5-BROMOURACIL: A NEW INHIBITOR OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE[X]

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In a previous study (Vardanis and Hochster, 1961) it was shown that extracts of the phytopathogenic, crown-gall tumor-inducing organism Agrobacterium tumefaciens oxidize glucose predominantly via glucose-6-phosphate (G-6-P) dehydrogenase and the splitting reaction of 2-keto-3-deoxy-6-phosphogluconate (Entner and Doudoroff, 1952). For this organism at least, G-6-P dehydrogenation thus becomes the rate-limiting reaction for the entry of G-6-P into the above pathway. For purposes of future studies designed to lead to an understanding of the mechanism of tumor induction and control it was considered desirable to find an effective inhibitor of this enzyme. D-glucosamine-6-phosphate (Glaser and Brown, 1955) did not prove to be useful in this respect with extracts of A. tumefaciens. The present communication shows that 5-bromouracil (BU) acts as an effective inhibitor of the G-6-P dehydrogenase in this organism.

Agrobacterium tumefaciens, strains B_6 (pathogenic) and II BNV_6 (non-pathogenic) were kindly supplied by Dr. A. C. Braun, Rockefeller Institute, New York. Cell-free extracts were prepared and enzyme assays performed essentially as described previously (Vardanis and Hochster, 1961) except that tris-(hydroxymethyl)aminomethane buffer, 1 µmole G-6-P, 0.4 µmole TPN and a protein concentration (per cuvette) of 0.5 mg were used here. Due to the low solubility of BU in biological fluids, 10 mg were dissolved in 0.1 ml N/NaOH and this solution added to the reaction cuvette containing sufficient tris buffer to bring the final pH to 8.2. This permitted the addition of 40-50 µmoles BU

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which did not precipitate out again and allowed the pH to remain constant throughout.

In view of recent controversies regarding the purity of BU (Lipetz and Stonier, 1961) the following data were obtained with the sample of BU (Sigma Chemical Co.) used in this work: $\lambda_{max}^{H_2O}$ 276 mμ, $\epsilon = 7.20 \times 10^3$; $\lambda_{min}^{H_2O}$ 241 mμ, $\epsilon = 1.77 \times 10^3$; $\lambda_{max}^{pH\ 13}$ 291 mμ, $\epsilon = 6.92 \times 10^3$; $\lambda_{min}^{pH\ 13}$ 254 mμ, $\epsilon = 1.89 \times 10^3$; $\lambda_{280/260}$ 1.47, $\lambda_{250/260}$ 0.54 (pH 5.6). The material moved as a single, ultraviolet absorbing spot on chromatography in several solvent systems, and upon infra-red analysis showed a very strong, sharp band characteristic of the C-Br bond at a frequency of 645 cm^{-1} which remained unaltered by alkali treatment and by subsequent alteration of the alkaline solution with acid to an experimental pH of 8.2. These constants are in close agreement with those given in the literature (Colthup, 1950; Bellamy, 1958; Wang, 1959) and show that the substance used was, indeed, BU of high purity.

Results and Discussion

When BU (45 μmoles) was added to cuvettes containing all other prerequisite components for the measurement of G-6-P dehydrogenase activity, strong inhibitions were obtained as illustrated in Fig. 1, curve 2.

There was some variation in the extent of the inhibition with crude extracts from one experiment to the next but essentially similar results were obtained with $(NH_4)_2SO_4$ fractions in which the enzyme had been purified approximately 20 fold.

Infra-red studies have revealed not only that BU exists in different forms in acid and in alkali but also that at pH 8.2, at which these experiments were done, more than two forms probably exist. All attempts to correlate the inhibition with the particular molecular species of BU responsible for the inhibitory effect have remained unsuccessful so far. Thus, the concentration of the active form of BU (at this pH) is unknown and for this reason it has not yet been possible to set up meaningful kinetic experiments. It is possible that the active form of BU may be one portion of the molecular species which, at pH 8.2, is

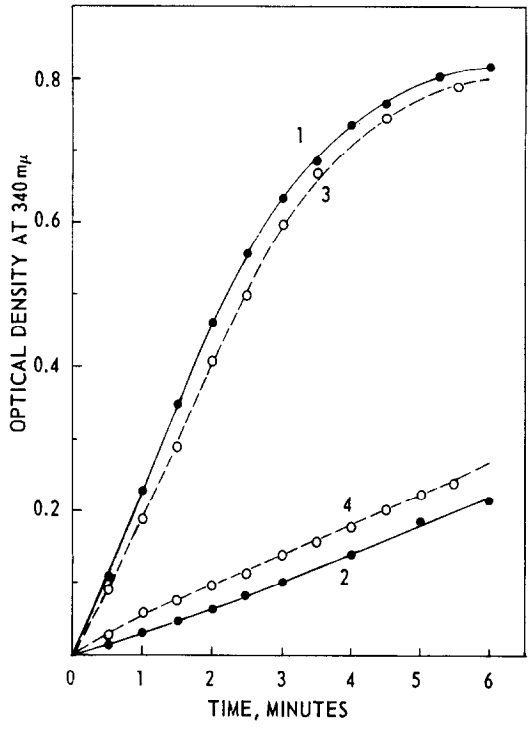

Fig. 1. Inhibition of A. tumefaciens G-6-P dehydrogenase activity by BU. Uninhibited and inhibited reactions of the enzyme of the pathogenic strain (B_6): curves 1, 2 and of the non-pathogenic strain (II BNV_6): curves 3, 4.

present in very small concentration. Thus the need for a high concentration of inhibitor is not surprising. Whereas it was found that increasing the pH of the reaction mixture led to increased inhibition by BU, the sharp drop in activity of the A. tumefaciens G-6-P dehydrogenase below 7.8 and above 9.0 did not permit the study to be extended into those pH ranges in which it might have been possible to study the effect of BU as a single molecular species. The use of lower concentrations of inhibitor produced a correspondingly lower percentage inhibition of the reaction.

While the BU effect described here is not limited to the pathogenic species of A. tumefaciens (c.f. Fig. 1, curves 1 and 2 with 3 and 4) identical experimental conditions with a highly purified G-6-P dehydrogenase from yeast resulted in only 10% inhibition while rat liver homogenate G-6-P dehydrogenase (1 mg protein) was shown to be inhibited approximately 30%.

The 6-phosphogluconate dehydrogenase, phosphohexose isomerase and polynucleotide phosphorylase of A. tumefaciens were not inhibited by the same concentrations of BU.

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