The Stability of Acyl Carrier Protein in Escherichia coli*

(G. L. P.), and funds from the National Science Foundation
(1.5 to 1.7 g) were taken up in 20 ml of 0.02 M triethylamine·HCl
(pH 7.5) and 10^{-2} M β-mercaptoethanol, broken in a French
pressure cell (Aminco Instrument Company), and centrifuged
at 12,000 × g for 30 min. The supernatants were stored in
liquid nitrogen. ACP was isolated via a published procedure
(13) on a small scale omitting the second DEAE-cellulose step.
All operations were carried out at room temperature. Only
the peak tube of the last purification step was utilized to obtain
the specific radioactivity (disintegrations per min of 3H per mg of
protein) of purified ACP.

Dilution Experiment—E. coli Ilv 453 was kindly provided by
II. E. Umbarger. This organism is a K-12 strain and has a
deficiency in the ilv E locus. A lesion was introduced in the pan
region using N,N'-nitrosoarginine (14) and penicillin
selection (15). The new lesion was “tight,” and no growth was

ACP, the existence of a small class of E. coli proteins which
exhibit high turnover rates (7–10), and the apparent importance
of protein turnover in the control of protein concentrations in
mamalian cells (11), we undertook a study of the stability of
the protein portion of holoACP. Our observations indicate
that ACP is a stable protein in E. coli.

METHODS AND MATERIALS

Pulse Experiment—E. coli M-99, a gift from P. R. Vagelos
and previously employed by him in the study of ACP metabolism
(3), is a β-alanine auxotroph. These cells were grown from a
small inoculum at 30° in 500 ml of a medium modified from
Pardee et al. (12) containing (at pH 7.0) 15 g of (NH₄)₂SO₄,
65 g of K₂HPO₄, 0.5 g of MgSO₄·7H₂O, and 50 g of glycerol per
5 liters of tap water and supplemented with 1.22 μCi (46.5 μg)
of β-[1-¹⁴C]β-alanine per 500 ml of medium. At mid-log (A₆₀₀ =
0.77) 0.288 μg of L-[4,5-²H]leucine (1.0 mCi) was added. After
5 min 1.0 g of β-leucine (unlabeled and dissolved in 50 ml of
medium) was added to the culture. After an additional 5 min
the culture was chilled, harvested by centrifugation, and resus-
pended in 100 ml of unsupplemented medium which was finally
added to 3130 ml of medium prewarmed to 36.5° and supple-
mented with 186 μg of β-[1-¹⁴C]β-alanine (4.9 μCi) and 10 g of
β-leucine. These experiments were carried out in a New Bruns-
wick fermentor which was stirred at 850 rpm and aerated at
a rate of 8 liters per min. Samples were withdrawn at timed
intervals, chilled, harvested by centrifugation, and frozen.

ACP Purification from Pulse Experiment—The frozen cells
(1.5 to 1.7 g) were taken up in 20 ml of 0.02 M triethylamine·HCl
(pH 7.5) and 10^{-2} M β-mercaptoethanol, broken in a French
pressure cell (Aminco Instrument Company), and centrifuged
at 12,000 × g for 30 min. The supernatants were stored in
liquid nitrogen. ACP was isolated via a published procedure
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The Stability of Acyl Carrier Protein in Escherichia coli

(Received for publication, December 26, 1972)

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SUMMARY

Two techniques have been employed to examine the turn-
over of the protein portion of acyl carrier protein, the con-
jugated protein which is the acyl carrier for fatty acid bio-
synthesis in Escherichia coli. The protein was shown to be
metabolically stable, in contrast to its covalently bound 4'-
phosphopantetheine prosthetic group which was previously
found to undergo turnover at the rate of 4% per min (Powell,
G. L., Elowsom, J., and Vagelos, P. R. (1969) J. Biol. Chem.
244, 5616–5624).

Acyl carrier protein, a small molecular weight protein (mol
wt 8847) possessing a covalently bound 4'-phosphopantetheine
prosthetic group, is the specific carrier of acyl groups during
fatty acid biosynthesis in Escherichia coli (1). It appears to be
localized in the bacterial membrane (2). Earlier work suggested
that CoA is the biosynthetic precursor for the 4'-phospho-
pantetheine prosthetic group of ACP (3), and subsequently the
enzymes catalyzing the synthesis of holoACP from apoACP
and CoA and that catalyzing the removal of the prosthetic
group were purified and partially characterized (4, 5). In vivo
kinetic radioisotopic tracer analysis of the metabolism of the
pantothenate compounds in E. coli has verified the precursor-
product relationship between CoA and ACP and revealed the
loss and subsequent replacement of the prosthetic group from
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obtained in the absence of pantothenate. This organism was cultured at 37°C in Vogel-Bonner mineral salts medium (16) containing 0.2% glucose, 2 μM pantothenate, 10⁻⁴ M L-isoleucine, 10⁻⁴ M L-valine, and 10⁻⁴ M L-leucine in a G-25 New Brunswick incubator-shaker at 180 rpm. Under these conditions neither pantothenate nor isoleucine was growth limiting (valine and leucine seem to spare the isoleucine), and good exponential growth into full stationary phase (A₆₅₀ ~ 1.2) could be obtained. The pantothenate meioties and the isoleucine residues in the protein of these cells were fully labeled (to the same radioisotopic specific activity as the medium) by growth from a small inoculum of cells in 250 ml of medium of the above composition containing a final concentration of 2 μM [¹⁴C]pantothenate (5.2 mCi per mmole) and 10⁻⁴ M [⁴, ⁵-³H]isoleucine (1.5 mCi per mmole).

Fully labeled cells (250 ml) were harvested at room temperature by centrifugation at mid-log (A₆₅₀ = 0.6), washed once with unlabeled medium, and resuspended in 600 ml of prewarmed medium (37°C) of composition similar to the above containing 2 μM [¹⁴C]pantothenate (5.2 mCi per mmole), but with 10⁻⁴ M unlabeled L-isoleucine, 10⁻⁴ M L-valine, and 10⁻⁴ M L-leucine. Samples of 50 ml were withdrawn immediately and at timed intervals thereafter, harvested by centrifugation, and the cell pellet immediately frozen at −80°C. The frozen cell pellet was taken up in 6.0 ml of 0.01 M Tris-HCl (pH 7.0), 10⁻³ M β-mercaptoethanol, and 0.05 M LiCl and ruptured in a French pressure cell at 20,000 p.s.i. The cell extract was then centrifuged at 40,000 × g. This supernatant solution contained 89% of the ³H and 103% of the ¹⁴C present before centrifugation. The major portion of this supernatant solution was loaded onto a column (1.5 × 4.5 cm) of DEAE-52 cellulose which had been previously equilibrated with 0.01 M Tris-HCl (pH 7.0), 10⁻³ M in β-mercaptoethanol. The pantothenate-containing compounds were then eluted with an increasing convex LiCl gradient using 70 ml of 0.7 M LiCl and 105 ml of 0.05 M LiCl both 0.01 M in Tris-HCl (pH 7.0) and 10⁻³ M in β-mercaptoethanol as previously described (6). The fractions containing ACP (determined by the characteristic conductivity and the ¹⁴C content of the fractions) were pooled and directly loaded onto a small (0.8 × 8.0 cm) column of A-25 DEAE-Sephadex which had been previously equilibrated with 0.02 M potassium phosphate (pH 6.2). Elution was accomplished using a linear gradient of 100 ml of 0.2 M LiCl and 100 ml of 0.6 M LiCl, both 0.02 M in potassium phosphate (pH 6.2) and 10⁻³ M in β-mercaptoethanol. A 25 DEAE-Sephadex was found to be superior in this procedure to the A-50 DEAE-Sephadex used previously for ACP purification (13). The fractions containing ¹⁴C were pooled and frozen for further studies. Two days were required to acquire the data for each pair of points.

Acrylamide Gel Electrophoresis of ACP—Fractions containing radioactively labeled ACP from a column chromatographic procedure were pooled, dialyzed overnight against 0.1 M urea and 10⁻³ M β-mercaptoethanol, and lyophilized in glass vessels which had been treated with 100 ml of toluene containing 0.1 ml of 1,3,3,3-tetramethyldisilazane and 10 ml of dichloromethylsilane, rinsed with methanol, and oven dried (the silylation was necessary for quantitative recovery of the radioactive ACP). The lyophilized material was then taken up in 300 ml of electrophoresis buffer containing 10⁻⁴ M dithiothreitol, 100 μl of glycerol, and a small amount of methylene blue as a marker. Electrophoresis was carried out in systems containing 35% acrylamide and 0.2% N,N'-diallyltartarate (17). One such system, running at pH 7.5 was modified from System II (18). The separating gel was made by increasing the acrylamide to 7.0 g and including 0.06 g of N,N'-diallyltartarate in place of the methylene-bisacrylamide. The stock solution was made up to 10.0 ml, and 4 ml of this stock solution were mixed with 1 ml of the buffer solution (46 ml of 1 n ICl, 0.85 g of trihydroxymethylaminomethane, and 0.45 ml of N,N,N'-tetramethylthlenediamine) and 3 ml of ammonium persulfate catalyst (14 mg/7.5 ml of water). An aliquot (0.80 ml) of this mixture was cast in 10-cm lengths of 6-mm glass tubing. The stacking gel was made with 1.85 g of N,N'-diallyltartardiamide in place of the methylene-bisacrylamide. The other solutions and procedures were unmodified.

The gels were removed from the glass electrophoresis tubes by first breaking the glass with the aid of a hammer, and the tough rubbery gels fractionated using a tuberulin syringe (no needle) and a razor blade much as described by Ward et al. (19). Twenty to 30 fractions were collected in scintillation vials. The gels were digested using 0.2 ml of 2% periodic acid at 37°C overnight. When 2 ml of Soluene were added followed by 10 ml of a toluene-based scintillation mixture, a homogeneous sample suitable for liquid scintillation radioassay was obtained. An equally satisfactory and less expensive method consisted of solubilization of the digested gels by shaking in toluene-Triton X-100 scintillation solution (20) containing 4% Cab-O-Sil. The above mixtures do not display the strong chemiluminescence observed following hydrogen peroxide digestion of methylene-bisacrylamide-containing gels. Moreover, the prolonged digestion in hydrogen peroxide required to digest the 30% acrylamide gels employed earlier by us can lead to over 60% loss of label from pantothenate-labeled ACP.

Other Assays and Procedures—The radioactivity of ¹⁴C- and ³H-containing samples was assayed in a toluene-Triton X-100 liquid scintillator solution (20) in the Packard Tri-Carb model 3320 liquid scintillation spectrometer or in Bray’s solution (21) using the Nuclear-Chicago 720 series liquid scintillation system. Settings were chosen to essentially exclude ³H from the ¹⁴C channel and to limit the ³H efficiency in the ¹⁴C channel. Calculations were performed in PL/1 with the aid of the IBM 360/50 digital calculator.

We found it advisable to verify the specific radioactivity of [³⁵P]pantothenate in vivo. We did this by estimating the CoA concentration (nanomoles per mg of cellular protein) in the crude extracts using an enzymatic assay for CoA (22). The disintegrations per min obtained in the CoA fraction isolated by DEAE-cellulose chromatography per mg of protein were then divided by the enzymatically estimated nanomoles of CoA to obtain the specific radioactivity in disintegrations per min per nmole. These corrected activities were employed in subsequent calculations. The purity of the [⁴H]isoleucine was verified by paper chromatography.

Protein concentration was estimated using the modified biuret procedure (23), the Lowry method (24) or, in the case of the very low concentrations encountered for the purified ACP, the spectrophotometric method of Waddell (25).

ACP was assayed by the malonyl-CoA-CO₂ exchange reaction as previously described (13).

Cell growth was followed spectrophotometrically at A₆₅₀ plate counts being used to establish the proportionality of this value to bacterial cell density.

Materials—A-25 and A-50 DEAE-Sephadex were purchased from Pharmacia. Whatman DE-52 DEAE-cellulose was purchased from Reeve-Angel Co. Soluene and Cab-O-Sil were obtained from Packard Instrument Co. N,N'-Diallyltartarate was purchased from Reeve-Angel Co. Soluene and Cab-O-Sil were obtained from Packard Instrument Co.
A number of studies on the turnover of bacterial cellular protein have been made and the results suggest that the average proteins are stable (average half-life of 30 days) in exponentially growing cells. This stability may decrease in non-growing cells to a rate of degradation and resynthesis of 5% per hour (26). More recent studies have indicated that a small population of proteins do turn over. The class of proteins with an enhanced turnover to the observed small population of proteins which apparently do turn over. The class of proteins with an enhanced turnover rate may be associated with the bacterial membrane (27). A high rate of turnover of mammalian membrane proteins has also been reported (28). ACP is apparently localized at the bacterial membrane in intact cells (2). However, the conditions of cell rupture described in our paper and probably in the former paper cells (7-9). ACP is a relatively abundant cellular protein in E. coli (0.5% of the soluble protein), and if it were to undergo turnover, it might be expected to make a substantial contribution to the observed small population of proteins which apparently do turn over. The class of proteins with an enhanced turnover rate may be associated with the bacterial membrane (27). A high rate of turnover of mammalian membrane proteins has also been reported (28). ACP is apparently localized at the bacterial membrane in intact cells (2). However, the conditions of cell rupture described in our paper (and probably in the former paper (27) as well) yield soluble extracts containing all of the [14C]pantothenate-containing compounds including ACP. ACP is a small, conjugated protein (mol wt = 8847) which is a specific coenzyme for lipid biosynthesis (1). Its covalently linked prosthetic group, 4'-phosphopantetheine, is removed and replaced at a rate of 4% of the total ACP pool per min, i.e. t1/2 = 16.9 min (6). Small proteins are usually turned over more slowly than large ones in both bacterial (27) and mammalian systems (29). Studies on the turnover of individual proteins in bacteria are few (30-33), and conjugated proteins in bacteria have not been studied in this way as far as we are aware.

The turnover or degradation of a protein in vivo may be experimentally demonstrated by showing the loss of constituent amino acids from that protein as further growth proceeds. This assay is most conveniently accomplished by incorporating radioactively labeled amino acids into the protein in intact cells, followed by periodic removal of samples of the cells during further growth and isolating the protein of interest in pure form. The amount of radioactivity in the isolated protein, relative to the mass of the protein isolated, gives a measure of the rate of breakdown of that protein ("Dilution Experiment"). Alternatively, following a pulse of labeled amino acid in a culture of E. coli growing logarithmically, cells were grown in an unlabeled medium. Assuming no recycling of label which results from protein catabolism, the specific radioactivity of a protein made constitutively should fall at a rate inversely related to the growth rate. For example, if the cell mass doubles, the specific radioactivity of a sample of pure protein would be halved.

We have carried out these two kinds of experiments with different strains of E. coli to characterize the rate of turnover of the protein of ACP in these cells. Although the experimental design and the purification procedures employed in each case were different, the conclusion from both was the same: the protein portion of ACP is metabolically stable.

Pulse Experiment—The pulse-labeling experiment has the advantage of accentuating the labeling of the proteins under-going turnover since the stable proteins would be labeled more slowly and only as a function of cell growth.

The 4'-phosphopantetheine prosthetic group of ACP of E. coli M-99 (auxotrophic for β-alanine) was labeled by growing these cells on β-14C]alanine. The protein portion of ACP was labeled by introducing L-[4,5-3H]leucine into the medium at mid-log growth, followed after 5 min by a large amount of unlabeled L-leucine. The culture was washed and resuspended in a fermentor containing medium supplemented with unlabeled L-leucine and β-14C]alanine of the same specific activity as before. Samples (1.5 to 1.7 g wet weight of cells) of this exponentially growing culture were withdrawn at timed intervals over an 8-hour period, and the ACP from each of these samples was purified using the five-step published procedure previously described (13). The β-14C]alanine was employed only as a marker in these experiments as ACP is the only known protein in E. coli which has a prosthetic group containing β-alanine (4'-phosphopantetheine). The ACP concentration after the final purification step was determined by protein assay (25) and the 3H content (l-leucine in the protein) measured. The values of 3H disintegrations per min per mg of ACP protein as a function of time are shown in Fig. 1. The theoretical line which most closely approximates the experimental points is that generated assuming that the 3H content of ACP remains constant while the ACP mass (milligrams of ACP protein) increases exponentially at the same rate as the mass of the cells. This behavior...
is that expected when ACP concentration is a constant fraction of cell mass (3) and when there is no turnover (see "Appendix"). The broken line in Fig. 1 corresponds to the expected loss of [3H]leucine from ACP if the protein moiety has a turnover rate equal to the already measured (6) rate of prosthetic group exchange. Thus it appears that the prosthetic group turnover or exchange does not involve a concomitant degradation of the protein portion of ACP.

In order for this conclusion to be valid the ACP isolated from the cells of the pulse-label experiment must be essentially homogeneous, although quantitative recovery is unimportant. If the isolated ACP contained a large amount of a contaminant which does not undergo turnover in E. coli (a condition met by the bulk of the protein of E. coli), then ACP turnover could be undetected. The evidence for the purity of the ACP in this experiment can be summarized as follows. First, the method used for ACP purification is known to yield pure ACP when utilized on a larger scale than in the present work. Second, the elution profile from the last step of purification shows a constant 3H:14C ratio in only those fractions which contain ACP. Contaminating protein which would have only 3H and not 14C label would alter the 3H:14C ratio unless it eluted simultaneously with ACP. Third, the isolated ACP was active (80% ± 30% of theory) in an enzymatic assay, the malonyl-CoA-CO₂ exchange reaction. The variability and lower-than-theoretical amount of active ACP could be due to the difficulties in assaying minute amounts of material, the presence of denatured ACP, or the presence of apoACP in the purified ACP preparations.

Dilution Experiment—The dilution experiment was carried out on a double auxotroph and has the advantage of providing an initial 3H:14C ratio which could be related directly to the relative specific activities of [3H]leucine and [14C]pantothenate in the medium (see "Appendix") and which provided an independent criterion for the purity of the ACP.

E. coli Ilv 453, auxotrophic for both pantothenate and isoleucine, was labeled by extensive growth on medium supplemented with [1-14C]pantothenate and L-[4,5-3H]isoleucine of known specific activity. At the beginning of the experiment, the exponentially growing cells were washed and resuspended in fresh medium supplemented with [1-14C]pantothenate of the same specific activity but containing only unlabeled L-isoleucine. Samples were taken immediately and at suitable intervals thereafter. The extracts prepared from the sampled cells were fractionated first on DEAE-cellulose. A typical separation is shown in Fig. 2. The second and last purification step, DEAE-Sephadex chromatography, is shown in Fig. 3. Only one 14C-containing peak is present; it is symmetrical and the 3H (isoleucine) to 14C (pantothenate) ratio is essentially constant across the peak. For a few of the samples, fractions representing 90% of the 14C radioactivity from A-25 DEAE-Sephadex chromatography were pooled and subjected to disc gel electrophoresis as described under "Methods and Materials." A typical result is shown in Fig. 4. A small 3H-containing peak was observed preceding
the main peak; however, only one peak of $^{14}$C radioactivity was obtained. The $^{3}$H:$^{14}$C ratio was nearly constant across the peak and the value of this ratio was close to that obtained from A-25 DEAE-Sephadex chromatography (see legend, Fig. 4).

Since a double auxotroph was employed, the specific radioactivity of the isoleucine and the pantothenate in the ACP should be the same as that in the medium; the $^{14}$C content permits a direct calculation of the $^{3}$H:14C radioactivity at zero time could be used to calculate the number of residues of isoleucine per pantothenate residue (see “Appendix”). The value obtained at $t = 0$ (see Fig. 5) corresponds to 5.36 isoleucine residues per pantothenate residue. The theoretical value based on amino acid analysis of ACP (1), sequence determinations (34), and now by synthesis (35) is 7.0. A value lower than theoretical would not be a sign of the presence of extraneous proteins but must reflect a certain amount of uncertainty in the values of the endogenous specific activity of the L-isoleucine since the endogenous specific activity of the $[^{14}$C]pantothenate was calibrated using the enzymic assay for CoA (see “Methods and Materials”).

The values of the logarithm of the $^{3}$H:14C ratio (see “Appendix”) as a function of time are plotted in Fig. 5. The theoretical line shown was calculated from the growth rate of the cells assuming that the ACP concentration in the cell is constant during exponential growth and that no $^{3}$H is lost from the ACP. The experimental data are again consistent with the suggestion that ACP is a stable protein and that it is not degraded during exponential growth.

We have demonstrated the stability of holoACP in contrast to apoACP which lacks the 4'-phosphopantetheine group. The two proteins are probably separated from one another upon DEAE-cellulose chromatography (35). However, apoACP and the holoACP must be rapidly interconverted, given the rapid turnover of the prosthetic group. Thus, the apoACP must also be stable or, if unstable, must make a very limited contribution to the demonstrated lack of turnover of the holoACP. It would be interesting to distinguish these possibilities under conditions in which apoACP was present in amounts comparable to holoACP.

When the iron content of ferritin, the free iron carrier in blood of mammals, is reduced, it exhibits a decreased stability (36). Similarly, when bacterial proteins are damaged (by introduction of genetic lesions) their turnover is also increased (32,33). Thus, it would be of interest to examine further the turnover of apoACP to ascertain whether the presence of the prosthetic group has any bearing on the stability of ACP.

We will examine the effects of pantothenate, nitrogen, phosphorus, and amino acid starvation on the turnover of this protein under conditions of growth and nongrowth as a model for the responses of this organism to such stresses. Similarly, current studies are concerned with the effect of inhibiting protein, DNA, and RNA synthesis on the turnover of ACP prosthetic group.

Earlier work demonstrated that the covalently bound 4'-phosphopantetheine prosthetic group of rat liver fatty acid synthetase also undergoes turnover, analogous to the situation in E. coli (37). Moreover, it was shown that the rate of prosthetic group exchange is much faster than the rate of protein turnover of all of the subunits of the fatty acid synthetase (38). It would appear that the turnover of the 4'-phosphopantetheine prosthetic group of fatty acid synthetase and of bacterial ACP must have some intrinsic biological significance which as yet is not understood. Since it is unlikely that the removal of the prosthetic group of ACP is an obligatory step in the de novo synthesis of a fatty acid molecule (37), this phenomena may represent an external control of either fatty acid or pantothenate metabolism.

Acknowledgments—We are indebted to P. Roy Vagelos for his interest and encouragement during this investigation. We appreciate the help of Joseph E. Sabaitis, Jr., in working out the conditions of the particular disc gel electrophoresis technique we used. Phaik Foon Tan and John DeLoach helped with the ACP purifications, and their help is gratefully acknowledged.

APPENDIX

In exponentially growing cells, the concentration of ACP within the cells is constant (1). Thus, one can relate the mass of ACP at any time, $t$, to the growth rate of the culture (conveniently estimated as the time required to double the cell mass) as follows:

$$S_{ACP} = (S_{ACP})_0 e^{\mu t}$$

where $(S_{ACP})_0 =$ mass of ACP at $t = 0$ and $\mu = 0.693$ per doubling time. Since ACP contains 1 mole of pantothenate (as 4'-phosphopantetheine) per mole of holoACP, one can conveniently relate the ACP mass to the $[^{14}$C]pantothenate content using the pantothenate specific radioactivity $A(^{14}$C).

$$[^{14}$C] = S_{ACP} \times A(^{14}$C)$$

Similarly for pure ACP the content of tritiated amino acid (seven isoleucines per mole of ACP) can also be related to ACP mass:

$$[^{3}$H] = S_{ACP} \times A(^{3}$H) \times 7$$

or at $t = 0$,

$$[^{3}$H]_0 = (S_{ACP})_0 \times A(^{3}$H) \times 7$$
where $A(\text{H}) = \text{amino acid specific radioactivity}$. If exponentially growing cells no longer incorporate (nor lose) $[3\text{H}]$iso-
leucine but continue $[\%]$pantothenate incorporation, the following changes in $3\text{H}:1\text{C}$ ratio for ACP are predicted:

$$
\frac{3\text{H}}{1\text{C}} = \frac{3\text{H}}{[\%] \times A(\text{H})} = e^{-\mu t}.
$$

From Equations 1 and 2 where $3\text{H}$ as used in Equation 4:

$$
\frac{3\text{H}}{1\text{C}} = \left(\frac{[\%]}{A(\text{H})}\right) \times 7 e^{-\mu t} = \frac{A(\text{H})}{A(\text{C})} \times 7 e^{-\mu t},
$$

also

$$
\log \left(\frac{3\text{H}}{1\text{C}}\right) = \log \frac{7 \times A(\text{H})}{A(\text{C})} - \mu t
$$

which should yield a straight line with slope $-\mu$ and intercept

$$
\log \frac{7 \times A(\text{H})}{A(\text{C})}
$$

If turnover occurs, i.e. if $3\text{H}$ is lost from ACP protein, the plot of log, $(3\text{H}:1\text{C})$ versus time would no longer be linear and would have a greater slope than $-\mu$. However, the intercept at $t = 0$ for pure ACP should remain as above.

The above equation is generally applicable to any protein possessing a prosthetic group which can be radioisotopically labeled. It is also valid for the pulse type experiment with the proviso that in Equation 6 the value of the intercept at $t = 0$ will be a function of the duration of the pulse and will not be equal to the specific activities of the medium. The slope will be equal to $-\mu$ if no turnover occurs.

REFERENCES

1. Prescott, D. J., and Vagelos, P. R. (1972) Advan. Enzymol. 36, 269–311
2. Van den Bosch, H., Williamson, J. R., and Vagelos, P. R. (1970) Nature 228, 338–341
3. Alberts, A. W., and Vagelos, P. R. (1966) J. Biol. Chem. 241, 3301–3304
4. Elovson, J., and Vagelos, P. R. (1968) J. Biol. Chem. 243, 3603–3611
5. Vagelos, P. R., and Larrabee, A. R. (1967) J. Biol. Chem. 242, 1776–1781
6. Powell, G. L., Elovson, J., and Vagelos, P. R. (1969) J. Biol. Chem. 244, 5616–5624
7. Pine, M. J. (1965) Biochim. Biophys. Acta 104, 439–456
8. Nath, K., and Koch, A. L. (1970) J. Biol. Chem. 245, 2889–2900
9. Nath, K., and Koch, A. L. (1971) J. Biol. Chem. 246, 6956–6967
10. Goldberg, A. I. (1970) Proc. Nat. Acad. Sci. U. S. A. 69, 422–426
11. Shimke, R. T., and Doyle, D. (1970) Annu. Rev. Biochem. 39, 920–976
12. Pardee, A. B., Jacob, F., and Monod, J. (1959) J. Mol. Biol. 1, 165–178
13. Maicens, P. W., Alberts, A. W., and Vagelos, P. R. (1969) Methods Enzymol. 14, 45–50
14. Adelberg, E. A., Mandel, M., and Chen, C. C. (1965) Biochem. Biophys. Res. Commun. 18, 788–795
15. Gorini, L., and Kaufman, H. (1960) Science 131, 604–605
16. Vogel, H. J., and Bonner, D. M. (1956) J. Biol. Chem. 218, 97–106
17. Anker, H. S. (1970) FASEB Lett. 7, 283
18. Garam, O. (1971) Methods Enzymol. 22, 565–578
19. Ward, S., Wilson, D. L., and Gilliam, J. J. (1970) Anal. Biochem. 38, 90–97
20. Patterson, M. A., and Greene, R. C. (1965) Anal. Chem. 37, 854–861
21. Bray, G. A. (1960) Anal. Biochem. 1, 279–285
22. Stoltzman, E. R. (1955) Methods Enzymol. 1, 590–599
23. Munkres, R. D., and Richards, F. M. (1965) Arch. Biochem. Biophys. 109, 466–479
24. Lowry, O. H., Roserbrook, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 189, 265–275
25. Waddell, W. J. (1956) J. Lab. Clin. Med. 48, 311–314
26. Maalé, O., and Kieldgaard, N. O. (1968) in Control of Macromolecular Synthesis, pp. 33–34, W. A. Benjamin, Inc., New York
27. Pine, M. J. (1970) J. Bacteriol. 103, 207–215
28. Dehlinger, P. J., and Schimke, R. T. (1971) J. Biol. Chem. 246, 2574–2583
29. Glass, R. D., and Doyle, D. (1972) J. Biol. Chem. 247, 5234–5242
30. Botman, D., and Spiegelman, S. (1942) J. Bacteriol. 48, 419–429
31. Hognest, D. S., Cohn, M., and Monod, J. (1955) Biochim. Biophys. Acta 14, 99–116
32. Goldschmidt, R. (1970) Nature 228, 1151–1154
33. Platt, T., Miller, J., and Weber, K. (1970) Nature 228, 1154–1156
34. Vanaman, T. C., Wark, S. J., and Hill, R. J. (1968) J. Biol. Chem. 243, 6290–6301
35. Hancock, W. S., Prescott, D. J., Marshall, G. R., and Vagelos, P. R. (1972) J. Biol. Chem. 247, 6224–6233
36. Drysdale, J. W., and Munro, H. N. (1966) J. Biol. Chem. 241, 3630–3637
37. Tweto, J., Liberati, M., and Larrabee, A. R. (1971) J. Biol. Chem. 246, 2469–2471
38. Tweto, J., Dehlinger, P. J., and Larrabee, A. R. (1972) Biochim. Biophys. Res. Commun. 48, 1371–1377
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*J. Biol. Chem.* 1973, 248:4461-4466.

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