JOURNAL OF BACTERIOLOGY, July 1973, p. 9-14
Vol. 115, No. 1

Induction Kinetics of the L-Arabinose Operon of *Escherichia coli*[1]

ROBERT SCHLEIF, WINAND HESS,[2] SOLOMON FINKELSTEIN, AND D. ELLIS

Graduate Department of Biochemistry and Department of Biology, Brandeis University, Waltham, Massachusetts 02154

Received for publication 22 March 1973

After addition of L-arabinose to growing *Escherichia coli*, the L-ribulokinase (EC 2.7.1.16) and L-arabinose isomerase (EC 5.3.1.4) first appear at about 0.7 and 1.4 min, respectively. These times are consistent with the distances of the genes from the ribonucleic acid polymerase initiation site in the operon. The kinetics of appearance of these enzymes as well as those of β-galactosidase (EC 3.2.1.23) in the same strain are consistent with a peptide elongation rate of no less than 14 amino acids per second. A measurement of the average peptide elongation rate made by measuring the kinetics of radioactive amino acid appearance in completed polypeptides yielded a rate of about 12 amino acids per s. Convenient assays of the arabinose isomerase and ribulokinase are also given.

The interval after addition of inducer to a culture of cells and the first appearance of induced enzyme reflects the following processes: entry of inducer into the cell, modification of inducer if necessary, inducer interaction with regulatory macromolecules, initiation of transcription, transcription of the gene, translation of the messenger, folding of the polypeptide, association of subunits, and further conformational changes before acquisition of enzymatic activity. Several of these processes are known to occur concurrently during induction of the *lac* operon of *Escherichia coli*. During transcription ribosomes begin translation, and the emerging polypeptide is free to begin folding (2, 6). Our present knowledge allows a rather accurate prediction of the times required for transcription and translation; thus, by comparison of this minimal interval to the interval actually found before newly induced enzyme appears, maximal upper limits may be placed on the times required for the other processes. In the *lac* operon, essentially all of the interval between inducer addition and the appearance of new enzymatic activity is occupied by the transcription-translation process (2). Similarly, in the work presented here on the induction kinetics of the arabinose operon, it is found that, although the L-ribulokinase and L-arabinose isomerases are detectably increased approximately 30 s later than required by transcription-translation, most of this 30-s time period is required for sufficient enzyme accumulation to be detectable above the background of the basal amounts of the enzymes.

MATERIALS AND METHODS

Growth medium and strain. $MnCl_2$ was added to minimal medium M9 (8) to give a concentration of 5×10^{-5} M; this contained 0.2% glycerol, 0.01% L-leucine, 0.01% L-threonine, and 0.001% thiamine and was used throughout the investigation. Cells had a doubling time of 90 min and were always used at a concentration of 2.5×10^8 cells per ml. The cells were strain JTL76, K-12 F^- *thr leu thi ara*$^+$ *lac*$^+$ derived from Pasteur strain RV Δlac_{74}. The arabinose genes of this strain were derived from strain RFS73, and the lactose genes were derived from strain RFS1 (8).

Assay of arabinose isomerase. Typically, 3 ml of culture were added to a (13 by 100 mm) tube containing 1 ml of M9 medium containing 400 μg of chloramphenicol per ml and incubated at 37 C for 10 min. After this incubation to allow complete folding of the newly synthesized polypeptides, 48 tubes were centrifuged at one time at 3,500 rpm for 15 min in a Sorval GSA rotor by the use of adapters, each holding 8 tubes. The supernatant fluid was aspirated off, and the cells were resuspended in 100 μliters of 0.1 M tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 7.7) containing 0.1 M L-arabinose, 0.01 M $MnCl_2$, and 100 μg of chloramphenicol per ml. Solutions of 0.2 M Tris buffer, 0.2 M L-arabinose, 200 μg of chloramphenicol per ml, and 0.02 M $MnCl_2$ were mixed shortly before use. Toluene (5 μliters) was added, and the cells were vigorously mixed for 10 s.

[1] Publication no. 899 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154.

[2] Present address: Department of Biology, University of California, San Diego, La Jolla, Calif. 92037.

After incubation at 37 C up to 20 h, the reaction was stopped by addition of 0.9 ml of 0.1 M HCl; 100 μliters of freshly dissolved 1.5% cysteine was added and, immediately after addition of 100 μliters of 0.12% carbazole (recrystallized from ethanol; Sigma Chemical Co.), 3 ml of 70% H_2SO_4 was added. After 20 min, color was read in a Bausch & Lomb Spectronic 20 spectrometer at 550 nm without transfer to another tube. Both HCl and H_2SO_4 addition were made with Lab Industries Repipetts.

Assay of L-ribulokinase. The enzyme was measured by production of ^{14}C-labeled ribulose phosphate separated from the uncharged substrates by chromatography on diethylaminoethyl (DEAE) paper. Substrate ^{14}C-labeled ribulose was synthesized in the assay mixture from L-[^{14}C]arabinose and excess arabinose isomerase. Cells centrifuged as described above were suspended in an equal volume of M9 medium and centrifuged, then resuspended in 100 μliters of assay mixture consisting of 0.05 M potassium phosphate buffer (pH 7.6), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.03 M magnesium acetate, 8 mM adenosine triphosphate, 5 mM NaN_3, 2 mM dithiothreitol, 300 μg of chloramphenicol per ml, 0.5 mg of streptomycin per ml, 0.01 M NaF, 0.02 ml of L-arabinose isomerase extract per ml of assay mixture prepared as described below, 10 μliters of L-[^{14}C]-arabinose (10,000 counts/min/μliter) per ml of assay mixture, and 5×10^{-4} M L-arabinose. A 5-μliter sample of toluene was added, and the cells were mixed vigorously for 30 s and left on ice 30 min before incubation at 30 C. The reaction rate was constant for at least 20 h. After incubation, strips of DEAE Whatman DE 81 paper (1.5 by 14 cm) folded lengthwise and then folded back 3 cm from one end were inserted into the incubation tubes and allowed to absorb the assay mixture, spotting the strip 3 cm from the end. The strip was removed, unfolded, and developed with water in ascending chromatography. The radioactivity in the first 9 cm was measured in a scintillation counter. The assay was proportional to ribulokinase added up to 5,000 counts/min of [^{14}C]-ribulose produced. No ribulokinase added gave a background of about 150 counts/min, and all incubations were adjusted to give between 1,000 and 5,000 counts/min of [^{14}C]ribulose phosphate.

Preparation of the L-arabinose isomerase. Strain RFS696 deleted of chromosomal arabinose genes, but lysogenic for λhy80 d*araB*$^-$ phage (10), was grown in 600 ml of YT (8) medium to an optical density at 550 nm of 1.0 (3×10^8 cells per ml, 1 g [wet weight] of cells per liter of culture); 7 ml of 20% arabinose was added, and the flask was heated to 42 C over a bunsen burner for 10 min, then grown for 3 h at 35 C. Cells were harvested by centrifugation, ground with 1.5 weights of alumina, suspended in 2 volumes of 0.05 M Tris buffer (pH 7.5), 10^{-4} M dithiothreitol, 0.02 M KCl, 0.01 M $MgSO_4$, 10^{-4} M EDTA; 0.02 mg of deoxyribonuclease I was added, and the extract was centrifuged for 90 min at 30,000 rpm in a Spinco angle 40 rotor. The extract was dialyzed against 100 volumes of the resuspension buffer, then chloramphenicol and streptomycin were added to a concentration of 100 μg of each per ml. Isomerase prepared as above keeps at least 6 months at 4 C.

Preparation of L-arabinose. A volume of L-[^{14}C]-arabinose containing 10 μCi (10 mCi/mmol, New England Nuclear) was diluted to 1 ml with 0.01 M Tris buffer, pH 7.6, and passed over a 1-ml DEAE column. Chloramphenicol and streptomycin were added to yield 100 μg of each per ml. The chromatography removed approximately 0.2% of nonarabinose radioactivity which otherwise remained at the origin of the DEAE paper in the kinase assay.

β-Galactosidase assay. Samples of cells, 0.2 ml, were added to 0.3 ml of M9 medium containing 500 μg of chloramphenicol per ml in tubes in a 37 C water bath. After 10 min, 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.002 M $MgSO_4$, and 0.2 M β-mercaptoethanol was added, and the samples were mixed vigorously after addition of 5 μliters of toluene and put in a 28 C bath. A volume of 0.2 ml of 0.1 M sodium phosphate buffer (pH 7.0), 0.001 M $MgSO_4$, 0.1 M β-mercaptoethanol, and 4 mg of orthonitrophenol-galactoside per ml was added, and the tubes were incubated until yellow color developed. A volume of 0.5 ml of 1 M Na_2CO_3 was added, and the absorbance at 420 nm was measured.

Measurement of peptide elongation rate. A volume of 120 μliters [^{3}H]proline (0.5 mCi per ml, 2 Ci per mmol) was added to 120 ml of cells. At the times indicated, 5-ml samples were pipetted into 5 ml of 10% trichloroacetic acid, 0.02 M sodium azide, and 200 μg of chloramphenicol per ml at 0 C. A volume of 1 ml was filtered, rinsed, and counted, and the remaining 9 ml was centrifuged, suspended in 0.15 ml of 0.01 M sodium phosphate buffer (pH 7.2), 0.14 M β-mercaptoethanol, 0.5% sodium dodecyl sulfate (SDS), and heated to 65 C for 10 min. After dialysis overnight against the same buffer, the sample was heated again to 65 C for 10 min. Bromophenol blue was added to 0.002%, and glycerin was added to 10%. The sample was then subjected to electrophoresis on 10% acrylamide, 3.3% ethylene diacrylate gels in 0.1 M sodium phosphate buffer (pH 7.2), and 0.2% SDS. After electrophoresis the gels were removed from their tubes, frozen, and sliced into 1-mm slices. Each slice was incubated at 55 C in 0.4 ml of 0.1% SDS for 4 h, and the radioactivity was measured in a scintillation counter after addition of 2.5 ml of aquasol (New England Nuclear). Molecular weights were calibrated with ribonucleic acid (RNA) polymerase; β′ = 160,000; β = 150,000; σ = 94,000; α = 40,000; bovine serum albumin = 68,000; lysozyme = 13,900; carboxypeptidase A = 34,600; rabbit serum = 23,500 and 50,000; ovalbumin = 45,000; and chymotrypsin = 25,000.

RESULTS

Induction kinetics of the lac operon. The arabinose enzyme induction kinetics can most easily be discussed by comparison to those for induction of β-galactosidase. Estimates of the rates of translation in *E. coli* are between 15 and 20 amino acids per second, and the corresponding coupled rates of transcription are 45 to 60 bases per s (3). Thus, at least 0.91 to 1.2 min must elapse after addition of inducer isopropyl-β-D-thiogalactoside (IPTG) before the appear-

ance of the active 1,100 amino acid β-galactosidase.

The first detectable increase in β-galactosidase occurs at 1.4 min (Fig. 1). After adding IPTG, β-galactosidase messenger is initiated at a high rate, and the kinetics observed here and previously are consistent with the polymerases initiating randomly in the time after IPTG addition (2). Thus, once sufficient time has elapsed for the polymerase which initiated first in the cells after IPTG addition to have completed transcription of the β-galactosidase gene, the number of polymerases completing transcription of this gene per unit time will be constant, and the coding potential will increase in proportion to time (t) for several minutes until the rate of messenger degradation becomes appreciable. The rate of enzyme synthesis is proportional to the amount of messenger, and the amount of enzyme is proportional to the integral of the rate which is t^2, thus the amount of enzyme present at t, E(t) minus the basal level E(0) is proportional to t^2, or $\sqrt{E(t) - E(0)} \alpha t$. Plotting the square root of the difference between the amount of enzyme at t and the basal level allows the use of all of the early points to determine more precisely the time at which induced enzyme first begins to appear. Such a plot (Fig. 2) shows that β-galactosidase activity first appears slightly before 1.2 min.

Induction kinetics of the arabinose operon. Figure 3 shows the arabinose operon, two of whose products were assayed in these experiments. The operator proximal ribulokinase is dimeric with two identical 50,000-dalton subunits (5), and the arabinose isomerase is hexameric with six identical 60,000-dalton subunits (7). Figures 4 and 5 show the induction kinetics of these two enzymes after addition of arabinose to the medium. On the basis of the transcription-translation times discussed above, induced kinase subunits should begin appearing between 0.39 and 0.50 min, and isomerase subunits should begin appearing between 0.83 and 1.1 min. The data, however, show that increases in both are detectable approximately 0.5 min later than predicted.

Most of the additional delay is attributable to

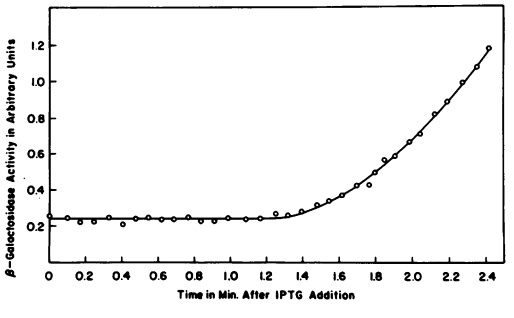

FIG. 1. *Induction kinetics of β-galactosidase after addition of 10^{-3} M IPTG. Samples of 0.2 ml were assayed as described in Materials and Methods.*

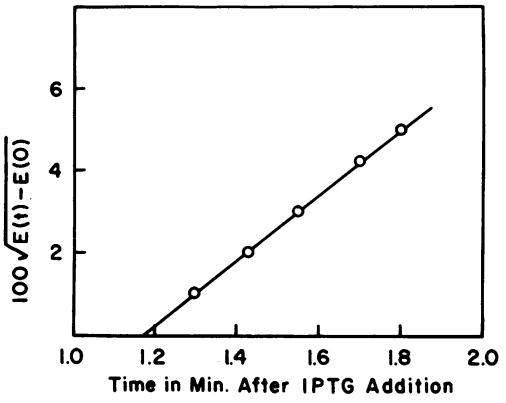

FIG. 2. *Data of Fig. 1 replotted as $100\sqrt{E(t) - E(0)}$.*

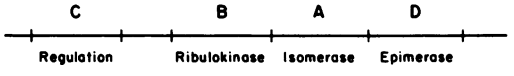

FIG. 3. *The L-arabinose operon of Escherichia coli (4, 10). C protein and arabinose lead to RNA polymerase transcription beginning between the C and B genes and proceeding across genes B, A, and D.*

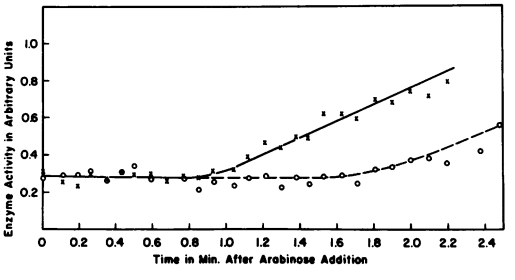

FIG. 4. *Induction kinetics of L-ribulokinase and L-arabinose isomerase in the same culture after addition of arabinose to 0.01 M at t = 0. Half of each 4-ml sample was used for the kinase assay, and half was used for the isomerase assay.*

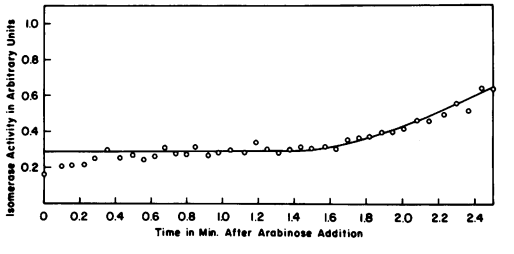

FIG. 5. *Induction kinetics of L-arabinose isomerase.*

the time required for sufficient new enzyme synthesis to be detectable above the basal level. Whereas β-galactosidase is inducible at about 1,000-fold above the basal level in this strain, the arabinose enzymes are inducible at only about 300-fold above basal level. This lower induction ratio increases the time required before new enzyme can be detected. The larger scatter of the arabinose enzyme assays precluded an extrapolation to true time of induction based on the shape of the initial increase as was possible for β-galactosidase. Additional effects could lead to delayed appearance of arabinose enzymes, isolated subunits of the arabinose enzymes may require appreciable time to associate, or acquire activity, or both. Thus, the time required for synthesis of the second kinase subunit or the sixth isomerase subunit could add times of 0.02 to 0.075 min to kinase synthesis and 0.10 to 0.38 min to isomerase synthesis, assuming that the average spacing between ribosomes on a messenger is between 40 and 80 nm and taking the translation rates as 15 amino acids per s as a minimum and 20% as a maximum.

Thus, our predicted induction kinetics of the arabinose enzymes closely agrees with those measured experimentally. In these experiments an uncertainty of no more than 0.30 min exists in the induction kinetics. Therefore, any additional times associated with control of the positively controlled arabinose operon in contrast to the negatively controlled lactose operon are less than 0.30 min.

The time required for arabinose to diffuse into the cells and to induce the arabinose operon significantly was undetectably small, less than 0.10 min. Varying the arabinose concentration over an eightfold range did not detectably alter the time required for new isomerase enzyme synthesis. Since the rate of diffusion into cells is proportional to the concentration difference across the membrane, doubling inducer concentration would halve the interval after inducer addition to the culture before the intracellular concentration was capable of significantly inducing the operon. As no variation of induction time was seen, the interval required for diffusion into the cells must be less than the 0.1-min reproducibility seen in these experiments. Similar experiments for the *lac* operon have shown that IPTG diffusion into cells is also rapid (2).

Peptide elongation rate. As a further check that the peptide elongation rate was normal in the cells used, the rate was measured directly by the method of Bremer and Yuan (1). The method was devised to measure the elongation rate of growing RNA chains and requires separation of chains according to size. Gausing (3) first applied this method to measurement of polypeptide elongation rates, utilizing the polypeptide size separation obtained on SDS polyacrylamide gels.

The basis of the method is as follows. Radioactive amino acid is added to cells in balanced growth. The number of polypeptide chains completed in the presence of label is proportional to time (t) and, for intervals shorter than the time required to synthesize completely a particular mass of polypeptide chain, the average length of labeled portion of each chain is also proportional to time. Thus, the increase of radioactive label in these completed chains is proportional to t^2. As total incorporation into all polypeptide chains is proportional to t, the fraction of label in any size of peptide increases proportional to t until label has been present for a time equal to the time required to synthesize that size of polypeptide. Thereafter, the fraction of radioactivity in that size completed peptide remains constant.

In practice, radioactive amino acid is added to the growing culture, and at various times thereafter samples are taken into buffer, stopping further protein synthesis. The polypeptides of each of these samples are separated according to size on SDS polyacrylamide gels, and the radioactivity in each size class is determined. Dividing the radioactivity in each size class by the total radioactivity on the gel yields the fraction of radioactivity in each size class.

Figure 6 shows the kinetics of incorporation of radioactive proline into protein, and Fig. 7 shows the resultant pattern of radioactivity found in gels of samples taken 0.1, 0.35, and 1.18 min after addition of radioactive proline. Figure 8 shows plots of the fraction of radioactivity in molecular weight classes 18,000 to 26,000, 26,500 to 35,500, and 35,500 to 47,500 as a function of time. These appear to show transitions from linear increase in fraction of radioactivity at 0.22, 0.31, and 0.43 min, respectively. After addition of radioactive amino acid, the nonradioactive intracellular amino acid is removed by dilution, displacing the ultimate incorporation of radioactive amino acid by the time required for utilization of an amount of amino acid equal to the amount in the pool. (Calculation shows the radioactive incorporation to be $FBt + BP(e^{-tF/P} - 1)$ where P is the pool size in mass units, F is the flow rate through the pool in mass units per unit time, and B is the specific activity of added amino acid.) Figure 8 shows the effective delay in this experiment is 0.05 min. In other experiments

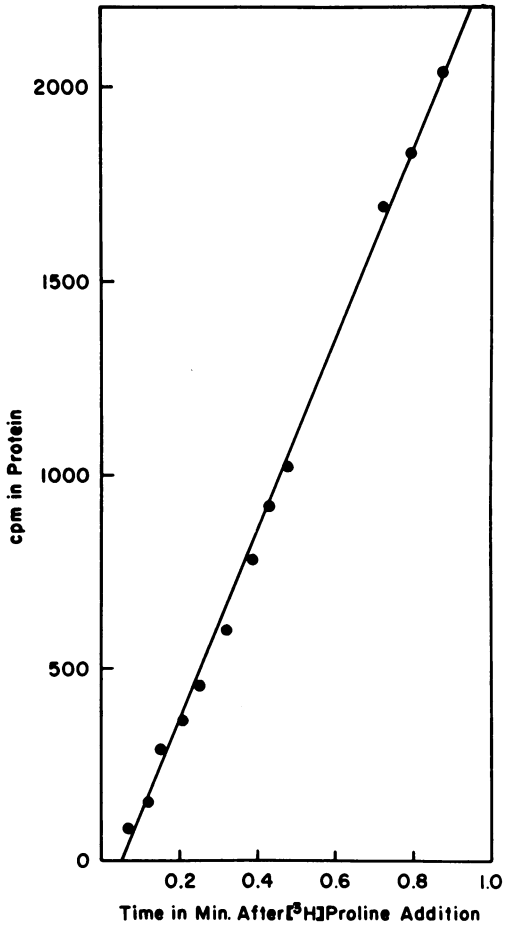

FIG. 6. *Incorporation of [³H]proline into protein as described in Materials and Methods.*

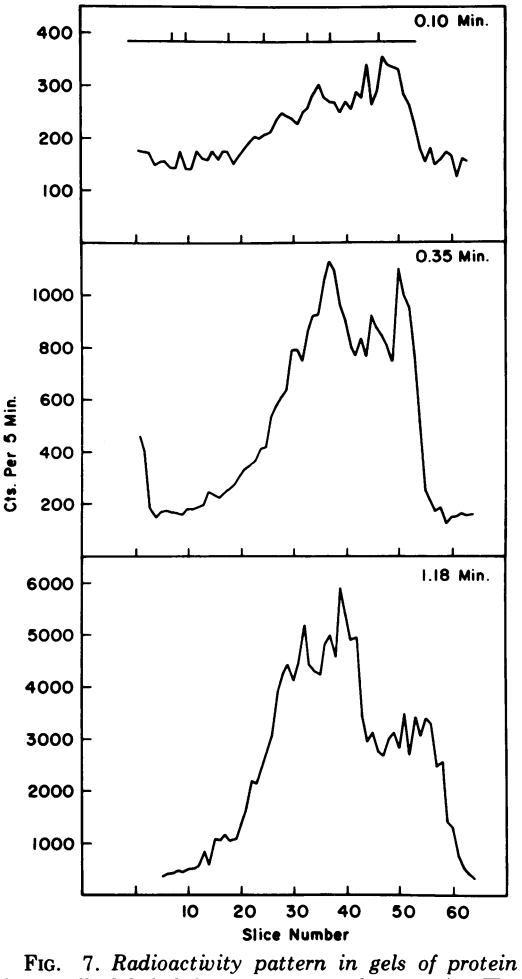

FIG. 7. *Radioactivity pattern in gels of protein from cells labeled for 0.1, 0.35, and 1.18 min. The marks show the migration positions corresponding to proteins of molecular mass (left to right) 150,000; 100,000; 70,000; 50,000; 30,000; 20,000 and the dye position.*

the delay has varied between 0.05 and 0.1 min. Subtracting this delay from the transition times found in Fig. 7 and calculating the average peptide elongation rate we find 10 to 13 amino acids per s.

DISCUSSION

In these experiments we measured the kinetics of appearance after induction of β-galactosidase, L-ribulokinase, and L-arabinose isomerase in the same cells growing under identical conditions. The shape of the initial increase of β-galactosidase activity allowed an estimation of the time at which β-galactosidase first began to increase. This time sets a lower limit on the rate of elongation of this peptide. Using this rate to estimate when L-ribulokinase and L-arabinose isomerase should appear yielded values approximately 0.5 min sooner than when the enzymes were detected to increase.

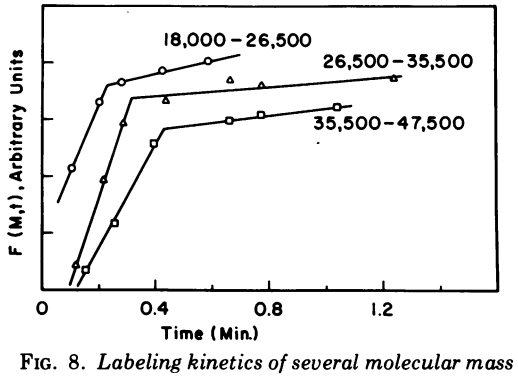

FIG. 8. *Labeling kinetics of several molecular mass ranges of proteins. The radioactivity in the molecular mass range divided by all radioactivity in the gel F(M,t) is plotted as a function of time).*

In the strain used in these experiments we find that β-galactosidase is inducible 1,000-fold above its basal level, whereas the arabinose enzymes are inducible only 300-fold above their basal levels. This lowered rate of induced enzyme synthesis increases the time required for a detectable amount of induced enzyme to accumulate. Hence, we conclude that the induction kinetics of the arabinose enzymes are consistent with the β-galactosidase induction kinetics and reflect a rate of no less than 14 amino acids per s.

The times required for enzymes to appear after induction allow lower limits to be set on elongation rates, but they do not directly determine upper limits. For example, elongation rates could be much higher than estimated and an appreciable time be required to initiate synthesis of the messengers. Therefore, a direct measurement was made on the average elongation rate of polypeptides in these cells. This used the method of Bremer and Yuan (1) devised to measure RNA elongation rates and which was applied by Gausing to measure protein elongation rates (3). In our cells, normal *E. coli* K-12 growing on minimal glycerol medium at 37 C, we found rates of 10 to 13 amino acids per s. This is below the lower limit on the rate of 14 amino acids per s determined from the induction kinetics. We do not understand the reason for the differences, but we believe that together our data show that the elongation rate in our cells was 14 ± 2 amino acids per s. This means almost all of the lag after inducer addition and the appearance of new enzyme is time required for transcription and translation. No more than 0.2 min can be required for initiation of messenger of the *lac* or *ara* operons.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant GM-18277, Career Development Award 1-K4-GM-38797 to R.F.S., and Training Grant GM-00212, all from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Bremer, H., and D. Yuan. 1968. RNA chain growth-rate in *Escherichia coli*. J. Mol. Biol. **38**:163-180.
2. Coffman, R. L., T. E. Norris, and A. L. Koch. 1971. Chain elongation rate of messenger and polypeptides in slowly growing *Escherichia coli*. J. Mol. Biol. **60**:1-19.
3. Gausing, K. 1972. Efficiency of protein and messenger RNA synthesis in bacteriophage T4-infected cells of *Escherichia coli*. J. Mol. Biol. **71**:529-545.
4. Gross, J., and E. Englesberg. 1959. Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1bt. Virology **9**:314-331.
5. Lee, N., and I. Bendet. 1967. Crystalline L-ribulokinase from *Escherichia coli*. J. Biol. Chem. **242**:2043-2050.
6. Leive, L., and V. Kollin. 1967. Synthesis, utilization and degradation of lactose operon mRNA in *Escherichia coli*. J. Mol. Biol. **24**:247-259.
7. Patrick, J., and N. Lee. 1969. Subunit structure of L-arabinose isomerase from *Escherichia coli*. J. Biol. Chem. **244**:4277-4283.
8. Schelif, R. 1969. An L-arabinose binding protein and arabinose permeation in *Escherichia coli*. J. Mol. Biol. **46**:185-196.
9. Schelif, R. 1972. Fine structure deletion map of the *Escherichia coli* L-arabinose operon. Proc. Nat. Acad. Sci. U.S.A. **69**:3479-3484.
10. Schleif, R., J. Greenblatt, and R. W. Davis. 1971. Dual control of arabinose genes on transducing phage λdara. J. Mol. Biol. **59**:127-150.