MOLECULAR PHARMACOLOGY, 12, 191-193

Aurintricarboxylic Acid Is a Nonspecific Enzyme Inhibitor

MINOU BINA-STEIN[1] AND THOMAS R. TRITTON[2]

Department of Chemistry, Yale University, New Haven, Connecticut 06520

(Received May 6, 1975)

SUMMARY

BINA-STEIN, MINOU & TRITTON, THOMAS R. (1976) Aurintricarboxylic acid is a nonspecific enzyme inhibitor. *Mol. Pharmacol.*, 12, 000-000.

Aurintricarboxylic acid (ATA) has been previously categorized as a drug which inhibits enzymes of polynucleotide metabolism. In this paper we present evidence that ATA does not show specificity toward this class of enzymes only. Rather, the drug will bind to and inhibit most enzymes regardless of their specific catalytic function.

INTRODUCTION

Aurintricarboxylic acid is a triphenylmethane dye which has been extensively studied as an inhibitor of various aspects of protein synthesis in both prokaryotic and eukaryotic organisms (1-16). Initially Grollman and Stewart (1) showed that the drug inhibited mRNA translation by preventing the formation of the initiation complex between mRNA and ribosomes. A later report from the same laboratory (2) gave evidence that ATA[3] inhibits polypeptide chain elongation as well as initiation. Other workers, investigating the various individual steps of protein synthesis, also found that ATA is an inhibitory drug (3, 4). It was also demonstrated (15) that the dye causes breakdown of rabbit reticulocyte polysomes and that it inhibits RNAse V function (14). Recently Blumenthal and Landers (5) showed that ATA inhibits Qβ replicase, *Escherichia coli* RNA polymerase, and T7 RNA polymerase, and summarized the available data by predicting that ATA will act to inhibit any nucleic acid-binding enzyme. An excellent review of much of this literature is available (17).

ATA is a negatively charged molecule at neutral pH (Fig. 1). It seems likely, then, that the dye might bind by simple electrostatic interactions to any protein which contains positively charged residues. In fact, it was reported almost 20 years ago that ATA binds to albumin (18). We reasoned that the dye might inhibit any enzyme either by nonspecific complex formation, causing the enzyme to adopt a conformation unfavorable for catalysis of its particular reaction, or by direct competition for the active site (as seems reasonable in nucleic acid-binding enzymes).

Exploratory equilibrium dialysis experi-

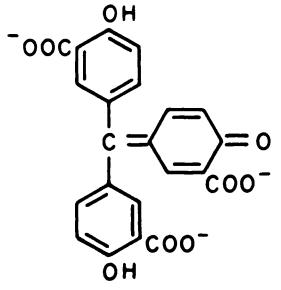

FIG. 1. *Aurintricarboxylic acid*

[1] Present address, National Institutes of Health, National Cancer Institute, Bethesda, Maryland 20014.

[2] Postdoctoral Fellow of the National Institutes of Health. Present address, Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510.

[3] The abbreviation used is: ATA, aurintricarboxylic acid.

ments showed that ATA binds to all the proteins mentioned in the legend to Fig. 2 (data not shown). We then tested the activity of these enzymes in the presence of ATA (Fig. 2). Five of these proteins (glucose 6-phosphate dehydrogenase, lysozyme, trypsin, chymotrypsin, and asparaginase) do not interact with nucleic acids, and all but one (asparaginase) are inhibited to a greater or lesser extent by the drug at concentrations equivalent to or less than those required to inhibit protein synthesis. Two enzymes which do specifically interact with nucleic acids, but which had not previously been investigated, were also studied. One of these (DNase I) was strongly inhibited by ATA but, surprisingly, the other (seryl-tRNA synthetase) was not.

There are several significant conclusions from these observations. First, ATA is not specifically inhibitory toward nucleic acid-binding enzymes, as previously believed. The drug binds to all the proteins tested and inhibits most, but not all, of the enzymatic activities. Furthermore, it does not inhibit all polynucleotide-binding enzymes, as shown by the full activity of seryl-tRNA synthetase in the presence of high concentrations of the dye. Thus it is not safe to assume that ATA can be used as a general inhibitor of enzymes of nucleic acid metabolism. ATA can still be useful experimentally, because polypeptide chain initiation is preferentially inhibited at drug concentrations too low to have an effect on elongation (1). It is important to bear in mind, however, that in any enzymatic system ATA may interfere with the normal mechanism by the simple fact of binding to the enzyme.

Thus, although ATA certainly has pharmacological activity, our results show that this activity most likely results from inhibition of a wide spectrum of enzymes.

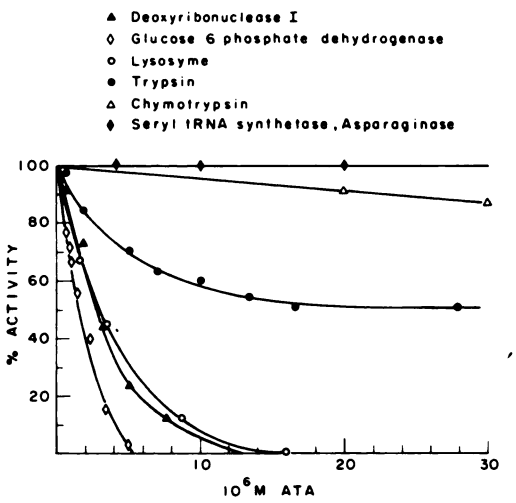

FIG. 2. *Inhibition of various enzymes by ATA*

The dye was added either prior to or after the reaction was initiated. The same degree of inhibition was observed in either case. Lysozyme, trypsin, chymotrypsin, deoxyribonuclease (DNase I), and glucose 6-phosphate dehydrogenase were obtained from Sigma Chemical Company. Activity assays for each were performed as described in the Worthington Enzyme Manual (Worthington Biochemical Corporation, 1972). Seryl-tRNA synthetase was a gift from Dr. Frantisek Kalousek and was assayed according to Söll *et al.* (19). Asparaginase was donated by Dr. R. E. Handschumacher and assayed as described (20). ATA was used as the ammonium salt (Sigma) and may be a mixture of compounds or isomers.

ACKNOWLEDGMENTS

We thank Dr. R. E. Handschumacher for purified *E. coli* asparaginase, Dr. L. Lachman for teaching us the asparaginase assay method, and Dr. F. Kalousek for a gift of *E. coli* seryl-tRNA synthetase. We are also grateful to Dr. Alan Cooper for stimulating conversations, and to Dr. Julian Sturtevant and Dr. Donald Crothers for providing laboratory space.

REFERENCES

1. Grollman, A. P. & Stewart, M. L. (1968) *Proc. Natl. Acad. Sci. U. S. A.*, 61, 719–725.
2. Stewart, M. L., Grollman, A. P. & Huang, M. T. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, 68, 97–101.
3. Weissbach, H. & Brot, N. (1970) *Biochem. Biophys. Res. Commun.*, 39, 1194–1198.
4. Siegelman, F. & Apirion, D. (1970) *Bacteriol. Proc.*, 149.
5. Blumenthal, T. & Landers, T. A. (1973) *Biochem. Biophys. Res. Commun.*, 55, 680–687.
6. Webster, R. E. & Zinder, N. D. (1969) *J. Mol. Biol.* 42, 425–439.
7. Mathews, M. B. (1971) *FEBS Lett.*, 15, 201–208.
8. Baglioni, C., Jacobs-Lorena, M. & Meade, H. (1972) *Biochim. Biophys. Acta*, 277, 188–197.
9. Dettman, G. L. & Stanley, W. M., Jr. (1973) *Biochim. Biophys. Acta*, 299, 142–147.
10. Horez, W. & McCarthy, K. S. (1971) *Biochim. Biophys. Acta*, 228, 526–535.

11. Roberts, W. K. & Coleman, W. H. (1971) *Biochemistry*, **10**, 4304–4312.
12. Lebleu, B., Marbaix, G., Werenne, J., Burny, A. & Huez, G. (1970) *Biochem. Biophys Res. Commun.*, **40**, 731–739.
13. Marcus, A. & Bewley, D. P. (1970) *Science*, **167**, 1735–1736.
14. Keewano, M., Schlessinger, D. & Apirion, D. (1970) *Nature*, **226**, 514–516.
15. Huang, M. T. & Grollman, A. (1972) *Mol. Pharmacol.*, **8**, 111–127.
16. Borgese, N., Mok, W., Kreibich, G. & Sabatini, D. D. (1974) *J. Mol. Biol.*, **88**, 559.
17. Apirion, D. & Dohner, D. (1975) in *Antibiotics III* (Gottlieb, D., ed.), pp. 327–339, Springer, New York.
18. Lindenbaum, A. & Schubert, J. (1956) *J. Phys. Chem.*, **60**, 1663–1664.
19. Söll, D. G., Cherayil, J. D. & Bock, R. M. (1967) *J. Mol. Biol.*, **29**, 97–112.
20. Jackson, R. C. & Handschumacher, R. E. (1970) *Biochemistry*, **9**, 3585–3590.