Differential Inhibition of Host and Viral Thymidylate Synthetases by Polyglutamates*

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The ability of folate analogues to inhibit host and viral thymidylate synthetases was measured using the corresponding *Escherichia coli* and T2-phage-induced enzymes. In the absence of Mg²⁺, 6 × 10⁻⁷ M pteroylhexaglutamate inhibited the T2-phage-induced synthetase by 50%, but at least 100-fold greater levels of this compound were necessary to inhibit the *E. coli* synthetase by this amount. At 2.5 × 10⁻⁸ M pteroylhexaglutamate, at least 80% inhibition of the T2-phage synthetase could be obtained with little or no inhibition of the *E. coli* enzyme. The pteroylmonoglutamate was about 2 orders of magnitude less inhibitory towards the T2-phage enzyme than the pteroyltri- to -heptaglutamates. However, upon addition of Mg²⁺ to the assay mixture, the inhibition produced by pteroylhexaglutamate was essentially reversed, with the *E. coli* synthetase now increasingly inhibited by this compound and the T2-synthetase only minimally impaired. Methotrexate and N6-formyl-2-amino-4-hydroxyquinazoline, although inhibitory to both enzymes in the presence or absence of Mg²⁺, did not show this differential selectivity.

These results suggest that certain folate analogues may be useful in distinguishing between a host and an infecting organism’s thymidylate synthetase and could thus provide an additional means of screening for potential chemotherapeutic agents.

The success of any chemotherapeutic approach to disease is usually a function of the enzymological differences between the host and a foreign organism or tissue. In the case of neoplastic disease, these differences are unfortunately mostly quantitative, thus limiting the extent to which a drug can be used against a specific tumor without being detrimental to normal tissue.

A greater degree of flexibility has been found, however, in the treatment of bacterial and parasitic disease since critical enzymes common to both host and infecting agent can often be selectively impaired as a result of differences in their physical and chemical properties. Thus, in the case of dihydrofolate reductase, the bacterial enzyme has been found to be several orders of magnitude more susceptible to inhibition by some drugs, such as trimethoprim, than the corresponding animal enzyme (1, 2). A similar strategy has been undertaken with parasitic diseases because of the differential sensitivity of host and parasite dihydrofolate reductases (3-5). Unfortunately, the number of enzymes, such as dihydrofolate reductase, which are in critical metabolic loci and are subject to selective inhibition, are limited.

Our recent studies on thymidylate synthetase suggest that this enzyme might be exploited also, although its most commonly employed inhibitors, the nucleotide analogues of dUMP, do not provide the necessary selectivity to be effective when used singly as chemotherapeutic agents. In contrast, it will be shown here that a marked differential response between a host enzyme, *Escherichia coli* thymidylate synthetase, and its viral counterpart, T2-phage-induced thymidylate synthetase, can be obtained with unreduced folate analogues of the enzyme’s second substrate, 5,10-methylenetetrahydrofolate, suggesting a potentially more productive route for chemotherapy with this enzyme. Aside from the practical application of these studies, they suggest a means of distinguishing kinetically between various thymidylate synthetases which do not appear to be available with the currently employed nucleotide analogues.

MATERIALS AND METHODS

The *E. coli* thymidylate synthetase was purified by a modification of the procedure of Friedkin and Donovan (6) to a specific activity of 1.5. The T2-phage-induced synthetase was purified to a specific activity of 2.5 as described previously (7). Both enzymes were about 25% pure based on the specific activities of the homogeneous enzymes. A unit is defined as the amount of enzyme required to produce 1 pmol of H₂pteroylmonoglutamate/min at 30°C using the assay procedure of Wahba and Friedkin (8). The polyglutamates used in these studies were synthesized as described earlier (9), while the N6-Formyl-2-amino-4-hydroxyquinazoline was kindly provided by Dr. Joseph R. Bertino, Yale University School of Medicine. Methotrexate was a generous gift of Lederle Laboratories, Pearl River, N. Y.

RESULTS AND DISCUSSION

As shown in a wide range of studies (10-14), many 5-substituted derivatives of dUMP are inhibitors of thymidylate synthetase. Some, such as 5-nitrodeoxyuridine, appear to be effective as anti-herpes virus agents, possibly as a result of their conversion to 5'-deoxynucleotides and their subsequent impairment of a specific thymidylate synthetase (15-17). The mechanism of action of such drugs, though, is not clear, since an animal virus thymidylate synthetase has not been clearly identified, particularly one which is more sensitive to these compounds than the host enzyme. Even in those cases where some selectivity in inhibition has been demonstrated (13), they are only marginal relative to the 10⁴-fold difference in sensitivity observed between animal and bacterial dihydrofolate reductases (1, 2).

An alternative approach to the selective inhibition of thymidylate synthetase was suggested from studies with 2-amino-4-hydroxyquinazolines (18) and polyglutamates (19), since both groups of compounds are fairly effective inhibitors of this enzyme. Further studies were encouraged in the latter case, as some degree of selectivity could be shown with respect

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to enzyme source and the number of γ-glutamyl residues associated with the pteroyl moiety (20). Thus, the folylpolyglutamates were 10 to 200 times more inhibitory towards the synthetases than folate, with the enzymes from these sources being inhibited in the following order: Lactobacillus casei > E. coli > T2-phage. More recent studies have shown that when the E. coli and T2-synthetases are assayed in the absence of Mg2+ an almost complete inhibition of the T2-enzyme could be produced with little inhibition of the E. coli enzyme being noted (Fig. 1A). The concentration of pteroylhexaglutamate required to inhibit the T2-enzyme by 50%, 6 x 10^{-11} M, was at least 100-fold lower than that required to impose the same degree of inhibition on the E. coli synthetase. However, upon adding Mg2+ to the reactions, the inhibition of the T2-enzyme by pteroylmonoglutamate was completely prevented at levels which were extremely inhibitory to this enzyme in the absence of Mg2+. In addition, the E. coli synthetase was now even more subject to inhibition than the T2-synthetase (Fig. 1B). A similar but less dramatic response was obtained with methotrexate in that the T2-synthetase was inhibited to a greater extent than the E. coli synthetase when Mg2+ was omitted (Fig. 2A). However, in this instance, the inhibition could not be reversed by Mg2+ (Fig. 2B). As in the case of the folylpolyglutamates, the E. coli synthetase was inhibited to a lesser degree in the absence of Mg2+. It was reported earlier (20) that NaCl could reverse most of the inhibition of the L. casei synthetase by the folypolyglutamates. The similarity in the capacity of NaCl and MgCl2 to reverse the observed inhibition suggested that the two salts are acting in the same manner. However, while Mg2+ can stimulate the E. coli synthetase rather dramatically (Fig. 3), NaCl elicits little or no response with this enzyme and the T2-synthetase is much less responsive to activation by either salt. It is not possible from these studies to determine whether reversal of the folypolyglutamate inhibition is influenced by the electrostatic interaction of the cations with the glutamate residues or is due to a salt-mediated conformational alteration of the enzyme which impairs folate binding. Thymidylate synthetase appears to possess multiple folate binding sites (21), a property which could explain some of the observed differences between the viral and bacterial synthetases, particularly if the concept of differential folate binding to substrate and inhibitor sites is introduced. Recent kinetic studies have shown the T2-synthetase to be inhibited noncompetitively in the presence of Mg2+, with a K_i of 8 x 10^{-7} M.1 A more comprehensive comparative analysis of the kinetics of inhibition of these enzymes by folate analogues, in the presence and absence of Mg2+, should more clearly define the properties of the folate binding sites. In any case, the folyl moiety is essential for inhibition, as p-aminobenzoylhexaglutamate was completely inactive at concentrations where the corresponding folyl derivative was very inhibitory.

Regardless of the mechanism of inhibition and its reversal, the manner in which the host and viral synthetases are affected by the folypolyglutamates suggests that related analogues can be developed which may be effectively exploited for chemotherapeutic purposes. Whether additional analogues to those described here might be as effective in vivo as these are in vitro is not known, but the enzymes’ marked differential response to the inhibitors suggests that the folate site may be a more fruitful target for selective chemotherapy than the nucleotide site. Even in cases where distinctive kinetic differences between parasite and host enzyme have not been observed, such as with the Plasmodium berghei and reticulocyte thymidylate synthetases (22), the physical properties of the

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1 G. F. Maley, F. Maley, and C. M. Baugh, unpublished results.
enzymes are sufficiently different to suggest that, under appropriate conditions, a selective degree of inhibition might be found. Thus, it is possible that if folylpolyglutamates had been used in these studies, characteristic kinetic differences between the synthetases would have been seen.

REFERENCES

1. Burchall, J. J., and Hitchings, G. H. (1965) Mol. Pharmacol. 1, 126-136
2. Burchall, J. J. (1973) J. Infect. Dis. 128, 5437-5441
3. Ferone, R., Burchall, J. J., and Hitchings, G. H. (1969) Mol. Pharmacol. 5, 49-59
4. Ferone, R. (1977) Bull. World Health Org. 55, 291-298
5. Van den Bossche, H. (1978) Nature 273, 626-630
6. Friedkin, M., and Donovan, E. (1972) Adv. Enzyme Regul. 10, 133-142
7. Galivan, J., Maley, C. F., and Maley, F. (1974) Biochemistry 13, 2282-2289
8. Wahba, A. J., and Friedkin, M. (1961) J. Biol. Chem. 236, PC11-12
9. Krumdieck, C. L., and Baugh, C. M. (1969) Biochemistry 8, 1568-1572
10. Heidelberger, C. (1973) in Cancer Medicine (Holland, J. F., and Frei, E., eds) pp. 788-791, Lea and Febiger, Philadelphia
11. Kampf, A., Pillar, C. J., Woodford, W. J., and Mertes, M. P. (1976) J. Med. Chem. 19, 909-915
12. Wataya, Y., Santi, D. V., and Hansch, C. (1977) J. Med. Chem. 20, 1469-1473
13. Kampf, A., Barfknecht, R., Shaffer, P. J., Osaki, S., and Mertes, M. P. (1976) J. Med. Chem. 19, 903-908
14. Edelman, M. S., Barfknecht, R. L., Huet-Rose, R., Boguslawski, S., and Mertes, M. P. (1977) J. Med. Chem. 20, 669-673
15. DeClercq, E., Descamps, J., Huang, G.-F., and Torrence, P. F. (1978) Mol. Pharmacol. 14, 422-430
16. Matsuda, A., Wataya, Y., and Santi, D. V. (1978) Biochem. Biophys. Res. Commun. 84, 654-659
17. Mertes, M. P., Cheng, C.-T.-C., DeClercq, E., Huang, G.-F., and Torrence, P. F. (1978) Biochem. Biophys. Res. Commun. 84, 1054-1059
18. Bird, O. D., Vaitkus, J. W., and Clark, J. (1970) Mol. Pharmacol. 6, 573-575
19. Kissiuk, R. L., Gaumont, Y., and Baugh, C. M. (1974) J. Biol. Chem. 249, 4100-4103
20. Kissiuk, R. L., Gaumont, Y., Baugh, C. M., Galivan, J. H., Maley, G. F., and Maley, F. (1979) in Developments in Biochemistry (Kissiuk, R. L., and Brown, G. M., eds) Vol. 4, pp. 431-435, Elsevier/North Holland, Amsterdam
21. Dolnick, B. J., and Cheng, Y.-C. (1978) J. Biol. Chem. 253, 3563-3567
22. Reid, V. E., and Friedkin, M. (1973) Mol. Pharmacol. 9, 74-80
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