	Alkaline phos	IIa1	0.6	1.39	1.25
	F84	PEP carboxylase	IIa3	11.5	1.03	1.06
	G51	ATPase, α	Ia4	10.4	1.28	0.74
	G74	Pyr formate-lyase, α	Ia2	0.5	1.07	1.23
	I26	rProtein L1	Ic	2.3	0.78	0.92

[a] The serial numbers correspond to the numbers in Fig. 1.

[b] Proteins are listed with alpha-numeric designations as described by Pedersen et al. (21).

[c] Biochemical identification of proteins F35.8, F43.8, B46.7, D84, F39.7, F46.6, F84, G51, and I26.0 was reported previously (4, 23). The others were identified by Teresa Phillips by comigration with purified proteins donated by the following: B58.3, phosphotransferase transport system enzyme I from E. B. Waygood; G54.7, pyruvate kinase I from M. Malcovanti; G70 and G74, pyruvate formate-lyase from J. Knappe; I33.5, glyceraldehyde-3-phosphate dehydrogenase from D. Fraenkel. Abbreviations used: F P-kinase, I, fructose-6-phosphate kinase, activity I; Pyr kinase, I, pyruvate kinase, activity I; Pyr formate-lyase, α and β, pyruvate formate-lyase, α and β subunits; Glyceral P-dehydro, glyceraldehyde-3-phosphate dehydrogenase; ATPase, α and β, ATPase (Mg^{2+}-Ca^{2+}), α and β subunits; PTS, EI, phosphotransferase system enzyme I; EF-G, protein chain elongation factor G; Asp aminotrans, aspartate aminotransferase; Alkaline phos, alkaline phosphatase; PEP, phosphoenolpyruvate; rProtein, ribosomal protein.

[d] See reference 21 for a description of the classification.

[e] These values were measured by determining the total radioactivity in the individual spot on a gel from cells labeled with [U-^{14}C]glucose. The methods for this determination and the value for EF-G, from which all others are calculated, are from Pedersen et al. (21).

[f] In most gels, spot C40.3 contains two individual proteins. The data shown reflect the behavior of the dominant member of the pair, but must be regarded as approximate.

etate and glycerol). For all save one polypeptide, anaerobic growth on glucose minimal medium led to maximum production. The exception, G31.5, had an anaerobic level exceeded only by its aerobic level with acetate as sole carbon and energy source.

Time course of polypeptide synthesis after shifts in oxygen supply. Changes in growth rate were gradual after aerobic/anaerobic shifts and led eventually to a net 30 to 40% change in rate. A shift to anaerobiosis resulted in a 20-min lag in bacterial growth (Fig. 2, line A). After a shift to aerobiosis, on the other hand, growth continued at the anaerobic rate for about 25 min, then quickly accelerated to the aerobic growth rate (Fig. 2, line B).

To examine the time course of induction and repression of the anaerobiosis-induced polypeptides during these transitions, samples of cultures were pulse-labeled at time intervals during

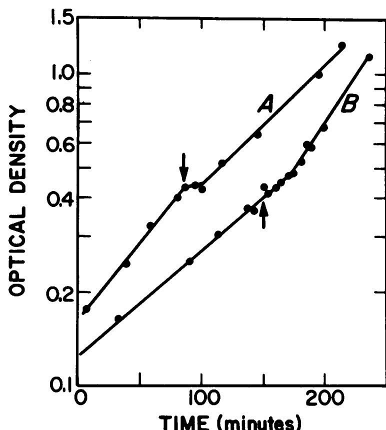

FIG. 2. Growth response of *E. coli* K-12 to shifts in oxygen supply. (A) Shift from aerobic to anaerobic conditions; (B) shift from anaerobic to aerobic. The times of the shifts are indicated by arrows.

the first hour after an abrupt shift in oxygen supply. Rates of synthesis of individual polypeptides at various times after the shift, expressed relative to their preshift rates, were calculated (Fig. 3). Figure 3A gives data for polypeptides G70 (pyruvate formate-lyase, β-subunit) and H97.3. Their response was characterized by an immediate, large change in rate of synthesis, followed by gradual recovery. The peak response for these proteins was far beyond their postshift steady-state rate of synthesis.

Figure 3B gives the time course for 11 other polypeptides, including the 4 known Embden-Meyerhof enzymes. After a 10-min lag, the synthetic rates of these proteins rapidly adjusted to the level of, or slightly greater than, the postshift steady state in the shift to anaerobiosis. The opposite shift yielded a time course similar to that of the polypeptides shown in Fig. 3A.

The proteins represented in Fig. 3C, A15.5, A48, B11.8, and B76, shared an unusually sluggish response to shifts in oxygen availability. Their response to the shift to anaerobiosis was particularly unproductive and in striking contrast to the other anaerobiosis-induced polypeptides.

The responses of four control polypeptides, B46.7, B58.3, F39.7, and G74, are shown in Fig. 3D. The control proteins generally responded little to either shift. Some, however, exhibited significant transient changes in rate of synthesis after one or the other shift.

DISCUSSION

The physiological alterations associated with the transfer of an aerated culture of *E. coli* to anaerobiosis are extensive. Most notable are changes in the routes of carbon source utilization and energy metabolism. After the depletion of oxygen in a glucose-grown culture, for example, the contribution of the hexose monophosphate pathway to glucose consumption decreases from approximately 25% to 5 to 8% (6, 17). At the same time the total rate of glucose consumption probably triples (14), placing a greatly increased demand on the Embden-Meyerhof pathway.

Simultaneously, the routes of pyruvate metabolism undergo equally dramatic changes. The pyruvate dehydrogenase complex is repressed (29) and inhibited (9, 26), and pyruvate is metabolized by pyruvate formate-lyase. This enzyme, which is activated by a complex mechanism requiring anaerobiosis (11, 12), provides acetyl coenzyme A from pyruvate without reduction of NAD^+, a feature advantageous to fermentative metabolism. Finally, the role of the tricarboxylic acid cycle as a source of reducing equivalents for respiration is minimized under anaerobic conditions, again through enzyme repression and inhibition, and the rapid flow of intermediates through glycolysis is then diverted to fermentative pathways.

Since the respiratory chain is inactive, ATP synthesis during anaerobic growth is primarily by substrate-level phosphorylations, and membrane energization requires either ATP hydrolysis (8) or energy coupling much less efficient than by aerobic mechanisms (5).

These alterations in carbon and energy metabolism are accomplished by the combined effects of enzyme activity modulation and induction/repression controls. Some metabolic effectors of enzyme activity have been identified, and regulatory circuits have been proposed and verified (25). The overall contribution of protein induction and repression to the adaptation to anaerobiosis, however, has not previously been assessed.

The present study has revealed 18 polypeptides whose levels are increased by anaerobiosis. Their combined weight fraction during aerobic growth is 81.1, and it increases to 276.8 during anaerobiosis. Although individual polypeptides were induced as much as 11-fold, overall the adaptation to anaerobiosis involves a modest change in the level of a set of polypeptides produced (and presumably functional) during aerobic growth. There may be other polypeptides, not included in the 18, that are induced anaerobically, but our scan has been thorough, and overlooked polypeptides would have to be of quite minor abundance and exhibit only small induction ratios.

Five of the polypeptides have been definitively identified. Not surprisingly, they are all enzymes of intermediary metabolism. Others have

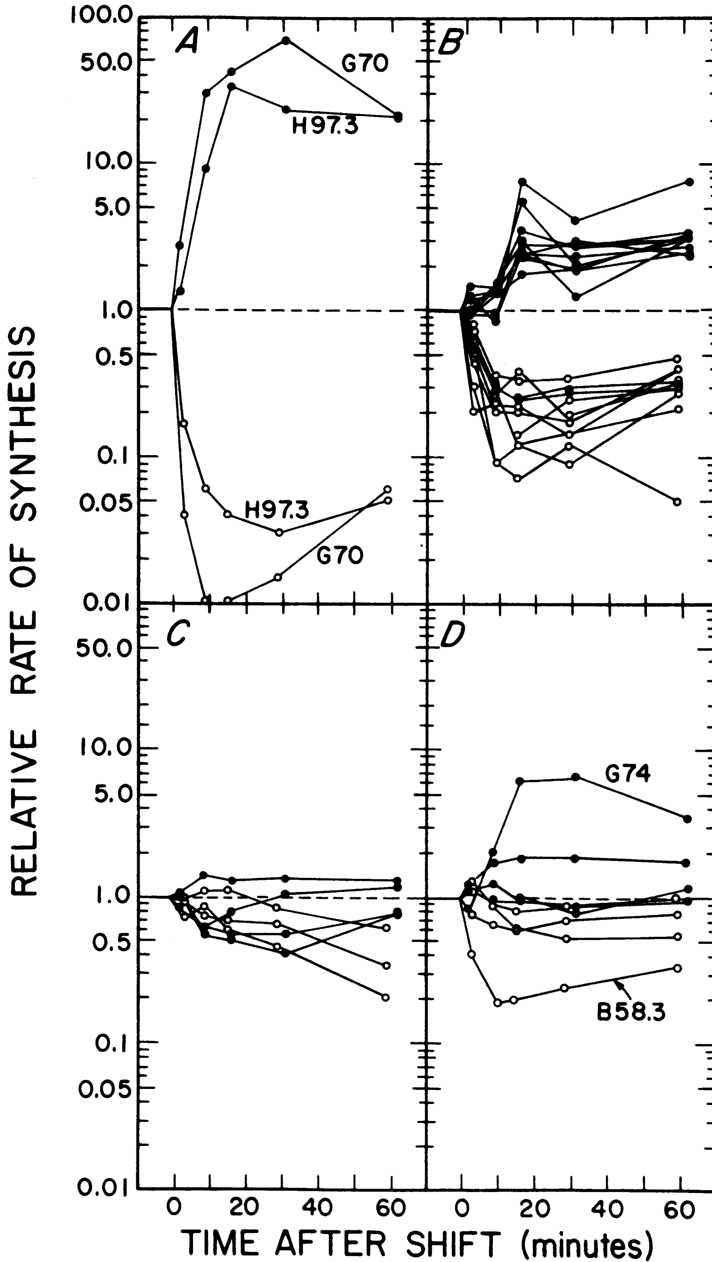

FIG. 3. Relative rates of synthesis of individual proteins at various times after shifts in oxygen supply. (●) Rates after a shift from aerobic to anaerobic; (○) rates after a shift from anaerobic to aerobic. All values are normalized to the measured preshift rate of synthesis. (A) Proteins G70 and H97.3; (B) proteins B15.0, C40.3, C43.1, F35.8, F38, F43.8, F50.5, G31.5 (● only), G54.7, and I33.5; (C) proteins A15.5, A48, B11.8 (● only), and B76; (D) control proteins B46.7, B58.3, F39.7, and G74. Data for polypeptides E79 and G41.3 are not shown.

been identified only tentatively, including proteins E79 and G41.3, which are missing in a mutant lacking the genes encoding acetate kinase and phosphotransacetylase. All Embden-Meyerhof or fermentative enzymes that have been located on our gels are included in the 18, but the biochemical identification of most is unknown. Obvious possibilities include the remaining glycolytic and fermentative enzymes.

The cellular specific activities of a number of enzymes of intermediary metabolism have been studied with respect to changes in oxygen sup-

ply. Small increases in the activities of the Embden-Meyerhof enzymes fructose-6-phosphate kinase (10, 13, 24, 28), fructose-1,6-diphosphate aldolase (10, 13, 24, 28), phosphoglucose isomerase (10), and pyruvate kinase (13, 16, 24) have been observed during anaerobic growth. Our results are in accord with these, and collectively they demonstrate that the dramatic increase in the rate of glucose consumption during anaerobiosis cannot be explained by changes in enzyme levels alone. It is notable that components of the glucose phosphotransferase transport system, enzyme I (control protein B58.3) and HPr (not shown), have the same level irrespective of oxygen supply.

The glycolytic enzymes we have identified differ widely in aerobic metabolic regulation, presumably the result of differences in amphibolic roles associated with their position in the pathway. Their uniform response to oxygen supply, both steady state and transient, and to nitrate indicate that they are sensitive to both common and unshared metabolic signals.

The activation cycle of pyruvate formate-lyase has been studied in detail by Knappe and his co-workers (11, 12). The enzyme is totally inactive in air. Activation when cells are transferred to anaerobiosis involves an unusually complex mechanism. The enzyme is modified in an unknown way during activation. The purified enzyme produces two spots on two-dimensional gels, G74 and G70 (each of which has multiple isoelectric forms), which correspond to the α and β subunits, respectively (see Fig. 1; J. Knappe, personal communication). The relationship between these subunits is not known, either in catalysis or regulation. Our data indicate that G70 (β) responds dramatically to changes in oxygen supply, both transiently and in steady state, presumably by the modification reaction. The substrate (or product) of this reaction is not evident on our gels; G74 (α) is an unlikely candidate since it has the same level in aerobic and anaerobic cultures.

The time course of synthesis of the anaerobiosis-induced polypeptides after abrupt shifts in oxygen supply has been used in this study as an indirect probe of the different metabolic signals to which the polypeptides respond, as well as their temporal relationships. A similarity in response to these shifts is of course not proof that the same effector is involved, nor is it unimaginable that two proteins might respond with much different kinetics to the same signal. Nonetheless, these measurements can greatly simplify the analysis of a complex mixture of proteins which share a common regulatory behavior, and are often able to distinguish direct from indirect effects.

The temporal response found with proteins G70 and H97.3 (Fig. 3A) achieves the desired postshift concentration of protein much more rapidly than would an immediate change in synthetic rate to only the postshift rate. It can be estimated, for instance, that protein H97.3 reaches a concentration equivalent to its anaerobic steady-state level approximately 75 min after a shift to anaerobiosis. Assuming no protein turnover, it would require 160 min to achieve the same end were there no overshoot in synthetic rate.

The short but marked lag in response for nine other polypeptides, including the known Embden-Meyerhof enzymes (Fig. 3B), suggests that the signal(s) to which they respond is elicited only after, and perhaps as a result of, other adjustments made more immediately after the shift.

The extremely sluggish nature of the third type of response (Fig. 3C) implies the gradual accumulation (or depletion) of a regulatory element, such as a metabolite or protein, as adaptation is achieved. It is of interest that the four proteins that display this behavior, A15.5, A48, B11.8, and B76, also share a dramatic repression by nitrate during anaerobic growth.

Protein H97.3 is also repressed by nitrate. Repression by both oxygen and nitrate may be the result of separate but coincident metabolic signals, or may indicate the presence of a more general signal associated with respiratory chain activity. This important distinction, which applies equally to many of the proteins discussed in the accompanying paper, will be the subject of future studies.

A more precise examination of the anaerobiosis-induced proteins, their interrelationships, and the metabolic signals to which they respond is underway.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-17892 from the National Institute of General Medical Sciences. M.W.S. was supported in part by Public Health Service Predoctoral Genetics Training Grant GM-07544, awarded to the Department of Human Genetics, The University of Michigan.

LITERATURE CITED

1. **Ames, G., and K. Nikaido.** 1976. Two-dimensional gel electrophoresis of membrane proteins. Biochemistry **15:**616–623.
2. **Andrews, S., G. B. Cox, and F. Gibson.** 1977. The anaerobic oxidation of dihydroorotate by *Escherichia coli* K-12. Biochim. Biophys. Acta **462:**153–160.
3. **Aranki, A., and R. Freter.** 1969. Use of anaerobic glove boxes for the cultivation of strictly anaerobic bacteria. Am. J. Clin. Nutr. **25:**1329–1334.
4. **Bloch, P. L., T. A. Phillips, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 81 *Escherichia coli* proteins. J. Bacteriol. **141:**1409–1420.
5. **Boonstra, J., J. A. Downie, and W. N. Konnings.** 1978. Energy supply for active transport in anaerobically grown

Escherichia coli. J. Bacteriol. 135:844–853.

6. **Csonka, L. N., and D. G. Fraenkel.** 1977. Pathways of NADPH formation in *Escherichia coli*. J. Biol. Chem. 252:3382–3391.
7. **Flavin, M.** 1975. Methionine biosynthesis, p. 457–503. *In* D. M. Greenberg (ed.), Metabolism of sulfur compounds, vol. VII, Metabolic pathways, 3rd ed. Academic Press, Inc., New York.
8. **Haddock, B. A., and C. W. Jones.** 1977. Bacterial respiration. Bacteriol. Rev. 41:47–99.
9. **Hansen, R. G., and U. Henning.** 1966. Regulation of pyruvate dehydrogenase activity in *Escherichia coli* K12. Biochim. Biophys. Acta 122:355–358.
10. **Hespell, R. B.** 1976. Glycolytic and tricarboxylic acid cycle enzyme activities during intraperiplasmic growth of *Bdellovibrio bacteriovorus* on *Escherichia coli*. J. Bacteriol. 128:677–680.
11. **Knappe, J., H. P. Blaschkowski, P. Grobner, and T. Schmitt.** 1974. Pyruvate formate-lyase of *Escherichia coli*: the acetyl-enzyme intermediate. Eur. J. Biochem. 50:253–263.
12. **Knappe, J., and T. Schmitt.** 1976. A novel reaction of S-adenosyl-L-methionine correlated with the activation of pyruvate formate-lyase. Biochem. Biophys. Res. Commun. 71:1110–1117.
13. **Kotlarz, D., H. Garreau, and H. Buc.** 1975. Regulation of the amounts and of the activity of phosphofructokinase and pyruvate kinase in *Escherichia coli*. Biochim. Biophys. Acta 381:257–268.
14. **Krebs, H. A.** 1972. The Pasteur effect and the relations between respiration and fermentation, p. 1–34. *In* P. N. Campbell and F. Dickens (ed.), Essays in biochemistry, vol. 8. Academic Press, Inc., New York.
15. **Lutkenhaus, J. F.** 1977. Role of a major outer membrane protein in *Escherichia coli*. J. Bacteriol. 131:631–637.
16. **Malcovanti, M., and H. L. Kornberg.** 1969. Two types of pyruvate kinase in *Escherichia coli* K-12. Biochim. Biophys. Acta 178:420–423.
17. **Model, P., and D. Rittenberg.** 1967. Measurement of the activity of the hexose monophosphate pathway of glucose metabolism with the use of [^{18}O] glucose. Variations in its activity in *Escherichia coli* with growth conditions. Biochemistry 6:69–79.
18. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. 119:736–747.
19. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007–4021.
20. **O'Farrell, P. H., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133–1142.
21. **Pedersen, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *E. coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell 14:179–190.
22. **Pertierra, A. G., and R. A. Cooper.** 1977. Pyruvate formation during the catabolism of simple hexose sugars by *Escherichia coli*: studies with pyruvate kinase-negative mutants. J. Bacteriol. 129:1208–1214.
23. **Phillips, T. A., P. L. Bloch, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 55 additional *Escherichia coli* proteins. J. Bacteriol. 144:1024–1033.
24. **Reichelt, J. L., and H. W. Doelle.** 1971. The influence of dissolved oxygen concentration on phosphofructokinase and the glucose metabolism of *Escherichia coli* K-12. Antonie van Leeuwenhoek J. Microbiol. Serol. 37:497–506.
25. **Sanwal, B. D.** 1970. Allosteric control of amphibolic pathways in bacteria. Bacteriol. Rev. 34:20–39.
26. **Schwartz, E. R., L. O. Old, and L. J. Reed.** 1968. Regulatory properties of pyruvate dehydrogenase from *Escherichia coli*. Biochem. Biophys. Res. Commun. 31:495–500.
27. **Smith, M. W., and F. C. Neidhardt.** 1983. Proteins induced by aerobiosis in *Escherichia coli*. J. Bacteriol. 154:344–350.
28. **Thomas, A. D., H. W. Doelle, A. W. Westwood, and G. L. Gordon.** 1972. Effect of oxygen on several enzymes involved in the aerobic and anaerobic utilization of glucose in *Escherichia coli*. J. Bacteriol. 112:1099–1105.
29. **Yamamoto, I., and M. Ishimoto.** 1975. Effect of nitrate reduction on the enzyme levels in carbon metabolism in *Escherichia coli*. J. Biochem. 78:307–315.