PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 273-276 (1976)

Elevated Methionine-tRNA Synthetase Activity in Human Colon Cancer[1] (39526)

JAMES P. KUSHNER,[2] DAVID BOLL, JOSEPH QUAGLIANA, AND SHERMAN DICKMAN

Departments of Medicine and Biochemistry, University of Utah College of Medicine, Salt Lake City, Utah 84132

The incorporation of all amino acids into proteins requires two steps: first the formation of aminoacyladenylates and then the attachment of the aminoacyl moiety to a specific transfer RNA (tRNA). For each amino acid both of these reactions are catalyzed by an aminoacyl-tRNA synthetase that is specific for that amino acid (1). The reactions may be written as follows.

1. Amino acid + ATP + enzyme $\rightleftharpoons$ aminoacyladenylate·enzyme + PP_i.
2. Aminoacyladenylate·enzyme + tRNA $\rightleftharpoons$ aminoacyl-tRNA + AMP + enzyme.

However, in both prokaryocytic and eukaryocytic cells the initiation of protein synthesis requires the participation of a special aminoacyl-$tRNA_f$. The tRNA involved in initiation ($tRNA_f^{met}$) differs structurally from the tRNA involved in the insertion of methionine into growing polypeptides ($tRNA_m^{met}$) (2, 3) but the synthesis of both Met-tRNAs is catalyzed by the same enzyme, Met-tRNA synthetase (4).

Many studies have demonstrated high rates of protein synthesis in rapidly growing tumors (5) and, in some animal models, protein synthesis increases in normal tissue prior to the development of neoplastic transformation (6). Increased activity of aminoacyl-tRNA synthetases would be expected in conjunction with increased protein synthesis, and DelMonti and Cini have demonstrated increased activity of a number of aminoacyl-tRNA synthetases, including Met-tRNA synthetase, in rat hepatomas as compared to normal rat liver (7). Enzyme activities correlated with the growth rates of the tumor.

In the studies presented herein, Met-tRNA synthetase activity was measured in a human neoplasm. Colon cancer was chosen as the test tumor since a large amount of normal colon is generally excised at the time of surigcal resection of the tumor. Thus it is possible to compare enzyme activity in the tumor with the adjacent normal tissue. In addition, experiments were done to study the inhibitory effects of a synthetic aminoalkyladenylate, methioninyladenylate, on mammalian Met-tRNA synthetase.

Materials and methods. Human colons containing well-differentiated adenocarcinomas were obtained at the time of surgical resection. Normal colonic mucosa was separated from the underlying tissue by sharp dissection, rinsed in chilled saline, and immediately homogenized in 0.25 *M* sucrose which contained 0.01 *M* Tris, pH 8.0, 0.5 *M* KCl, and 0.01 *M* $MgCl_2$. The homogenate was centrifuged 90 min at 67,000*g*. The most superficial, intraluminal portion of the carcinomas was separated from the underlying fibrous stroma of the tumors by sharp dissection and then similarly processed. The particle-free supernates were collected with a syringe and the protein content determined by a modification of the procedure of Lowry *et al.* (8) using beef serum albumin as a standard. Aliquots of the supernatants (1–20 μl) were then incubated with a mixture of yeast tRNAs (1 m*M*; Calbiochem, La Jolla, Calif.), [^{3}H]methionine (2.7 μ*M*, 10,000 cpm; New England Nuclear, Boston, Mass.), ATP (5 m*M*); Sigma Chemical Co., St. Louis, Mo.), dithioerythritol (5 m*M*), KCl (100 m*M*), $MgCl_2$ (10 m*M*), and Tris buffer (50 m*M*), pH 7.0.

Solutions were incubated for 15 min at 37°, then quickly chilled and 75 μl were transferred to a paper circle 23 mm in diam-

[1] This work was supported in part by USPHS Research Grants AM-04489, 00803, and CA 14252, Southwest Oncology Group Grant CA 13282-03, and the Veterans Adminstration.

[2] Author to whom reprint requests should be addressed, at Division of Oncology/Hematology, University of Utah College of Medicine, 50 N. Medical Drive, Salt Lake City, Utah 84132.

eter (Whatman 3MM). This was immediately plunged into ice-cold 5% trichloroacetic acid (TCA). After 15 min, the pad was washed thoroughly with cold 5% TCA and then with ethanol:ether (1:1). The pad was then immersed in cold ether for 15 min, dried, placed in a vial with 10 ml of a scintillation cocktail [Omnifluor (New England Nuclear) in toluene, 4 g/liter], and counted in a liquid scintillation counter. The specific activity of Met-tRNA synthetase was then calculated and expressed as nanomoles of Met-tRNA formed per minute per milligram of protein. Under these conditions the amount of product formed is proportional to enzyme concentration and to time.

In experiments done to study the inhibitory effects of methioninyladenylate, the source of Met-tRNA synthetase was a particle-free homogenate of rabbit liver or human colon tumor which was prepared as above. Methioninyladenylate was prepared by a modification of the method of Cassio *et al.* (9). Incubation mixtures were as above but in addition contained methioninyladenylate in concentrations ranging from 2.16×10^{-8} to 2.16×10^{-5} *M*. Experiments were done utilizing two concentrations of [^{3}H]methionine, 2.7 and 5.4 *M*. All assays were performed in duplicate.

Results. Met-tRNA synthetase activity in normal and carcinomatous colonic mucosa. The Met-tRNA synthetase activity of normal colonic mucosa and colonic cancers from five patients is shown in Table I. The protein concentration of the particle-free extracts from the normal and cancerous tissues was similar (normal mean = 12.4 mg/ml; carcinoma mean = 11.23 mg/ml). In each case specific enzymatic activity in the cancer exceeded that in the adjacent normal mucosa by a factor of at least 2:1. The mean specific actiity of Met-tRNA synthetase in the tumor tissue was approximately four times that of the normal mucosa. This is a highly significant difference ($P < 0.05$, two-tailed).

Inhibition of mammalian Met-tRNA synthetase by methioninyladenylate. Methioninyladenylate proved to be a potent inhibitor of rabbit hepatic Met-tRNA synthetase. In assays utilizing 2.7 *M* methionine as the substrate there was detectable inhibition of Met-tRNA synthetase activity at concentrations of methioninyladenylate of 10^{-8} *M* (Table II). Concentrations of methioninyl adenylate of 10^{-5} *M* almost completely inhibited enzymatic activity (Table II). As expected with a competitive inhibitor, a higher substrate concentration (5.4 *M* methionine) partially blocked the inhibitory effect of methioninyladenylate at 2.2×10^{-8} *M*, the lowest concentration studied (Table II). Similar results were obtained with extracts of normal colonic mucosa and carcinomatous mucosa (83% inhibition at 2.2×10^{-6} *M* methioninyladenylate).

Discussion. The reaction catalyzed by the enzyme Met-tRNA synthetase can be considered as the first commited step of protein

TABLE II. INHIBITION OF Met-tRNA SYNTHETASE BY METHIONINYLADENYLATE.[a]

Methioninyladenylate (*M*)	[^{3}H]Met-tRNA formed[b] from 2.7 *M* Met	Inhibition (%)	[^{3}H]Met-tRNA formed[b] from 5.4 *M* Met	Inhibition (%)
0	112.0		162.0	
2.2×10^{-8}	98.6	12	161.0	1
2.2×10^{-7}	76.1	23	127.0	22
2.2×10^{-6}	22.3	80	41.3	75
2.2×10^{-5}	3.6	97	6.8	96

[a] Met, methionine.
[b] Nanomoles per assay tube.

TABLE I. SPECIFIC ACTIVITY OF Met-tRNA SYNTHETASE IN EXTRACTS FROM NORMAL COLONIC MUCOSA AND COLONIC CANCERS[a]

	Case number					
	1	2	3	4	5	Mean
Colonic cancer	0.32	0.25	1.26	0.48	0.60	0.58
Normal mucosa	0.11	0.12	0.29	0.15	0.08	0.15
Cancer/normal	2.9	2.1	4.3	3.2	7.5	3.9

[a] Specific activity reported as nanomoles of Met-tRNA per minute per milligram of protein. Met, methionine.

biosynthesis. Methionine is not available for any other metabolic pathway once it is attached to the initiator tRNA. Rapidly proliferating cells require increased rates of protein synthesis, and thus the findings reported herein, i.e., increased activity of Met-tRNA synthetase in human colon cancer as compared with normal colonic mucosa, support the concept that the amount and relative concentration of aminoacyl-tRNAs play a significant rate-controlling function in cell growth (10). It is very likely that Met-tRNA synthetase activity is increased in other human neoplasias as well. Tumor tissue is known to take up methionine rapidly and this fact is utilized in the employment of ^{75}Se-labeled methionine scanning in the detection of a variety of human tumors (11, 12).

Previous studies have shown that methioninyladenylate inhibits the activity of Met-tRNA synthetase in both bacterial and avian cells *in vitro* (13, 14). This inhibitory effect results in a nonlethal condition of suspended growth which can be reversed by the addition of methionine to the culture media (13, 14). Our results indicate that mammalian Met-tRNA synthetase is also inhibited by methioninyladenylate. This finding suggests the possibility of synchronizing populations of tumor cells by the sequential administration of methioninyladenylate and methionine. Attaining inhibitory concentrations of methioninyladenylate *in vivo* may be difficult but, were it possible, the synchronization effect could enhance the cytotoxic capabilities of cycle active antitumor drugs.

Summary. Methionine-tRNA synthetase catalyzes the formation of methionine-tRNA (Met-tRNA) which is necessary for the initiation of protein biosynthesis. This study compares Met-tRNA synthetase activity in extracts of human colon cancer and normal colonic mucosa.

Particle-free supernatants were prepared from homogenates of tumors and adjacent normal mucosa. These preparations were incubated with ATP, a mixture of yeast tRNAs, Mg^{2+}, K^{+}, dithioerythritol, and [^{3}H]methionine. Specific activities (nanomoles of [^{3}H]Met-tRNA per milligram of protein) of Met-tRNA synthetase from tumors and normal mucosa were measured and compared.

Five patients were studied and in each the tumor sample had a higher specific activity than its normal counterpart. The mean specific activity of Met-tRNA synthetase in the tumor tissue was approximately four times that of the normal mucosa. Methioninyladenylate, when added to the assay tubes, proved to be a potent inhibitor of Met-tRNA synthetase.

Since protein biosynthesis begins with the formation of Met-tRNA, the synthetase may be a rate-limiting enzyme governing protein biosynthesis in tissues. High specific activity of the enzyme would be expected to correlate with rapid cell growth. Inhibition of the enzyme with methioninyladenylate may prove a useful method of regulating protein biosynthesis in tumor cells.

The authors express their appreciation to Sylvia Buorge-Berman for technical assistance and to Drs. Leroy Townsend and Arthur Lewis for the synthesis of methioninyladenylate.

1. Chapeville, F., and Rouget, P., "Frontiers of Biology" (L. Bosch, ed.), vol. 27, p. 5. North-Holland (London), Amsterdam.
2. Piper, P. W., and Clark, B. F. C., Nature **247,** 516 (1974).
3. Simseh, M., RajBhandary, U. L., Boisnard, M., and Petrissant, G., Nature (London) **247,** 518 (1974).
4. Rudland, P. S., and Clark, B. F. C., *in* "Frontiers of Biology" (L. Bosch, ed.), Vol. 27, p. 55. North-Holland, Amsterdam (1972).
5. Potter, V. R., *in* "Cancer Medicine" (J. F. Holland and E. Frei, eds.), p. 178. Lea & Febiger, Philadelphia (1973).
6. Sheth, N. A., Bhide, S. V., J. Nat. Cancer Inst. **51,** 1977 (1973).
7. Del Monte, V., and Cini, G., FEBS Lett. **20,** 33 (1972).
8. Campbell, P. N., and Sargent, J. R., *in* "Techniques in Protein Biosynthesis" (P. N. Campbell and J. R. Sargent, eds.), Vol. 1, P. 299. Academic Press, New York (1967); Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
9. Cassio, D., Lemoine, F., Waller, J. P., Sandrin, E., and Boissones, R. A., Biochemistry **6,** 827 (1967).
10. Lotfield, R. B., and Eigner, E. A., in "Regulatory Mechanisms for Protein Synthesis in Mammalian

Cells" (A. San Pietro, M. R. Lamborg, and F. T. Kennly, eds.), p. 231. Academic Press, New York (1968).
11. Haynie, T. P., Svoboda, A. C., and Zuidema, G. D., J. Nuclear Med. **5,** 90 (1964).
12. Henera, N. E., Gonzales, R., Schwarz, R. D., Diggs, A. M., and Belsky, J., J. Nuclear Med. **6,** 792 (1965).
13. Cassio, D., Robert-Gero, M., Shire, D. J., and Waller, J. P., FEBS Lett. **35,** 112 (1973).
14. Robert-Gero, M., Lawrence, F., and Vigier, P., Biochem. Biophys. Res. Commun. **63,** 594 (1975).

Received April 27, 1976. P.S.E.B.M. 1976, Vol. 153.