EFFECT OF GOUGEROTIN ON THE PROTEIN SYNTHESIS IN THE MOUSE LIVER *

Hyogo Sinohara and Howard H. Sky-Peck

Department of Biochemistry, University of Illinois College of Medicine, and Department of Medicine, Presbyterian-St. Luke's Hospital, Chicago, Ill.

Received October 27, 1964

It has been shown that protein synthesis in mammalian systems is essentially the same as in bacteria. However, there are certain differences between them. For example, chloramphenicol inhibits bacterial protein synthesis, whereas it has no effect in usual mammalian systems (Nathans et al., 1962). Recently, it has been reported that gougerotin, a new antibiotic isolated from Streptomyces gougerotii, which has the structure of 1-(N-sarcosyl-1-cytosinyl)-3-D-serylamido-1, 3-dideoxy-β-D-allopyranuro amide (Iwasaki, 1962), inhibits protein synthesis in the same manner as puromycin in a cell-free system of Escherichia coli (Clark and Gunther, 1963). However, its effects in mammalian systems have not been studied. The present paper describes the effect of gougerotin on protein synthesis in the mouse liver.

Microsomes, ribosomes, and pH5 precipitable protein fraction were prepared according to the procedure of Takanami (1961). Amino acyl transferase which catalizes the transfer of amino acids from soluble RNA to ribosomes was purified by the method of Takanami and Okamoto (1960).

---

* This investigation was supported by Public Health Service Research Grant No. CA 03060-08 from the National Cancer Institute.

C^{14}-amino acyl soluble RNA was prepared as described previously (Sinohara and Sky-Peck, 1964). Radioactivity was determined in a thin-window gas flow counter and corrected to infinite thinness from a self-absorption curve. C^{14}-algal protein hydrolyzate (1.38 mC/mg) was purchased from New England Nuclear Corporation. Gougerotin was generously supplied by Dr. A. Miyake, Takeda Chemical Industries, Ltd., Japan.

TABLE I

Effect of Gougerotin on the Incorporation of C^{14}-Amino Acids into Proteins in a Cell-free System

Conditions	Counts/min	Inhibition (%)
Complete	13,700	
+ Gougerotin 4×10^{-4} M	3,280	80
+ Gougerotin 2×10^{-4} M	4,080	74
+ Gougerotin 1×10^{-4} M	4,970	67
+ Gougerotin 2×10^{-5} M	9,540	31
- Energy *	712	

Incubation mixture contained in a final volume of 1.0 ml ; 1.5 μmole ATP, 0.1 μmole GTP, 10 μmoles phosphoenolpyruvate, 50 μg of pyruvate kinase, 50 μmoles Tris-HCl (pH 7.8), 33 μmoles KCl, 6.5 μmoles $MgCl_2$, 0.5 μC C^{14}-amino acid mixture, 5 mg microsomal protein, and 3 mg supernatant protein. The tubes were incubated at 37° for 30 min. The reaction was stopped by the addition of 10% trichloracetic acid containing 20 nonlabeled amino acids (0.05 M each). The protein precipitate was prepared for counting by the method of Siekevitz (1952).

* ATP, GTP, phosphoenolpyruvate and pyruvate kinase were omitted.

Table I shows that at slightly higher concentrations than those used in puromycin experiment (Yarmolinsky and de la Haba, 1959), gougerotin inhibits amino acid incorporation into proteins. This decrease, however, is not due to the inhibition of amino acid activation. As can be seen in Table II and III, gougerotin had no effect on this reaction.

TABLE II

Effect of Gougerotin on the Activation of Amino Acids

Incubation Time (min)	Hydroxamic acid formed (μmole/ml)		
	—Amino Acids	Complete	Gougerotin
15	0.07	0.47	0.47
30	0.10	0.95	0.93

Incubation mixture contained in a final volume of 1.0 ml ; 9 μmoles ATP, 9 μmoles $MgCl_2$, 1400 μmoles NH_2OH, 2 μmoles each of 20 amino acids, 100 μmoles Tris-HCl (pH 7.8), 4 mg pH5 precipitable protein. The treated samples contained 0.4 umole of gougerotin. Tubes were incubated at 37°. Hydroxamic acid formed was determined by the method of Beinert et al. (1953).

TABLE III

Effect of Gougerotin on the Incorporation of C^{14}-Amino Acids into Soluble RNA

Incubation Time (min)	Counts/min/mg RNA	
	Control	Gougerotin
20	1,110	1,091
40	2,342	2,350

Incubation mixture contained in a final volume of 1.0 ml ; 9 μmoles ATP, 9 μmoles $MgCl_2$, 0.2 umole each of 20 nonlabeled amino acids, 0.3 μC of C^{14}-amino acids, 100 μmoles Tris-HCl (pH 7.8), and 3 mg pH5 precipitable protein. The treated sample contained 0.4 μmoles of gougerotin. Tubes were incubated at 37°. The reaction was terminated by adding 1 ml of 1.2 M $HClO_4$ and the radioactivity of soluble RNA was determined by the method of Nemeth and de la Haba (1962). The values were corrected from minus ATP blank.

Table IV shows that gougerotin, like puromycin, blocks protein synthesis by inhibiting the transfer of amino acids from soluble RNA to proteins.

TABLE IV

Effect of Gougerotin on the Transfer of C^{14}-Amino Acids from Soluble RNA to Ribosomes

Conditions	Counts/min	Inhibition (%)
Control	4,050	
Gougerotin	1,010	74
Trichloracetic acid at zero time	94	

Incubation mixture contained in a final volume of 1.0 ml ; 1.5 µmole ATP, 0.3 µmole GTP, 5 µmoles phosphoenolpyruvate, 50 µg pyruvate kinase, 8 µmoles $MgCl_2$, 40 µmoles KCl, 50 µmoles Tris-HCl (pH 7.8), 0.2 mg C^{14}-amino acyl RNA (12,350 counts/min), 2.1 mg ribosomal protein, 0.6 mg transfer enzyme. Treated sample contained 0.4 umole of gougerotin. Tubes were incubated at 37° for 15 min. Proteins were counted by the method of Siekevitz (1952).

Although these results suggest that the inhibitory mechanism of gougerotin is very similar to that of puromycin, it is not likely that their mode of action is the same. Nathans et al. (1963) studied various analogues of puromycin and found that only aromatic L-amino acid analogues which are linked by an amide bond to 3'-amino group of 3'-deoxyribose, have much activity. They also showed that neither D-amino acid analogues nor nonaromatic L-amino acid analogues were active and that both the 2'- and 5'-isomers of puromycin were ineffective. Gougerotin does not seem to satisfy these specificities.

According to the recent concepts (Slapikoff et al., 1963; Nirenberg and Leder, 1964; Arlinghaus et al., 1964), amino acyl transfer from soluble RNA to the formation of protein which involves several steps. It is likely that puromycin inhibits one of these steps while gougerotin blocks a different site. These antibiotics may be useful to elucidate the details of the final steps of protein synthesis. Preliminary experiments in intact mice suggested that gougerotin in vivo (40 mg/Kg body weight) blocked the transfer of labeled amino acids from ribosomes

to microsomal membranes (deoxycholate soluble portion from microsomes), which resulted in the marked inhibition of glucosamine-C^{14} incorporation into microsomal membrane accompanied by the accumulation of UDP-N acetylglucosamine. UDP-N-acetylglucosamine participates in the formation of glycoproteins on the microsomal membrane (Sinohara and Sky-Peck, 1964). This effect *in vivo* is different from that of puromycin *in vivo*, which blocks the activation of amino acids (Nemeth and de la Haba, 1962).

REFERENCES

Arlinghaus, R., Shaeffer, J. and Schweet, R., Proc. Natl. Acad. Sci. U.S., 51, 1291 (1964).

Beinert, H., Green, D.E., Hele, P., Hift, H., von Korff, R.W. and Ramakrishnan, C.V., J. Biol. Chem., 203, 35 (1953).

Clark, J.M. Jr. and Gunther, J.K., Biochim. Biophys. Acta, 76, 636 (1963).

Iwasaki, H., Yakugaku Zasshi, 82, 1393 (1962).

Nathans, D., von Ehrenstein, G., Monro, R. and Lipmann, F., Federation Proc., 21, 127 (1962).

Nathans, D., Allende, J.E., Conway, T.W., Spyrides, G.J. and Lipmann, F., in Vogel, H.J., Bryson, V. and Lampen, J.O., (Editors), Informational Macromolecules, p.349, Academic Press, N.Y. (1963).

Nemeth, A.M. and de la Haba, G., J. Biol. Chem., 237, 1190 (1962).

Nirenberg, M. and Leder, P., Science, 145, 1399 (1964).

Siekevitz, P., J. Biol. Chem., 195, 549 (1952).

Sinohara, H. and Sky-Peck, H.H., Biochim. Biophys. Acta, in press (1964).

Slapikoff, S., Fessenden, J.M. and Moldave, K., J. Biol. Chem., 238, 3670 (1963).

Takanami, M., Biochim. Biophys. Acta, 51, 85 (1961).

Takanami, M. and Okamoto, T., Biochim. Biophys. Acta, 44, 379 (1960).

Yarmolinsky, M.B. and de la Haba, G.L., Proc. Natl. Acad. Sci. U.S., 45, 1721 (1959).