A MODEL FOR CALCULATING MESSENGER RNA HALF-LIFE: SHORT LIVED MESSENGER RNA IN THE INDUCTION OF MAMMALIAN δ-AMINOLEVULINIC ACID SYNTHETASE

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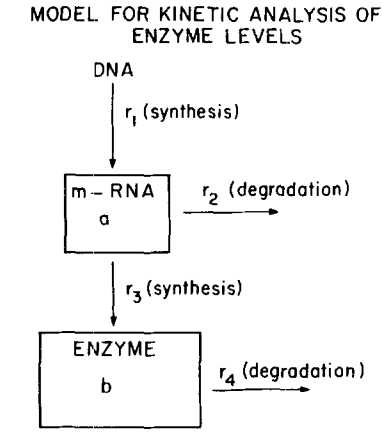
The turnover rates of messenger RNA (m-RNA) have been studied in bacteria (Levinthal et al., 1962; Hartwell and Magasanik, 1963) and in mammalian tissues (Marks et al., 1962; Seed and Goldberg, 1963; Revel and Hiatt, 1964; Scott and Bell, 1964). Bacterial m-RNA appears to have a rapid turnover (Levinthal et al., 1962), whereas most of rat liver m-RNA is probably stable for at least 40 hours (Revel and Hiatt, 1964). Previously employed techniques for estimating m-RNA half life have utilized the measurement of the time between the inhibition of RNA synthesis caused by actinomycin D and the decrease of protein synthesis (Levinthal et al., 1962; Reich et al., 1962; Hartwell and Magasanik, 1963; Seed and Goldberg, 1963; Scott and Bell, 1964). The present communication describes the use of a simple mathematical model for determining the half life of the m-RNA involved in the synthesis of a specific enzyme. An example is presented of an inducible enzyme which is synthesized from short lived m-RNA in rat liver. Previous studies have demonstrated that the enzyme employed in the present study (δ-aminolevulinic acid synthetase or ALA synthetase), the first and rate controlling enzyme in porphyrin biosynthesis, can be induced in mammalian liver by several compounds (Granick and Urata, 1963; Tschudy et al., 1964; Marver et al., 1965).

A simplified model of the genetic control of enzyme synthesis is presented in Fig. 1. If one assumes first order kinetics for the degradation of a specific m-RNA and for the synthesis and degradation of the enzyme



FOLLOWING ACTINOMYCIN D:

$$b = \frac{k_3 a_0 e^{-k_2 t}}{k_4 - k_2} + \left(b_0 - \frac{k_3 a_0}{k_4 - k_2}\right) e^{-k_4 t}$$

Fig. 1

(ALA synthetase in this case) produced by it, equations can be derived which describe the amount of enzyme present under different conditions.

Let a = concentration of a specific m-RNA present at any time

b = concentration at any time of the enzyme made by above m-RNA

k_2 = first order rate constant for the degradation of the specific m-RNA

k_3 = first order rate constant for the synthesis of the enzyme by its m-RNA

k_4 = first order rate constant for degradation of the enzyme

Then at any time t, the rate of degradation of m-RNA = $r_2 = k_2 a$; the rate of synthesis of enzyme = $r_3 = k_3 a$; the rate of degradation of enzyme = $r_4 = k_4 b$. If it is assumed that actinomycin at a certain dosage reduces the rate of synthesis of m-RNA (r_1 in Fig. 1) to 0 (at the time defined as t_0 for the following equations) and puromycin at a particular dosage reduces r_3 to 0, then it can be shown that k_2 and k_4 can be determined by using these compounds independently. After administration of puromycin k_4 is determined from the relationship $k_4 t_{\frac{1}{2}} = .693$ where $t_{\frac{1}{2}}$ is the experimentally determined half life

of the enzyme. Once k_4 is known it is possible to determine k_2 by the use of actinomycin. If actinomycin reduces r_1 to 0 at time t_0 then

1) $db/dt = k_3a - k_4b$

2) $a = a_0e^{-k_2t}$ where a_0 = amount of m-RNA present at t_0

3) $db/dt = k_3a_0e^{-k_2t} - k_4b$, for which the solution is

4) $$b = \frac{k_3a_0}{k_4 - k_2} e^{-k_2t} + \left(b_0 - \frac{k_3a_0}{k_4 - k_2}\right) e^{-k_4t}$$

Thus, after administration of actinomycin the curve of enzyme activity should contain two exponential components with slopes k_2 and k_4. Since k_4 is determined from puromycin data, k_2 can be obtained either graphically or by numerical solution. Graphic solution is achieved by accurately determining the shape of the curve of enzyme activity after administration of actinomycin and resolving the curve into its individual exponential components. One component has a slope k_4 (determined from puromycin data) and the other slope represents k_2. Such a graphic solution is applicable even when the level of enzyme is changing at the time of administration of actinomycin. For a numerical solution of k_2 it is necessary to know the value of k_3a_0. This is obtained by administering actinomycin at a time when the enzyme level is not changing, i.e., when $k_3a_0 = k_4b$. The present studies were performed with ALA synthetase which was induced in rat liver by administration of allylisopropylacetamide (AIA) (Tschudy et al., 1964; Marver et al., 1965). The curve of enzyme activity was measured after administration of actinomycin at the plateau portion of the curve of induction of ALA synthetase. At this time $k_3a_0 = k_4b$ and hence both a graphic and numerical solution for k_2 are possible. In another study actinomycin was administered during the rising portion of the induction curve of ALA synthetase.

Methods

ALA synthetase was induced in female Sprague Dawley rats (110-130 g) fasted 48 hours by a single subcutaneous injection of AIA (300 mg/kg in all

studies but one in which 150 mg/kg was used). Puromycin (100 mg/kg was injected intraperitoneally 12 hours after AIA, following which the curve of decline of ALA synthetase was measured. Actinomycin D (1.5 mg/kg) was administered subcutaneously to two groups of rats at 5 hours and 14 hours respectively after AIA. ALA synthetase was measured in liver homogenates as described previously (Tschudy et al., 1965). Each point in the curves presented below represents the ALA synthetase value from the pooled liver homogenate of 4-6 rats.

Results and Discussion

In the two experiments presented in Fig. 2, puromycin was administered

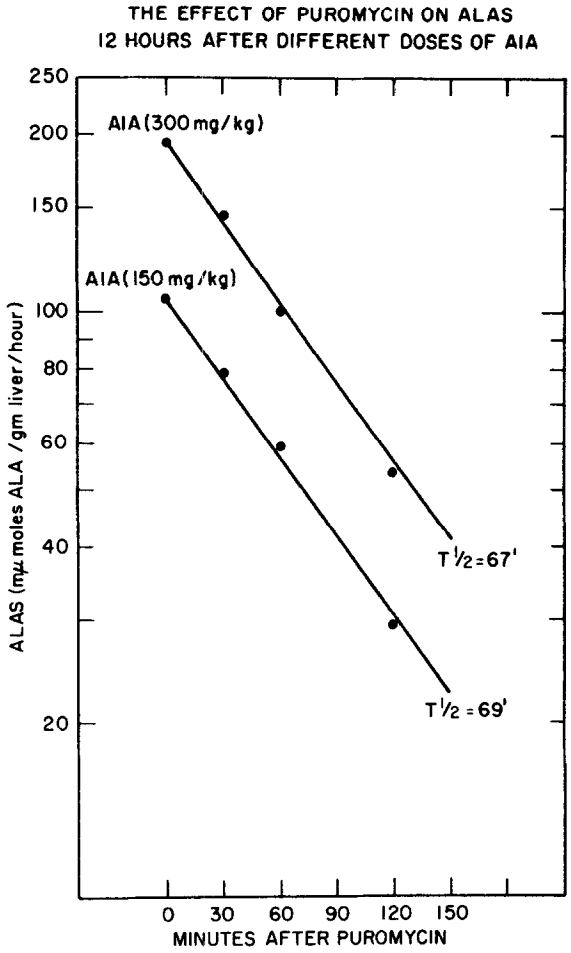


Fig. 2

12 hours after different doses of AIA (300 mg/kg and 150 mg/kg), which had induced different levels of ALA synthetase (approximately 2:1 ratio). It is seen that the half life of the induced enzyme is independent of the level of the enzyme, thus demonstrating that the degradation of the enzyme is a first order process, as assumed in the mathematical analysis above. A number of experiments performed with puromycin gave a half life of ALA synthetase of 67-72 minutes. A half life of 72 minutes would correspond to a k_4 of .577/hr.

The effect of actinomycin D given at two different times during the induction of ALA synthetase is presented in Fig. 3. The solid line repre-

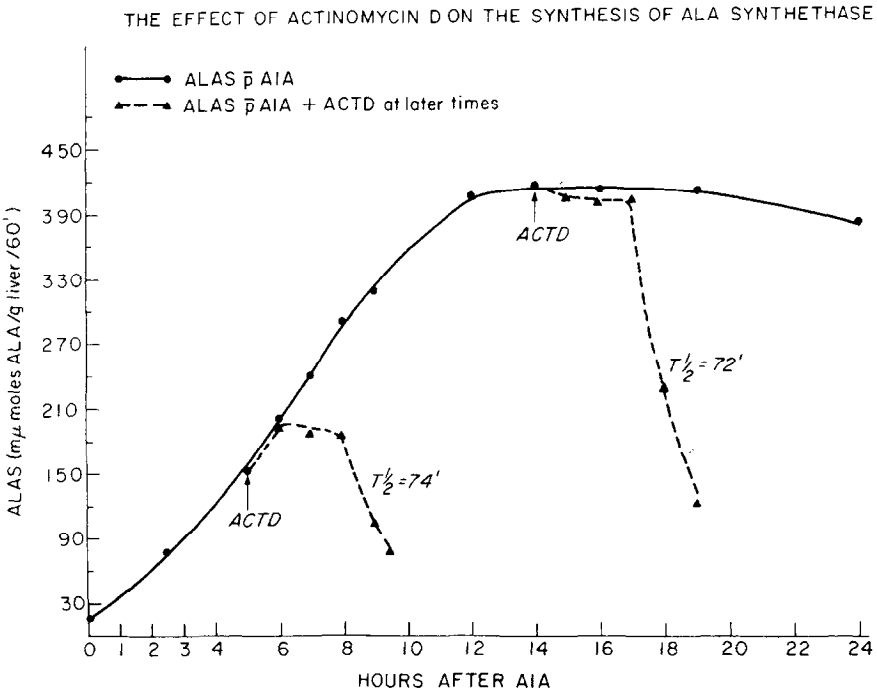


Fig. 3

sents the curve of induction of ALA synthetase after AIA administration whereas the dotted lines represent the curves of ALA synthetase when actinomycin D was given 5 or 14 hours after AIA.

Examination of the curve of ALA synthetase activity when actinomycin D

was given 14 hours after AIA reveals two components. This curve is plotted semilogarithmically in Fig. 4. Since db/dt = 0 at t_0, $k_3 a_0 = k_4 b$ under the

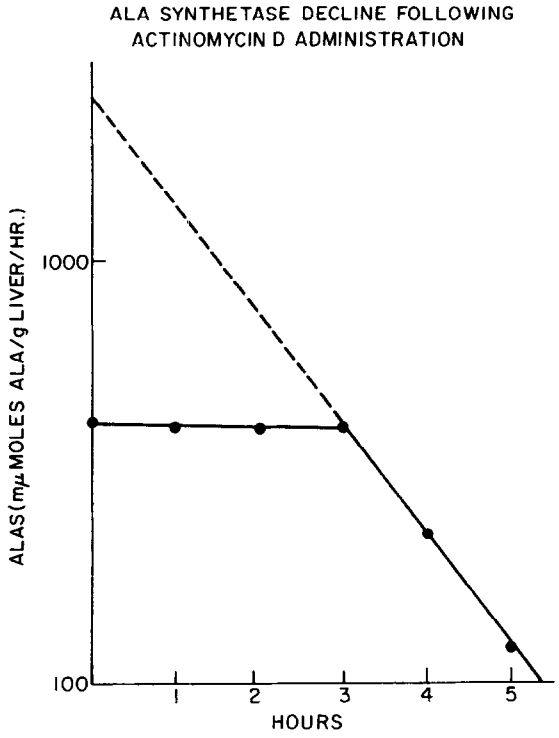


Fig. 4

conditions of this study. If one uses the value of k_4 = .577 from the puromycin studies and the observed value of b at t_0, $k_3 a_0$ can be calculated and substituted in the equation for b, allowing a numerical solution for k_2. It is found that when values of k_2 of .57/hour or .60/hour are substituted in the equation for b (along with k_4 = .577), the experimental curve is reproduced almost exactly (differences less than 1%).

A numerical solution for k_2 can also be achieved from the curve of b when actinomycin D was given 5 hours after AIA. If it is assumed that the brief early rise of this curve represents delay in achieving the effect of actinomycin D and that t_0 = 6 hours after administration of AIA, a curve of shape similar to that analyzed above can be evaluated. Since t_0 is at a time when the slope of the curve of b is very close to linear, db/dt can be

accurately determined and k_3a_0 can be calculated from the relationship $db/dt = k_3a_0 - k_4b$. When $k_4 = .577$ and the calculated value of k_3a_0 is substituted, it is again found that values of .57/hour or .60/hour fit the experimental curve almost exactly (less than 1% difference). A value of $k_2 = .60$/hour would correspond to a half life of 1.15 hours or 69 minutes for the m-RNA involved in the synthesis of ALA synthetase.

Graphic analysis of the two curves by resolution into individual exponential components is less accurate in the present studies than the above numerical solutions because only a small number of points are available for the second component. Such analysis gave a half life of the m-RNA of about 43-54 minutes.

It is possible that some or all of the early relatively flat portion of the curve of b after actinomycin (Fig. 3) could be related to pharmacologic factors such as the time involved in its absorption and delivery to the site of action. It can be shown that if this were the case, the same solution for k_2 will apply. If t_0 is delayed for a period up to 3 hours after administration of actinomycin D, the experimentally observed curve is closely approximated (within 5%) by a value of $k_2 = .60$.

Thus the present data are strongly suggestive of a half life of the m-RNA for ALA synthetase of about 40-70 minutes. Previous studies of Revel and Hiatt (1964) indicated that the bulk of cytoplasmic rat liver m-RNA is stable for at least 40 hours. However, there may be marked variation in the stabilities of different m-RNA's within the same cell. The m-RNA for ALA synthetase may be part of a small fraction of relatively unstable m-RNA in rat liver.

It should be emphasized that the use of the above model is based on the assumptions that at the dosage used puromycin specifically blocks the ability of m-RNA to stimulate protein synthesis and actinomycin D specifically blocks the synthesis of m-RNA. These assumptions may not be entirely correct, in that certain other effects of these compounds may also be manifested. However, if the assumed effects are the major ones, the present data clearly

indicate a half life for ALA synthetase m-RNA which is relatively short and which allows for short term control of hepatic heme synthesis by changes in the level of this enzyme.

References

Granick, S. and Urata, G., J. Biol. Chem., 238, 821 (1963).
Hartwell, L. H. and Magasanik, B., J. Mol. Biol., 7, 401 (1963).
Levinthal, C., Keynan, A., and Higa, A., Proc. Nat. Acad. Sc., 48, 1631 (1962)
Marks, P., Burka, E. R. and Schessinger, D., Proc. Nat. Acad. Sc., 48, 2163 (1962).
Marver, H. S., Tschudy, D. P., Perlroth, M. G. and Collins, A., Fed. Proc., 24, 721 (1965).
Reich, E., Franklin, R. M., Shatkin, A. J. and Tatum, E. L., Proc. Nat. Acad. Sc., 48, 1238 (1962).
Revel, M. and Hiatt, H. H., Proc. Nat. Acad. Sc., 51, 810 (1964).
Scott, R. B. and Bell, E., Science, 145, 711 (1964).
Seed, R. W. and Goldberg, I. H., Proc. Nat. Acad. Sc., 50, 275 (1963).
Tschudy, D. P., Welland, F. H., Collins, A. and Hunter, G., Jr., Metabolism, 13, 396 (1964).
Tschudy, D. P., Perlroth, M. G., Marver, H. S., Collins, A., Hunter, G., Jr., and Recheigl, M., Jr., Proc. Nat. Acad. Sc., 53, 841 (1965).