Biochemical Pharmacology, Vol. 22, pp. 2488–2490. Pergamon Press, 1973. Printed in Great Britain.

Inhibition of arginyl-transfer ribonucleic acid (t-RNA) protein transferase from baby hamster kidney cells

(*Received* 12 *March* 1973; *accepted* 20 *April* 1973)

In a previous communication,[1] we reported the presence in cultured mammalian cells of arginyl-tRNA protein transferase, an enzyme which catalyzes the transfer of arginine from arginyl-tRNA to the amino terminal of specific proteins present in the cultured cells. We also reported that hemoglobin and hemin inhibited the transferase reaction. The following data add two additional potent inhibitors of the transferase reaction, dextran sulfate (DxS) and aurintricarboxylic acid (ATA). The implications of this inhibition are discussed.

Baby hamster kidney (BHK) cells were grown and harvested as previously described.[1] The pelleted cells were resuspended in 2 vol. of an ice-cold solution containing 250 mM sucrose, 150 mM Tris buffer, pH 7·8, 100 mM mercaptoethanol, 30 mM KCl, 5 mM $MgAc_2$ and 0·1 mM EDTA and broken by 10 strokes with both the loose and tight fitting pestles of a Dounce homogenizer. The suspension of broken cells was centrifuged for 10 min at 600 *g*. The recovered supernatant fluid from this centrifugation was spun at 10,000 *g* for 20 min. The supernatant fluid was again recovered and centrifuged at 39,000 rev/min for 2 hr in a Spinco 50 Ti rotor. The resulting supernatant fluid, which contained the arginyl-tRNA protein transferase and endogenous acceptor protein, was used for the experiments presented below.

The following procedures have been shown[1] to measure the addition of arginine to the amino terminal of endogenous acceptor proteins as catalyzed by arginyl-tRNA protein transferase. The incubation mixtures contained, in addition to a portion of the high-speed supernatant fluid, 100 mM Tris buffer (pH 7·8), 100 mM mercaptoethanol, 65 mM KCl, 15 μM ^{14}C-arginine (20 Ci/mole), 3 mM $MgCl_2$, 3 mM ATP, 12 mM creatine phosphate, approximately 10 units/ml of creatine phosphokinase, 30 μg/ml of cycloheximide and, where appropriate, the compound to be tested for inhibition. Samples were analyzed for ^{14}C-arginine incorporation into protein by precipitation with 5% trichloroacetic acid containing 0·2% l-arginine. The precipitated material was sedimented at 10,000 *g* for 10 min and the precipitates were resuspended in 5% trichloroacetic acid–0·2% arginine solution. After heating at 90° for 30 min to degrade arginyl-tRNA, the precipitates were collected and washed by the standard filter disk procedure. For measurement of arginyl-tRNA formation, the 90° heat step was omitted in replicate samples and the arginyl-tRNA formation was calculated as the difference in recovered radioactivity between the unheated and heated samples. Radioactivity was measured by liquid scintillation.

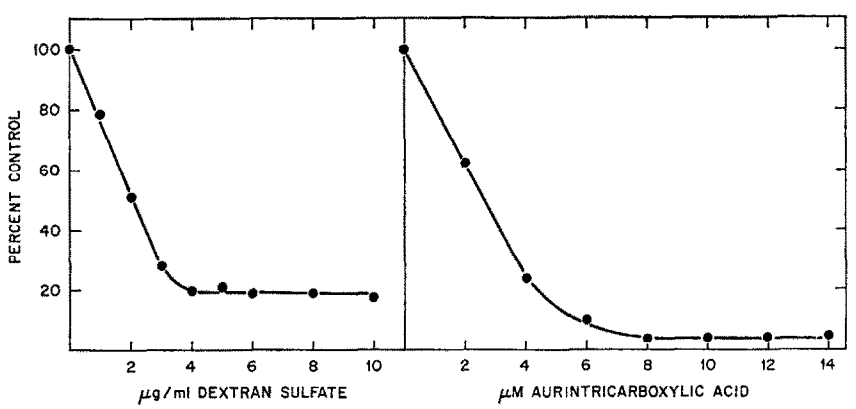

Fig. 1. Inhibition of arginine incorporation into protein. Incubations were for 60 min at 37°. Reactants were as described in the text and contained the indicated concentrations of inhibitors. High-speed supernatant fluid protein concentration was 1·3 mg/ml in the DxS experiment and 1·0 mg/ml in the ATA experiment. Control arginine incorporation, which was approximately linear during the 60-min incubation, was 3,411 cpm/mg protein/hr in the DxS experiment and 2945 cpm/mg protein/hr in the ATA experiment.

Dextran sulfate was obtained from Pharmacia, aurintricarboxylic acid from Sigma Chemical Company, and polynucleotides from Miles Laboratories, Inc.

Figure 1 demonstrates apparent inhibition of arginyl-tRNA protein transferase by various concentrations of DxS and ATA. In this particular experiment, the DxS even at higher concentration does not appear to give complete inhibition. Other experiments (see Table 1), however, show complete inhibition by DxS in the same concentration range.

It is possible that the site of inhibition of DxS and ATA is partly or entirely at the arginyl-tRNA synthetase step, since the ^{14}C-arginine in the incubation mixture must first be transferred to tRNA, as arginyl-tRNA is the actual substrate for the transferase. To test this, addition of arginine to tRNA by arginyl-tRNA synthetase was measured in the presence of concentrations of DxS or ATA which markedly inhibited transfer of arginine to protein. The results are in Table 1. At levels of both compounds which gave complete inhibition of the transferase reaction, little if any inhibition of arginyltRNA formation was found, thus indicating that the inhibition was specific at the concentrations used for the transferase.

TABLE 1. INHIBITION OF ARGININE INCORPORATION*

Inhibitor	Per cent of control incorporation into	
	Protein	tRNA
DxS (8 μg/ml)	0	81
ATA (10 μM)	0	105

* Incubations were for 15 min at 37° with or without inhibitor as described in the text. High-speed supernatant fluid protein concentration was 0·5 mg/ml in the DxS experiment and 0·4 mg/ml in the ATA experiment. Control incorporation of arginine into protein (hot acid-insoluble product), which was linear during the incubation period, was 2080 cpm/mg protein/hr in the DxS experiment and 2220 cpm/mg protein/hr in the ATA experiment.

The polyanions, poly A, C and U, were also tested. These were poor apparent inhibitors of the transferase. These polynucleotides gave 20–25 per cent maximal inhibition at concentrations of 25 μg/ml of phosphate and higher.

These data are of considerable interest for two reasons. First, it is always of value in the study of an enzyme to have a specific inhibitor. Second, that these two particular compounds inhibit arginyl-tRNA protein transferase leads to some interesting possibilities concerning the role of the enzyme in cellular functioning, when considered in light of some previous findings.

Grollman *et al.*[2–4] and others (reviewed in reference 5) have demonstrated in bacterial and mammalian cell-free systems that ATA binds to polysomes, 80 S and 40 S particles as well as inhibiting the binding of poly U and poly C to ribosomes. Their data indicate that ATA inhibits the initiation of protein synthesis by preventing attachment of mRNA to the ribosome. A similar mode of action may be true for DxS[6,7] Leibowitz and Soffer[8] report that there is an endogenous acceptor protein associated with the 30 S ribosomal unit in *Escherichia coli*, which has a similar transferase, but for leucine and phenylalanine rather than arginine (leucyl, phenylalanyl-tRNA protein transferase). Evidence from this laboratory (unpublished observations) suggests that at least a fraction of the endogenous cellular acceptor protein in mammalian cells is associated with ribosomes.

Taken together, these observations lead to the speculation that the endogenous arginine acceptor protein and the arginyl-tRNA protein transferase are involved in modulation of ribosomal function. Leibowitz and Soffer[8] have made a similar proposal with regard to the leucine, phenylalanine transferase in *E. coli*. Obviously, this is at present very speculative. The inhibition by ATA and DxS may be fortuitous as may the seeming association of endogenous acceptor protein with ribosomes. In addition, ATA may not even be the inhibitory component present in the commercially obtained material, which has been found to be relatively impure.[4] Work is in progress to test this hypothesis.

Acknowledgments—I wish to acknowledge the fine technical assistance of Mrs. Evelyn B. Lentz and Mr. Frank Valente. This work was supported by Public Health Service Grants GM19110 and CA10748.

Department of Pharmacology,
Yale University School of Medicine,
333 Cedar Street,
New Haven, Conn. 06510, U.S.A.

BARRY GOZ

REFERENCES

1. B. Goz and P. Voytek, *J. biol. Chem.* **247,** 5892 (1972).
2. A. P. Grollman and M. L. Stewart, *Proc. natn. Acad. Sci. U.S.A.* **61,** 719 (1968).
3. M. L. Stewart, A. P. Grollman and M. T. Huang, *Proc. natn. Acad. Sci. U.S.A.* **68,** 97 (1971).
4. M. T. Huang and A. P. Grollman, *Molec. Pharmac.* **8,** 111 (1972).
5. S. Pestka, *A. Rev. Microbiol.* **25,** 487 (1971).
6. A. Korner, *Biochim. biophys. Acta* **174,** 351 (1969).
7. R. Ascione, R. B. Arlinghaus and G. F. Vande Woude, in *Protein Biosynthesis in Nonbacterial Systems* (Eds. J. A. Last and A. I. Laskin), p. 59. Marcel Dekker, New York (1972).
8. M. J. Leibowitz and R. L. Soffer, *Proc. natn. Acad. Sci. U.S.A.* **68,** 1866 (1971).

Biochemical Pharmacology, Vol. 22, pp. 2490–2492. Pergamon Press, 1973. Printed in Great Britain.

The long-term influence of sterigmatocystin on mouse and rat liver nuclear deoxyribonucleases

(*Received* 21 *September* 1972; *accepted* 27 *March* 1973)

Normally the highest acid DNAase (deoxyribonuclease) cellular activity levels are found in lymphatic and neoplastic tissues while the lowest levels are found in spermatozoa and erythrocytes which do not replicate.[1] In contrast to the pattern in normal tissue, a lack of correlation between growth rate and acid DNAase activity was found in a series of malignant animal tissues.[2–4] Considerable increase in activity of pancreatic DNAase was found in *in vitro* experiments after the administration of various potent carcinogens.[5,6]

The present study reports sequential changes in activity of nuclear acid and alkaline DNAases throughout the period of administration of a potent hepatocarcinogen, sterigmatocystin,[7,8] to a susceptible (rat) and resistant (mouse) animal species. The experimental animals were random-bred Onderstepoort Albino mice and Wistar-derived Albino rats from our own colony. Due to the difficulty of dosing mice, the long-term administration (371 days) of sterigmatocystin was achieved by mixing the toxin with the standard laboratory mashed ration at a level of 0·2 mg per mouse daily. Groups of animals were killed by decapitation at predetermined intervals; each group consisting of four treated animals and four controls. Specimens of hepatic tissue were obtained for histological examination and thereafter the livers were weighed and homogenized.

Albino male rats with body weights of 100 ± 5 g received sterigmatocystin, dissolved in dimethylsulphoxide (DMSO) per gastric tube. The experiment lasted 335 days and the animals were dosed once per week with 20 mg sterigmatocystin per kilogram of body weight except between days 140 and 170. Controls received the same volume of DMSO only. Two treated and two control animals were killed by decapitation at 3-weekly intervals. The livers were excised, a sample placed in 10% buffered formalin for histological examination and the remainder homogenised.

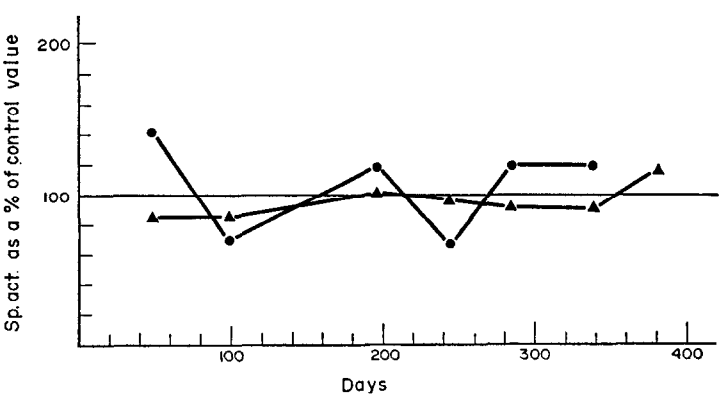

Fig. 1. Long-term influence of sterigmatocystin on the specific activities (in units/milligram of protein) of mouse liver nuclear acid DNAase (▲) and alkaline DNAase (●).