Effect of Hydroxyurea on T4 Ribonucleotide Reductase*

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SUMMARY

Phage T4-induced ribonucleotide reductase, purified to homogeneity, catalyzes the reduction of the four ribonucleotides CDP, UDP, ADP, and GDP to the corresponding deoxyribonucleotides. The enzyme is an order of magnitude more sensitive to hydroxyurea than the corresponding Escherichia coli enzyme. Fifty per cent inhibition occurs at 10 μM to 30 μM hydroxyurea. Inhibition is complete at a high concentration of the drug, and there is no differential effect on the four substrates. Treatment of T4 ribonucleotide reductase or its isolated subunits with hydroxyurea does not lead to their irreversible inactivation.

Hydroxyurea inhibits DNA synthesis in several prokaryotes (1-3) and eukaryotes (4-6). There is good evidence that the target of the drug is ribonucleotide reductase (EC 1.17.4.1), the enzyme which reduces the four common ribonucleotides to the corresponding deoxyribonucleotides. The drug inactivates the Escherichia coli ribonucleotide reductase in vitro by scavenging the free radical in the iron-containing enzyme subunit protein B2 (7-9).

Infection of E. coli with bacteriophage T4 induces the formation of a new ribonucleotide reductase, which has been thoroughly studied biochemically (10-13) and genetically (14, 15). T4 ribonucleotide reductase contains an iron- and radical-containing equivalent of protein B2, and is sensitive to hydroxyurea, a fact we had observed several years ago, but not yet reported. However, it has been shown that T4 DNA precursor synthesis is highly sensitive to hydroxyurea in vivo (16), and this was the basis for a powerful selection method for certain nuclease-deficient T4 mutants (17, 18).

In a recent communication, Yeh and Tessman investigated the effect of hydroxyurea on the reductase activity of extracts of T4-infected cells (19). They found that the drug inhibited the reduction of CDP and ADP, but had considerably smaller effect on the reduction of UDP and GDP. In the latter case, even concentrations as high as 0.5 mM gave only a partial inhibition. As it is our experience that T4 ribonucleotide reductase cannot stand a 0.5 mM concentration of anything except water, we have reinvestigated the effect of hydroxyurea on the pure T4 enzyme (Fig. 1). Contrary to Yeh and Tessman, we found that the drug was a potent inhibitor of the reduction of all four ribonucleotide substrates. Fifty per cent inhibition was obtained with 10 to 30 μM hydroxyurea, and complete inhibition at 200 μM concentration. The differences between the four substrates were negligible. Fig. 1 also shows the effect of hydroxyurea on the E. coli ribonucleotide reductase. A 10-fold higher concentration of the drug was required to inhibit the bacterial enzyme than the phage enzyme. This agrees well with in vivo observations made by Warner and Hobbs (16). The bacterial enzyme was completely inactive in 2 mM hydroxyurea, and again there was no difference between two substrates tested (Fig. 1).

The assay in Fig. 1 does not measure ribonucleotide reduction directly, but rather the disappearance of NADPH. One might argue that some component of the hydrogen donor

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system (thioredoxin or thioredoxin reductase) was inhibited by hydroxyurea. However, control experiments using 5,5'-di-thiobis(2-nitrobenzoic acid) (11), instead of ribonucleotide reductase as oxidant of thioredoxin, showed that even 2 mM hydroxyurea did not affect the hydrogen transport.

In summary, our results are clearly in conflict with those of Yeh and Tessman (19).

In an attempt to illuminate the mechanism of action of hydroxyurea on the T4 ribonucleotide reductase, a solution of the enzyme was incubated at room temperature in the presence of a high concentration (0.4 mM) of the drug. After 10 min and 30 min, aliquots of the solution were removed and diluted 10-fold into incubation mixtures, and the reactions were recorded. The observed rates (Table I) were about the same as expected in diluted (0.04 mM) hydroxyurea (cf. Fig. 1). This indicated that no irreversible inactivation of the enzyme occurred during incubation with the drug.

Similar results were obtained with the isolated T4 reductase subunit proteins B1 and B2 (13). Each subunit was incubated with an inhibitory concentration (0.15 mM) of hydroxyurea, and then diluted 10-fold into a complete incubation mixture containing an excess of the other subunit. Again, the observed activity was about the same as that expected in diluted (0.015 mM) hydroxyurea, and no inactivation had occurred during preincubation. These results contrast somewhat with the picture of hydroxyurea as a radical scavenger in protein B2 (9). We suggest an alternative explanation that would apply for the T4 enzyme: the radical becomes inactivated by hydroxyurea only during the catalytic process.

**REFERENCES**

1. Gale, G. R., Kendall, S. M., McLain, H. H., and DuBois, S. (1964) *Cancer Res.* 24, 1012-1019
2. Rosenkranz, H. S., Garro, A. J., Levy, J. A., and Carr, H. S. (1966) *Biochim. Biophys. Acta* 114, 501-515
3. Rosenkranz, H. S., and Levy, J. A. (1965) *Biochim. Biophys. Acta* 93, 181-183
4. Pfeiffer, S. E., and Tolmach, L. J. (1967) *Cancer Res.* 27, 124-129
5. Schwartz, H. S., Garofalo, M., Sternberg, S. S., and Philips, F. S. (1965) *Cancer Res.* 25, 1867-1870
6. Yarbro, J. W., Kennedy, B. J., and Barnum, C. P. (1965) *Proc. Nat'l Acad. Sci. U. S. A.* 53, 1030-1035
7. Krakoff, L. H., Brown, N. C., and Reichard, P. (1965) *Cancer Res.* 25, 1559-1565
8. Brown, N. C., Eliasson, H., Reichard, P., and Thelander, L. (1969) *Eur. J. Biochem.* 9, 512-518
9. Atkin, C. L., Thelander, L., Reichard, P., and Lang, G. (1973) *J. Biol. Chem.* 248, 7464-7472
10. Berglund, O., Karlstrom, O., and Reichard, P. (1969) *Proc. Nat'l Acad. Sci. U. S. A.* 62, 829-835
11. Berglund, O. (1972) *J. Biol. Chem.* 247, 7270-7275
12. Berglund, O. (1972) *J. Biol. Chem.* 247, 7276-7278
13. Berglund, O. (1975) *J. Biol. Chem.* 250, 7450-7455
14. Yeh, Y.-C., Dubovi, E. J., and Tessman, I. (1969) *Virology* 37, 615-623
15. Yeh, Y.-C., and Tessman, I. (1972) *Virology* 47, 767-772
16. Warner, H. R., and Hobbs, M. D. (1968) *J. Virol.* 3, 331-336
17. Goscin, L. A., and Hall, D. H. (1972) *Virology* 50, 84-94
18. Goscin, L. A., and Hall, D. H. (1973) *Virology* 56, 207-217
19. Yeh, Y.-C., and Tessman, I. (1978) *J. Biol. Chem.* 253, 1323-1324
20. Sjoberg, B.-M. (1972) *J. Biol. Chem.* 247, 8058-8062
21. Holmgren, A. (1968) *Eur. J. Biochem.* 6, 47-58
22. Thelander, L. (1973) *J. Biol. Chem.* 248, 1391-1401

**Table I**

| Protein       | Conditions of incubation | Activity* % |
|---------------|--------------------------|-------------|
| Native enzyme =                         | 0.4 mM hydroxyurea, 10 min  | 33          |
| (B1 + B2)     | 0.4 mM hydroxyurea, 30 min | 30          |
| B1           | 0.15 mM hydroxyurea, 10 min | 71          |
|              | 0.15 mM hydroxyurea, 30 min | 78          |
| B2           | 0.15 mM hydroxyurea, 10 min | 71          |
|              | 0.15 mM hydroxyurea, 30 min | 60          |

* Activity is given in per cent of that of a control incubated without hydroxyurea.