THE SELECTIVE EFFECT OF ACTINOMYCIN D ON FREE POLYRIBOSOMES OF MOUSE LIVER *

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Received June 24, 1969

SUMMARY

The administration of actinomycin D to fasted mice 12 hours before killing results in marked disaggregation of hepatic polyribosomes as determined by sucrose density gradients prepared from deoxycholate-treated postmitochondrial supernatants. This disaggregation of hepatic polyribosomes occurs in the free but not in the membrane-bound polyribosomes. Free, but not membrane-bound, polyribosomes of livers of actinomycin D-treated animals demonstrate decreased incorporation in vitro of ^{14}C-leucine into proteins.

Data on the half-life of mRNA in bacterial and mammalian cells have been mainly derived from studies using actinomycin D (1). This antibiotic has been considered to be a useful tool for such turnover studies since it rapidly inhibits RNA synthesis, resulting in little or no synthesis of new mRNA. On the other hand, existing mRNA continues to breakdown. The induced disaggregation of polyribosomes and exponential decay of protein synthesis in vivo have been used to estimate the half-life of mRNA (1).

Recently we have begun a series of studies on the influence of actinomycin D in vivo on hepatic polyribosomes and protein synthesis under experimental conditions associated with enhanced protein synthesis (2, 3). Since recent reports

* This investigation was supported by U.S.P.H.S. Research Grants AM-05908 from the National Institute of Arthritis and Metabolic Diseases, CA-07465 from the National Cancer Institute and Training Grant GM-135 from the National Institute of General Medical Sciences.

(4-7) have indicated a specificity of function of free and membrane-bound polyribosomes of the liver, we first decided to investigate the effect of actinomycin D in vivo on these two ribosomal fractions, studying polyribosomal profiles and each fraction's ability to synthesize protein in vitro. The results reported in this communication indicate that actinomycin D selectively affects free but not membrane-bound polyribosomes of mouse liver, causing disaggregation and decreased protein synthesis.

MATERIALS AND METHODS

Female mice of CFT strain from Carworth Farms were used. Animals were fed a commercial ration (Wayne Lab-Blox) and weighed 20-25 g at the start of the experiments. Diet was removed from all mice at 5 P. M. and 6 hours later one group received intraperitoneally actinomycin D (Dactinomycin - Merck, Sharp and Dohme), 50 µg/20 g body weight, in 0.2 ml saline and the second or control group received 0.2 ml saline. Animals had free access to water throughout. The following morning, 12 hours following the intraperitoneal injections, the animals were killed by decapitation. The livers were rapidly removed and freed from gall bladders. In every experiment livers from 6 to 8 animals were pooled in each group. Postmitochondrial supernatants (PMS), free and membrane-bound polyribosomes were prepared by the method of Blobel and Potter (8) with the modification that triton X100 was not used in the isolation of membrane-bound polyribosomes. Size distribution of polyribosomes of deoxycholate-treated PMS and of free and membrane-bound polyribosomes was determined by layering samples on linear 12 ml sucrose gradients (0.3 to 1.1 M sucrose containing TKM (0.05 M Tris, 0.025 M KCl and 5 mM $MgCl_2$)) and centrifuging in a Spinco SW 41 swinging bucket rotor in a model L2-65 Spinco ultracentrifuge at 38,000 rpm for 1 hour at 2° C (2).

In studies on incorporation in vitro, either PMS or polyribosomes (free or membrane-bound) of liver homogenates were used. The methodology using PMS was as described earlier (2). When polyribosomes were used for protein synthesis in vitro, the incubation mixture in a total volume of 1 ml, consisted of 0.2 ml of polyribosomes (0.7 to 1.0 mg) in TKM; 0.3 ml of cell sap prepared from livers of control animals in 0.125 M sucrose in TKM; and 0.5 ml of solution containing in μmoles 0.7 ATP, 0.28 GTP, 7.0 PEP, 1.5 Tris, pH 7.4, 7.5 NH_4Cl, 0.175 $MgCl_2 \cdot 6\ H_2O$, 0.05 each of 19 amino acids and 0.05 of ^{14}C-L-leucine (sp. act. 6.5 mc/mM), and 18 μg of pyruvate kinase. The samples were incubated for 10 minutes at 37° in a water bath kept shaking mechanically and the reaction was stopped by the addition of 2.0 ml of 10% TCA containing 2% Celite. Samples were then prepared for counting as described earlier (2).

RESULTS AND DISCUSSION

The sucrose density gradient patterns of postmitochondrial supernatants from livers of control and experimental animals are presented in Figure 1.

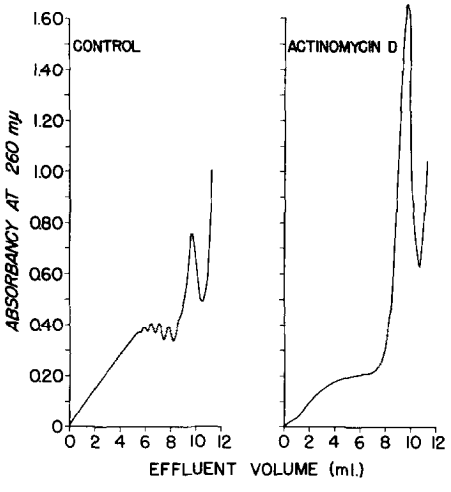

Figure 1.

Sucrose gradient patterns of polyribosomes prepared from deoxycholate-treated postmitochondrial supernatants of livers of control and actinomycin D-treated mice.

TABLE 1

INCORPORATION IN VITRO OF ^{14}C-LEUCINE INTO PROTEINS BY HEPATIC POLYRIBOSOMES OF CONTROL OR ACTINOMYCIN D-TREATED MICE

Group	Source of Hepatic Ribosomes		
	Postmitochondrial Supernatant	Polyribosomes	
		Free	Membrane-bound
	cpm/sample [x]	cpm/mg ribosomes	cpm/mg ribosomes
Control	(6) 2615 ± 464 *	(2) 4392 ± 728 *	(2) 5149 ± 707 *
Actinomycin D	(6) 1407 ± 331	(2) 2345 ± 534	(2) 5683 ± 583
% of Control [+]	52 ± 2.2 [#]	53 ± 3.6 †	110 ± 3.0 **

[x] In each experiment, samples of experimental group were corrected to RNA concentration of control group.

* Number of experiments in parentheses; values are mean ± S. E. of the mean.

[+] In each experiment, the experimental group was compared with its control group (100%).

[#] $P < 0.01$.

† $0.05 > P > 0.01$.

** $P > 0.05$.

Actinomycin D caused marked disaggregation of hepatic polyribosomes and these polyribosomes were functionally less active than those of control animals in their ability to incorporate in vitro ^{14}C-leucine into proteins (Table 1). Although a large proportion of polyribosomes became disaggregated 12 hours after treatment with actinomycin D, indicated by the shift from polyribosomes to monosomes (Fig. 1), some polyribosomes remained intact. Similar results have been reported by other investigators (9, 10). Such data would be compatible with the concept that actinomycin D may selectively affect one class of polyribosomes and not another. This is conceivable since mammalian cells, unlike bacterial cells, have

two classes of polyribosomes, free and membrane-bound (11). The results presented in Fig. 2 indicate that the free but not the membrane-bound polyribosomes of the actinomycin D-treated animals showed marked disaggregation. The patterns illustrated in Fig. 2 are representative of patterns obtained in three experiments using the modified method of Blobel and Potter (8).[x] Similar, but less satisfactory, patterns were also obtained in four experiments when two other methods (6, 12) with slight modifications were used for the isolation of free and bound polyribosomes.

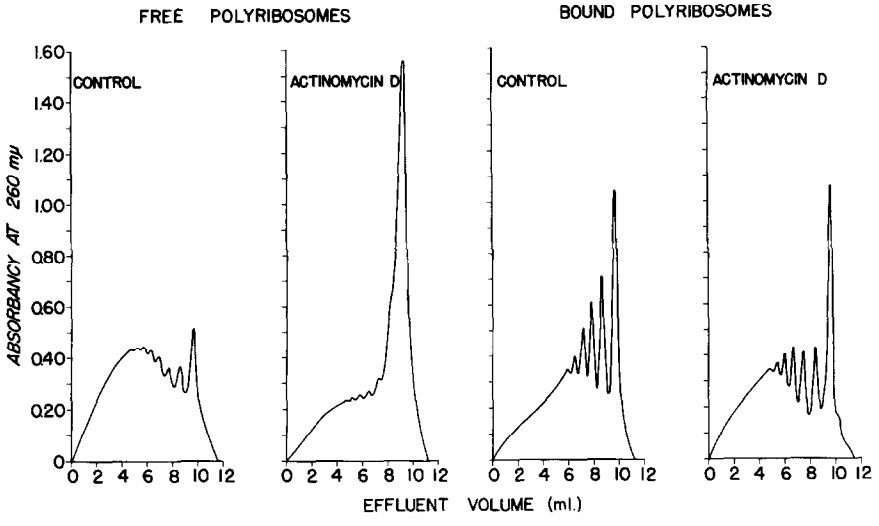

Figure 2.

Sucrose gradient patterns of hepatic free and membrane-bound polyribosomes of control and actinomycin D-treated mice.

The results of in vitro protein synthesis measured by ^{14}C-leucine incorporation into proteins using free and membrane-bound polyribosomes from

[x] In one experiment we compared the patterns of fasted rats and mice and observed that the sucrose gradient pattern of membrane-bound hepatic polyribosomes of control, overnight fasted, mice showed fewer heavy aggregates of polyribosomes and more monosomes than that of simultaneously prepared and similarly handled membrane-bound polyribosomes of normal, overnight fasted, rats. This is probably due to a difference in the effect of overnight fasting on mice and rats.

livers of control and actinomycin D-treated mice are presented in Table 1. The free polyribosomes of the experimental animals were functionally less active than those of their controls. These results are comparable to those obtained using polyribosomes in postmitochondrial supernatants (Table 1) in which the population of free polyribosomes outnumbers that of the membrane-bound polyribosomes (13). In contrast, the ^{14}C-leucine incorporation into proteins obtained using membrane-bound polyribosomes is similar in the control and experimental groups (Table 1).

In an earlier study Blobel and Potter (14) reported that actinomycin D treatment caused a disaggregation of free as well as of membrane-bound hepatic polyribosomes in the rat. However, they state that the disaggregation of free polyribosomes was more marked than that of membrane-bound polyribosomes. While our own work was in progress, Hill and Saunders reported in an abstract (15) that the administration of actinomycin D to rats disaggregated free but not membrane-bound hepatic polyribosomes.

In an attempt to explain the selective effect of actinomycin D treatment on free polyribosomes of mouse liver under our experimental conditions, two observations may be relevant. First, mRNA of polyribosomes is constantly being degraded by endogenous RNAase (16) and second, hepatic membrane-bound polyribosomes are considerably more resistant to exogenous RNAase than are free polyribosomes (14). Thus, under conditions where RNA synthesis is inhibited and little or no new mRNA is being formed, it is likely that marked disaggregation of free but not of membrane-bound polyribosomes may occur 12 hours after actinomycin D administration.

The results presented in this communication raise serious reservations regarding the use of actinomycin D for measuring half-life of mRNA in mammalian cells. In view of our results, earlier estimates of half-life of mRNA derived from experiments using actinomycin D are probably valid only when they are related

to free polyribosomes. At the present time our knowledge of the specificity of function of free and of membrane-bound polyribosomes is limited (4-7). Therefore, it is imperative that one should be cautious in the interpretation of mRNA half-life data based on actinomycin treatment.

REFERENCES

1. Manchester, K. L. in "The Biological Basis of Medicine," Vol. 2 (E. E. Bittar and N. Bittar, eds.) p. 228, Academic Press, New York (1968).
2. Sidransky, H., Sarma, D. S. R., Bongiorno, M. and Verney, E., J. Biol. Chem. 243, 1123 (1968).
3. Sidransky, H., Wagle, D. S. and Verney, E., Lab. Invest. 20, 364 (1969).
4. Redman, C. M., Biochem. Biophys. Res. Comm. 31, 845 (1968).
5. Hallinan, T., Murty, C. N. and Grant, J. H., Life Sci. 7, 225 (1968).
6. Takagi, M. and Ogata, K., Biochem. Biophys. Res. Comm. 33, 55 (1968).
7. Hicks, S. J., Drysdale, J. W. and Munro, H. N., Science 164, 584 (1969).
8. Blobel, G. and Potter, V. R., J. Mol. Biol. 28, 539 (1967).
9. Staehelin, T., Wettstein, F. O. and Noll, H., Science 140, 180 (1963).
10. Stewart, G. A. and Farber, E., Science 157, 67 (1967).
11. Bloemendal, H., Bont, W. S. and Benedetti, E. L., Biochim. Biophys. Acta 87, 177 (1964).
12. Benedetti, E. L., Bont, W. S. and Bloemendal, H., Lab. Invest. 15, 196 (1966).
13. Blobel, G. and Potter, V. R., J. Mol. Biol. 26, 279 (1967).
14. Blobel, G. and Potter, V. R., J. Mol. Biol. 26, 293 (1967).
15. Hill, R. B., Jr. and Saunders, E. H., Abstract 67, Sixty-sixth Annual Meeting of The American Association of Pathologists and Bacteriologists, San Francisco, March, 1969.
16. Eikenberry, E. F. and Rich, A., Proc. Nat'l. Acad. Sci. U. S. 53, 668 (1965).