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Elevated Serine Catabolism Is Associated with the Heat Shock Response in *Escherichia coli*

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The biochemical events associated with the heat shock response are not well understood in any organism, nor have the signals that initiate the induction of heat shock protein synthesis been identified. In this work, we demonstrate that the rate of serine catabolism of *Escherichia coli* cells grown in glucose minimal medium supplemented with serine is elevated three- to sevenfold when the growth temperature is shifted from 37 to 44°C. Elevations in growth temperature and mutations or treatments that lead to elevated basal rates of serine catabolism at 37°C result in the excretion into the culture medium of acetate derived from exogenous serine. Increases in the basal level of serine catabolism at 37°C do not per se induce a heat shock response but are associated with abnormalities in the pattern of induction of heat shock polypeptides following a temperature shift. We postulate that the events responsible for or resulting from the elevation in serine catabolism associated with a shift-up in temperature modulate the induction of 3 of the 17 heat shock polypeptides identified in *E. coli*. These observations suggest that heat shock diverts serine away from the production of glycine and C_1 units, which are required for initiation of protein synthesis and for nucleotide biosynthesis, and towards acetyl coenzyme A and acetate.

When *Escherichia coli* cells are shifted from 37°C to a higher but nonlethal temperature, they respond by inducing the synthesis of a group of polypeptides referred to as heat shock proteins (18). Synthesis of these polypeptides is under control of a heat shock regulatory protein encoded by the *htpR* gene (19). This polypeptide appears to be an alternate σ factor (σ^{32}) for RNA polymerase, which recognizes consensus sequences in the promoter regions of heat shock genes and directs their transcription (10). If cells are transformed with a plasmid containing the *htpR* coding sequence under control of the *tac* promoter, the synthesis of σ^{32} can be induced at 28°C by addition of isopropyl-β-D-galactopyranoside to the growth medium (11). Under these conditions, 10 of the 15 heat shock polypeptides measured were induced by isopropyl-β-D-galactopyranoside at 28°C as well as or better than by a shift to 42°C, and three were very poorly induced by isopropyl-β-D-galactopyranoside at 28°C (28). The latter three heat shock polypeptides were later shown to be abnormally induced in a mutant strain (*metK*) with reduced adenosylmethionine synthetase activity (16). At that time it was postulated that the induction of these three polypeptides required a metabolic signal in addition to the synthesis of the *htpR* gene product and that this metabolic signal was abnormal in the *metK* mutant strain. Such a signal must be induced independently of σ^{32}, or its generation must require both σ^{32} and a shift-up in temperature.

The *metK* strain is prototrophic at 37°C but shows an unexpected requirement for supplementation of glucose minimal medium with serine and isoleucine to sustain rapid growth at 44°C (16). We now demonstrate that the mutant strain shows an elevated rate of serine catabolism at 37°C. We provide evidence that the abnormalities in the heat shock polypeptide induction pattern in the *metK* strain can be mimicked by treatments of its isogenic parent with compounds that elevate serine catabolism at 37°C. All strains tested exhibit higher rates of serine catabolism at temperatures above 37°C, and in the mutant strain the diversion of endogenously synthesized serine becomes limiting for growth at 44°C.

MATERIALS AND METHODS

Bacterial strains. The majority of experiments presented in this paper were conducted with the isogenic strains RG (*metK*$^+$) and RG62 (*metK*) isolated by Greene et al. (9). These strains are prototrophic at 37°C. Experiments to determine whether activation of L-serine deaminase requires σ^{32} were conducted with the isogenic strains CG2044 (*htpR*$^+$ *trp*) and CG2046 (*htpR trp*) constructed by Grossman et al. (12).

Media and growth conditions. All cultures were grown aerobically in rotatory action shakers at either 37 ± 0.1 or 44 ± 0.2°C. The growth of cells was monitored at 420 nm, and the cells were grown to an optical density of 0.3 (~3 × 10^7 cells per ml) before the temperature was shifted by transfer from a bath at 37°C to one at 44°C. Cells were grown in 3-(*N*-morpholine)propanesulfonic acid (MOPS) minimal medium (17) supplemented with 10 μM thiamine, 0.4% glucose, and amino acids as indicated. The concentrations of amino acid supplements are those used in defined rich medium (29) unless noted otherwise. Tryptophan (100 μM) was added to glucose minimal MOPS medium for growth of strains CG2044 and CG2046.

Measurements of catabolism of exogenous labeled serine. Strains RG and RG62 were cultured in glucose minimal MOPS medium with supplementation as noted. When the strain was in exponential growth phase with an A_{420} of 0.3, L-[3-^{3}H]serine (17.5 dpm/pmol) was added to give a final concentration of 250 μM. Samples (2 ml) of the culture were withdrawn at 5-min intervals after addition of serine and filtered to remove the cells. When measurements of the rate of serine catabolism were required, the filtered medium was passed over an AG50X8 H^+ column (4-ml bed volume) to remove residual serine, and the column was washed with 10 ml of glass-distilled water. Portions of the eluate were

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counted to determine the concentration of labeled neutral and acidic products in the medium. In experiments in which analysis of the labeled products was performed, the culture was incubated with labeled serine either during exponential growth at 37°C or starting 5 min after a shift to 44°C. The incubation was continued for 30 min and then terminated by filtration of the cells. The filtrate was acidified and lyophilized, and the volatile fraction was collected in a cold trap immersed in dry ice-methanol. The volatile fraction could then be subjected to analysis on a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column which was eluted isocratically with 8 mM H_2SO_4. The eluate was monitored at 214 nm, 1-ml fractions were collected, and radioactivity was measured in a Beckman LS7500 scintillation counter.

Measurements of in vivo serine metabolism. Cultures were labeled with L-[3-^{3}H]serine for 30 min as described in the section above. The cells were then washed and disrupted by sonic treatment. Total soluble cellular protein was isolated by trichloroacetic acid precipitation after treatment with RNase-DNase. The precipitated protein was subjected to acid hydrolysis and derivatized with phenylisothiocyanate, and the phenylisothiocyanate amino acids were separated by reversed-phase high-pressure liquid chromatography (26). Each amino acid peak was collected separately for scintillation counting. The specific radioactivity of the tyrosine peak could not be determined because a radioactive impurity eluted at the same position.

Radioactive labeling of protein and resolution on two-dimensional gels for autoradiography. Samples of cultures of exponentially growing cells (A_{420}, ~0.3) were removed during growth at 37°C and again 5 min after a shift of the culture to 44°C. These samples (2 ml) were transferred to preheated vials containing L-[^{3}H]lysine (200 μCi; 83 Ci/mmol) and labeled for 5 min. Unlabeled lysine (16 μmol) was added for a 3-min chase. Extracts were prepared and processed for resolution on two-dimensional polyacrylamide gels (22) as previously modified (3). Gels were treated with 2,5-diphenyloxazole after dehydration in dimethyl sulfoxide, dried, and exposed to Kodak XAR film for 2 to 16 days at −70°C (4).

RESULTS

To explore the basis for the nutritional requirements of a *metK* strain at 44°C, we examined the metabolism of exogenous serine in mutant (RG62) and parent (RG) strains at 37 and 44°C. Preliminary experiments established that labeled intracellular products were insufficient to account for the uptake of labeled exogenous serine. Figure 1 shows the results of an experiment in which the label remaining in the medium during incubation of cells with labeled exogenous serine (250 μM) was analyzed. Serine taken up by the cells was converted to a mixture of neutral and acidic compounds that are excreted into the medium. The rate of excretion of these products at 37°C was elevated ~3-fold in the *metK* strain as compared with the rate in its isogenic parent, and in both strains the rate was further elevated after a shift of temperature from 37 to 44°C. The elevation associated with a temperature shift from 37 to 44°C was somewhat variable, and we have observed three- to sevenfold elevations for strain RG. About 75% of the serine taken up by the cells at 44°C was metabolized to excreted products. The differences in rates of catabolism of serine observed in these experiments were not merely due to differences in growth rates. Under these conditions, the *metK* strain had doubling times

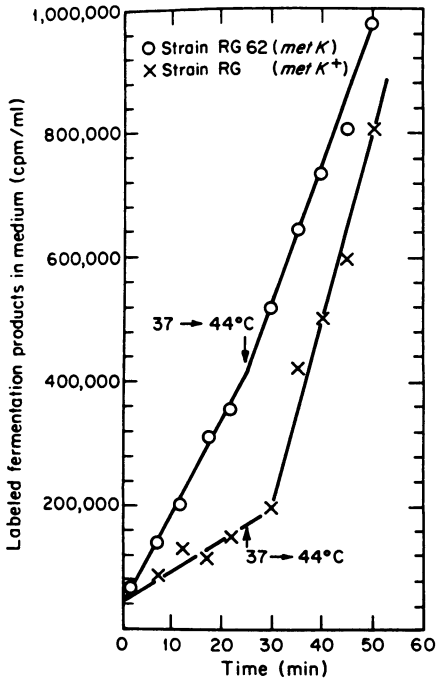

FIG. 1. Catabolism of exogenous serine by *E. coli* RG (*metK*$^+$) and RG62 (*metK*) before and after a shift from 37 to 44°C. The strains were cultured at 37°C in glucose minimal MOPS medium supplemented with thiamine. When the culture was in exponential growth phase, with an A_{420} of 0.3, L-[3-^{3}H]serine (17.5 dpm/pmol) was added to give a final concentration of 250 μM. Measurements of the rates of serine catabolism were carried out as described in Materials and Methods. At 25 min after the addition of serine, the cultures were shifted from 37 to 44°C and measurements were continued for another 25 min.

of 65 min at 37°C and 260 min at 44°C, while its isogenic parent had doubling times of 57 and 103 min, respectively (16). The increases in the rate of serine catabolism seen after shifts from 37 to 44°C were also not merely due to the effect of temperature on the reaction velocity. An experiment was performed in which one culture was shifted from 37 to 44°C for 5 min and then returned to 37°C, while another identical culture was maintained at 44°C after the shift. The rates of serine catabolism in the two flasks were identical for as long as measurements were made (25 min after the shift).

The labeled products excreted into the medium can be purified by lyophilization of the acidified medium and collection of the volatile fraction in a cold trap. About 85% of the labeled products are volatile and are recovered from the cold trap. Analysis of the purified volatile products with a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column indicated that label eluted in two peaks with retention times of 14.6 and 17.4 min, respectively. The compound eluting at 14.6 min had the same retention time as acetic acid, a normal product formed during aerobic metabolism of glucose, and, like acetate, was retained on the anionic exchange resin AG1X8 chloride at neutral pH but not at pH 2. Incubation of cells with L-[2,3-^{13}C]serine resulted in the formation of acetate labeled in both carbons as indicated by proton nuclear magnetic resonance spectroscopy (Fig. 2). The second peak eluted from the HPX-87H high-pressure liquid chromatography column in the same position as tritiated water or methanol. A series of experiments in which [3-^{3}H]serine was mixed with uniformly ^{14}C-labeled glucose, serine, or bicarbonate and incubated

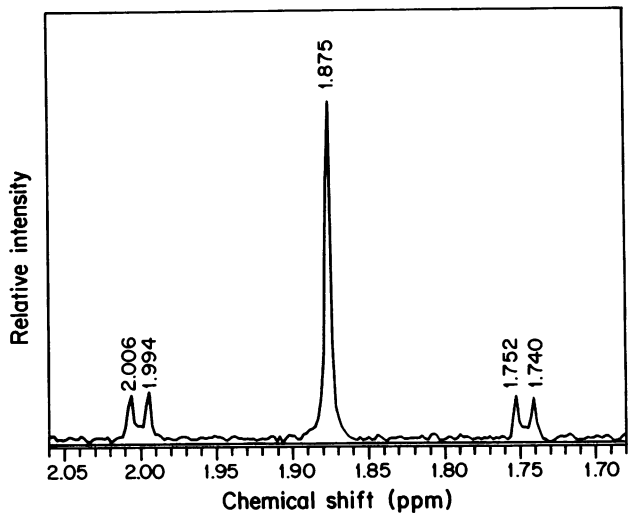

FIG. 2. Proton nuclear magnetic resonance spectroscopy of the volatile fraction obtained from the medium after incubation of strain RG with L-[2,3-$^{13}C_2$]serine. Strain RG was grown at 37°C in glucose minimal MOPS medium supplemented with thiamine, glycine, leucine, and isoleucine, and with 5 mM L-[2,3-$^{13}C_2$]serine. When the culture reached an A_{420} of ~4, the incubation was terminated by filtration and the medium was lyophilized after acidification with 400 μl of 1 N HCl. The volatile fraction was collected in a cold trap and reneutralized. The proton nuclear magnetic resonance spectrum of this sample was measured in a General Electric 500 MHz nuclear magnetic resonance spectrometer, with 10% D_2O as a lock, using the selective 1-3-3-1 pulse sequence described by Hore (14). No other ^{13}C-labeled compounds could be detected in the sample, although a peak corresponding to unlabeled methanol (presumably derived during shell freezing the sample in a dry ice-methanol bath) was also detected. The observed chemical shifts and coupling constants for acetate correspond closely to the reported values: δ = 1.88 (reported, 1.88); $^1J_{C-H}$ = 128 Hz (reported, 130 Hz); $^2J_{C-H}$ = 6 Hz (reported, 6.9 Hz for acetate *O*-methyl ester).

with cultures of *E. coli* growing at 44°C did not lead to appearance of ^{14}C in the peak eluting at 17.4 min, and we tentatively concluded that this peak was due to tritium released to the solvent during serine catabolism. Furthermore, oxidation of the purified volatile products with acidic aqueous sodium dichromate, which converts methanol to formic acid, did not affect the elution position of the second radiolabeled peak from the HPX-87H column, although formic acid elutes at 13.4 rather than 17.4 min. In glucose minimal MOPS medium, 50% of the excreted volatile label from L-[3-^{3}H]serine is associated with acetic acid, and 50% is released to the solvent; these ratios are the same for mutant and parent strains at each temperature.

We next examined intracellular products of serine in mutant and parent strains. Table 1 shows the incorporation of label from exogenous L-[3-^{3}H]serine into the amino acid residues of the total cellular protein isolated from cells grown in glucose minimal medium. The average specific radioactivity of the intracellular serine pool during labeling was presumably reflected directly in the specific radioactivity of the seryl residues incorporated into protein. The specific radioactivity of seryl residues in strain RG62 (*metK*) was ~4-fold lower than that in its isogenic parent, strain RG, suggesting that the synthesis of serine from unlabeled glucose proceeds at a higher rate in strain RG62. Since the experiments whose results are shown in Fig. 1 and Table 1 were performed under identical conditions, the rate of serine catabolism of strain RG62 at 37°C is probably 12-fold higher than that of strain RG, if the greater dilution of labeled serine by endogenously synthesized serine in the mutant strain is taken into account. Also, at 37°C, the mutant strain incorporated a greater fraction of the serine label into amino acids derived from pyruvate than its parent did, indicating a diversion of serine metabolism towards pyruvate in this strain. When the incorporation of label from serine into amino acid residues at 37 and 44°C is compared, both strains show an increase in the specific radioactivity of leucyl residues at 44°C but a general increase in the flux of label into all amino acids derived from pyruvate is not observed. In contrast, the temperature shift did result in substantial elevation in the rate of excretion of labeled acetate in the parent strain and smaller elevations in the *metK* strain (Fig. 1). These observations suggest that temperature shifts result in a rather specific channeling of exogenous serine into pathways leading to acetyl coenzyme A (which is directly incorporated into leucine but not other amino acids) and acetate. Since exogenous serine is required for rapid growth of the mutant strain at 44°C, endogenously synthesized serine must also be diverted towards acetate or acetyl coenzyme A or both, so that the availability of serine for other reactions becomes limiting for growth. In both strains, the increase in serine catabolism after a temperature shift is attained within 5 min. Induction of the heat shock response occurs equally rapidly, with the maximal rate of synthesis of heat shock polypeptides observable 5 to 10 min after a temperature shift (20).

TABLE 1. Incorporation of label from [3-^{3}H]serine into the amino acids of *E. coli* proteins[a]

Strain and temperature	Specific radioactivity (dpm/pmol) of serine pool	Specific radioactivity ratio[b] of:														
		Ala[c]	Cya	Asx	Glx[c]	Phe	Gly	His	Ile[c]	Lys[c]	Leu[c]	Met	Pro[c]	Arg[c]	Thr	Val[c]
RG (*metK*$^+$)																
37°C	5.08	0.137	0.924	0.035	0.058	0.029	0.043	0.340	0.211	0.084	0.190	1.573	0.095	0.071	0.022	0.216
44°C	6.14	0.169	0.742	0.015	0.070	0.029	0.034	0.298	0.142	0.072	0.306	1.614	0.079	0.068	0.024	0.261
RG62 (*metK*)																
37°C	1.42	0.356	0.751	0.041	0.186	0.073	0.046	0.167	0.189	0.148	0.283	1.559	0.165	0.126	0.085	0.424
44°C	1.47	0.333	0.908	0.023	0.169	0.096	0.035	0.279	0.286	0.183	0.404	0.906	0.209	0.192	0.096	0.434

[a] Cultures were grown in glucose minimal MOPS medium supplemented with thiamine. After cells were labeled for 30 min with [3-^{3}H]serine, the total cellular protein was isolated and the specific radioactivities of the component amino acids were determined as described in Materials and Methods.

[b] Ratio of specific radioactivity of indicated amino acid to that of serine.

[c] Amino acid derived from pyruvate and/or from acetyl-CoA or α-ketoglutarate formed from pyruvate. (α-Ketoglutarate dehydrogenase activity is repressed in *E. coli* grown aerobically with glucose as the carbon source [1].)

We wished to determine whether the elevated level of serine catabolism in strain RG62 (*metK*) is related to its abnormal heat shock polypeptide induction pattern. This strain shows constitutive synthesis of the *lysU* gene product, lysyl-tRNA synthetase form II, at 37°C (13) and greatly reduced levels of induction of two unidentified heat shock polypeptides, C14.7 and G13.5, on a temperature shift to 44°C (16). While nothing is known about the regulation of synthesis of the latter two proteins except that they are members of the heat shock regulon, there have been several studies of regulation of *lysU* expression. Synthesis of lysyl-tRNA synthetase form II has been shown to be elevated when glucose minimal medium is supplemented with glycyl-L-leucine and isoleucine (13). Since L-serine deaminase, which converts serine to pyruvate, is known to be induced by glycine and leucine (15, 23) and is also induced at elevated temperatures (21), we suspected that induction of *lysU* might be linked to activation of L-serine deaminase by glycyl-L-leucine. Supplementation of the medium with either glycyl-L-leucine or glycine plus leucine leads to marked growth inhibition if isoleucine is not included in the medium, and so we compared the rate of serine catabolism at 37°C of the parent strain in the presence of isoleucine (0.4 mM) alone with that in the presence of isoleucine, glycine (0.8 mM), and leucine (0.8 mM), or with isoleucine plus 3 mM glycyl-L-leucine. Both glycyl-L-leucine and glycine plus leucine resulted in an ~2-fold elevation of the rate of catabolism of labeled exogenous serine. A temperature shift from 37 to 44°C in the presence of glycine plus leucine resulted in only a 1.3× increase in the rate of serine catabolism after the shift (data not shown). In Fig. 3, patterns of protein synthesis are compared for cells of strain RG grown in the presence or absence of glycyl-L-leucine after labeling during exponential growth at 37°C (Fig. 3A and B) or after labeling 5 to 10 min following a shift of the cultures to 44°C (Fig. 3C and D). Addition of glycyl-L-leucine to the medium results in changes that resemble those seen previously in *metK* mutants grown in glucose minimal medium (16). During exponential growth at 37°C, these changes include constitutive synthesis of lysyl-tRNA synthetase form II, incresed synthesis of MetE (cobalamin-independent methionine synthase), and decreased synthesis of protein W, a factor required for the translation of phage message (7). The identities of these peptides were inferred from their coordinates in the gene-protein index (25). In contrast to *metK* mutants, which are deficient in adenosylmethionine synthetase, treatment of the *metK*$^+$ strain with glycyl-L-leucine does not appear to alter the synthesis of this polypeptide. Supplementation of the growth medium with glycyl-L-leucine also has a profound effect on the pattern of protein synthesis 5 to 10 min after shift of the culture from 37 to 44°C. The induction of synthesis of C14.7 and G13.5 is clearly depressed in the medium containing glycyl-L-leucine. Again, the resemblance to the protein synthesis pattern seen after a temperature shift of a *metK* strain grown in glucose minimal medium is striking (16). Similar alterations in the patterns of protein synthesis at 37°C and after a shift to 44°C were also observed in media supplemented with glycine, leucine, and isoleucine (data not shown). We conclude that the synthesis of 3 of the 17 identified heat shock polypeptides in *E. coli* is affected by factors responsible for or resulting from the elevation of serine catabolism after a temperature shift.

We have examined the effects of a temperature shift from 28 to 42°C on serine catabolism in an *htpR* strain (CG2046) and its isogenic parent (CG2044). Serine catabolism was elevated after the shift in both strains (Fig. 4). The rate of serine catabolism in the two strains did begin to deviate 15 or more min after the temperature shift, but we attribute these differences to the cessation of growth of the *htpR* strain at 42°C. Thus, the elevation in serine catabolism after a temperature shift appears not to require σ^{32} induction. A companion experiment conducted with strain RG in which the rate of serine catabolism was measured at 28°C and after a shift to 42°C (data not shown) permitted an estimate of the temperature dependence of the rate of serine catabolism in this strain when the strain was grown in glucose minimal MOPS. These data are shown in Fig. 5, along with data from a 37-to-44°C shift. The rate of serine catabolism rises sharply at temperatures above 37°C and appears to parallel the threshold for induction of the heat shock response (20).

DISCUSSION

Our studies suggest that the elevation in serine catabolism observed after a temperature shift from 37 to 44°C may constitute a metabolic signal linked to the induction of three of the heat shock polypeptides: lysyl-tRNA synthetase form II, C14.7, and G13.5. This signal appears to be generated independently of the synthesis of σ^{32}. If the rate of serine catabolism is elevated before the temperature shift, as occurs with strain RG62 in glucose minimal MOPS medium or with strain RG in medium supplemented with glycine and leucine or with glycyl-L-leucine, the *lysU* gene product is constitutively induced. In these cases a temperature shift results in a smaller-than-normal increase in the rate of serine catabolism after the shift, and C14.7 and G13.5 are poorly induced. It is not clear whether the linkages are direct (as would be the case if L-serine deaminase itself were the inducer) or indirect and mediated by metabolic changes causing or resulting from the activation of L-serine deaminase activity. Our results suggest that elevation in L-serine deaminase activity would increase the cellular pools of pyruvate or acetyl coenzyme A or both, and these pools may in turn be involved in the induction of this subset of heat shock proteins.

Our observations also help to explain some interesting observations made by Danchin and his co-workers in studying serine toxicity. These workers showed that high levels of serine in the medium lead to isoleucine restriction and that the toxicity of serine is exacerbated by inclusion of glycine, methionine, and leucine in the medium, forming the basis of the SGM plate test for *relA* mutants (5, 27). They noted further that serine toxicity is enhanced by elevated temperature. Given our results, the exacerbation of serine toxicity by heat or by glycine and leucine is probably linked to the elevated rate of serine catabolism induced by these agents. We have also shown that the presence of methionine in the medium increases the rate of serine catabolism at 37°C (data not shown). If pyruvate pools are expanded because of the elevated rate of serine catabolism induced by these agents, isoleucine restriction is likely to occur. Barak and his co-workers (2) have shown that the pyruvate/α-ketobutyrate ratio is critical in determining the ratio of acetolactate to acetohydroxybutyrate formed by the acetohydroxy acid synthase isozymes and thus that elevations in pyruvate pools may lead to isoleucine restriction. The presence of leucine in the medium will compound this problem by repressing the synthesis of the *ilvH* gene product (6), which is the acetohydroxy acid synthase isozyme responsible for initiating the synthesis of isoleucine in K-12 strains of *E. coli* (2).

The rationale for reprogramming serine metabolism after a temperature shift remains to be learned. Our results are

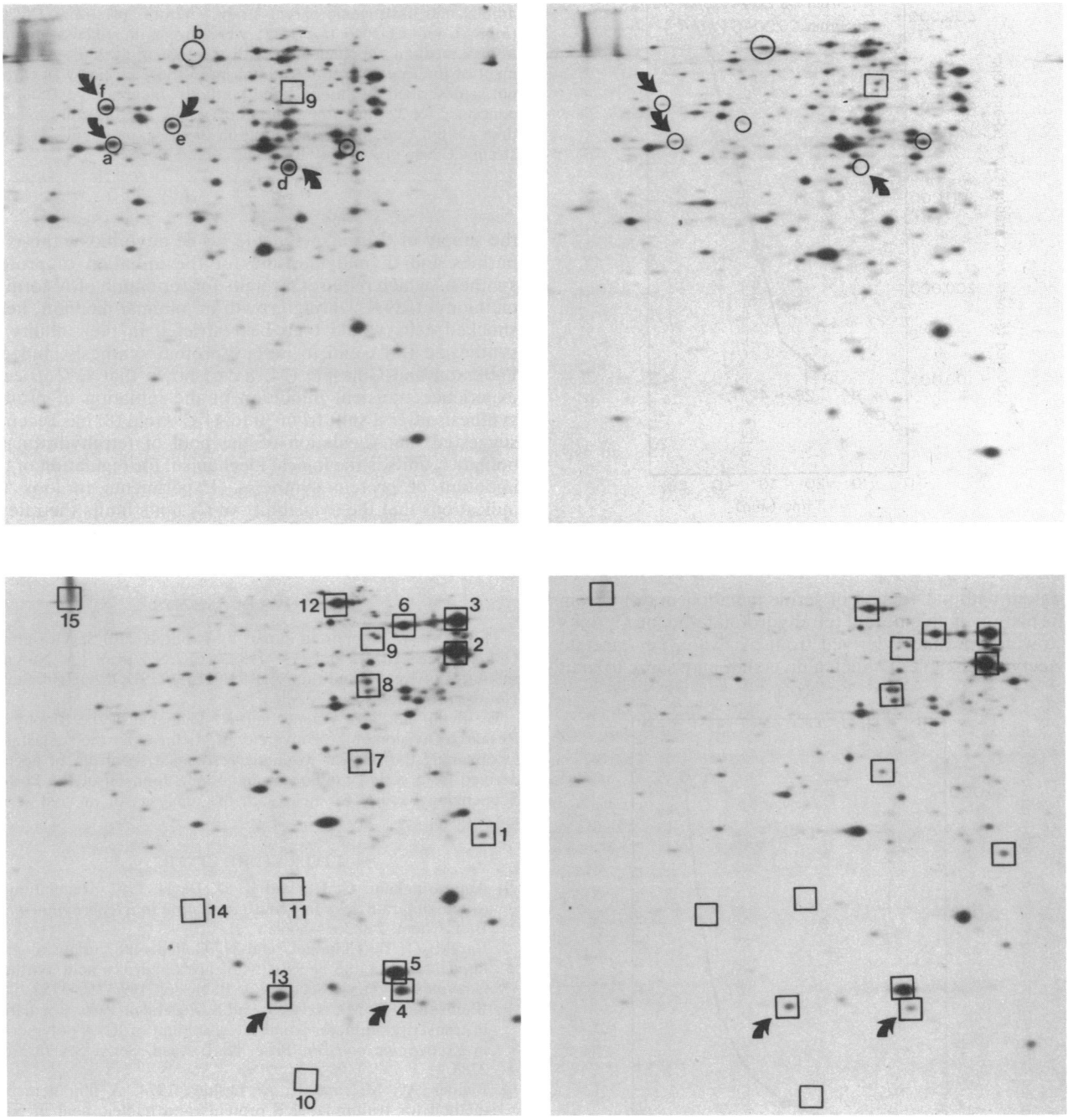

FIG. 3. Effect of glycyl-L-leucine on the synthesis of individual polypeptides in strain RG (*metK*$^+$) before and after a shift from 37 to 44°C. Polypeptides were separated by isoelectric focusing in the horizontal dimension (acidic proteins migrate to the right) and by electrophoresis in the presence of sodium dodecyl sulfate in the vertical dimension (small proteins appear at the bottom). (A and B) Cells grown in glucose minimal MOPS medium supplemented with thiamine and Ile (0.4 mM) at 37°C (A) or with thiamine, 3 mM glycyl-L-leucine, and 0.4 mM Ile at 37°C (B) Circles indicate polypeptides whose synthesis is altered in the presence of glycyl-L-leucine or in *metK* mutants or both. These include protein W (a factor required for translation of phage message [7]) (a), cobalamin-independent methionine synthase (the *metE* gene product) (b), and adenosylmethionine synthetase (the *metK* gene product) (c). The square labeled 9 indicates the polypeptides of lysyl-tRNA synthetase form I (the only labeled peptide in this square in panel A and the lower spot in the square in panel B) and form II (upper spot in the square in panel B). Polypeptides with decreased levels of synthesis in the presence of glycyl-L-leucine are indicated by arrows. (C and D) Cells from the culture grown in glucose minimal MOPS supplemented with Ile (shown in panel A) (C) and from the culture grown in the presence of glycyl-L-leucine (shown in panel B) (D) labeled 5 to 10 min after a shift to 44°C. The boxes indicate heat shock polypeptides, which are numbered in panel C, and correspond to α-numerics from the gene-protein index (25), as follows: 1, B25.3; 2, B56.5 (*groEL*); 3, B66.0 (*dnaK*); 4, C14.7; 5, C15.4 (*groES*); 6, C62.5; 7, D33.4; 8, D48.4; 9, D60.5 (*lysU*); 10, F10.1; 11, F21.5; 12, F84.1; 13, G13.5; 14, G21.0; 15, H94.0 (*lon*).

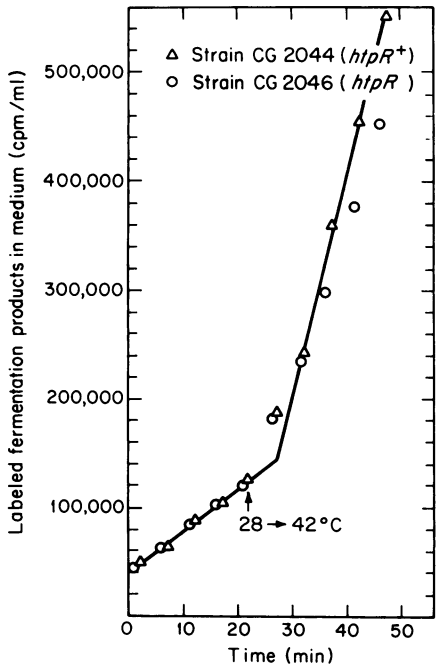

FIG. 4. Rate of excretion of fermentation products derived from exogenous serine before and after a shift from 28 to 42°C by an *htpR* mutant and its isogenic parent strain. Cultures of strains CG2044 (*htpR*$^+$) and CG2046 (*htpR165*) were grown in glucose minimal MOPS medium supplemented with tryptophan at 28°C. Measurement of the rates of serine catabolism in these strains was carried out as described in Materials and Methods. Incubation at 28°C was continued for 22 min after addition of serine, and the cultures were then shifted to 42°C for the duration of the experiment. At 42°C, strain CG2046 grows only for about 30 min.

consistent with a diversion of serine metabolism away from the formation of glycine and tetrahydrofolate-bound C_1 units and towards products derived from pyruvate, i.e., acetate and acetyl coenzyme A. Such a diversion may serve to limit the supply of C_1 units available for de novo biosynthesis of purines and thymidylate and for the initiation of protein synthesis which requires C_1 units for formation of *N*-formylmethionyl tRNA. During growth in minimal medium, heat-shocked cells would then be restricted in their ability to synthesize DNA and to initiate protein synthesis. Indeed, Patterson and Gillespie (24) have shown that *E. coli* cells experience transient inhibition of the initiation of protein synthesis after a shift from 30 to 44°C. Gold (8) has recently suggested that regulation of the pool of tetrahydrofolate-bound C_1 units is the logical mechanism for regulation of the initiation of protein synthesis. Experiments to look for indications that the availability of C_1 units limits the rate of growth of heat-shocked cells are now in progress in our laboratories.

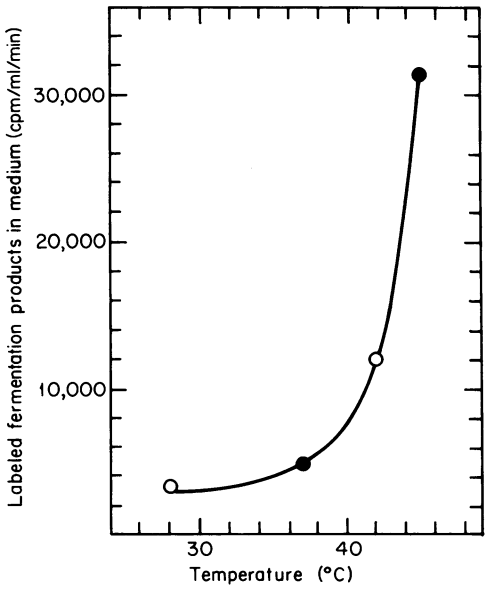

FIG. 5. Temperature dependence of the rate of serine catabolism to form volatile excretion products. All measurements were performed using prototrophic strain RG, and the rate of excretion was determined from the slopes of plots of serine catabolism versus time such as those shown in Fig. 1 and 4. Data are given for catabolism in glucose minimal MOPS, except that the measurements at 28 and 42°C were made in medium supplemented with 0.1 mM tryptophan. Symbols: ○, rates of serine catabolism measured during exponential growth at 28°C and after a shift to 42°C; ●, rates of serine catabolism measured during exponential growth at 37°C and after a shift to 44°C.

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