ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 306, No. 2, November 1, pp. 518–520, 1993

Effects of Paraquat on *Escherichia coli:* Sensitivity to Small Changes in pH of the Medium—A Cautionary Note[1]

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Received May 20, 1993, and in revised form July 13, 1993

Uric acid appears to protect *Escherichia coli* against the growth-inhibiting effect of paraquat, but this is actually due to acidification of the medium and does not occur when the pH of the medium is readjusted to neutrality. Any compound which lowers the pH of the medium will thus diminish the effect of paraquat on *E. coli*, whether that effect is inhibition of growth or adaptive induction of members of the soxRS regulon. © 1993 Academic Press, Inc.

Uric acid has been reported to protect *Escherichia coli* against the toxicity of PQ^{2+} [3] (1). Although attributed to the scavenging of free radicals by urate (1), it appeared possible that this protective effect might actually have been due simply to the acidity of uric acid. This explanation seemed possible because previous work had demonstrated that with *Escherichia coli* B lowering the pH of the medium dramatically diminished uptake and retention of PQ^{2+} (2). It seemed likely that pH would similarly influence uptake of PQ^{2+} into *E. coli* K12, although K12 strains do not retain PQ^{2+} as do B strains (3).

Our attention was recently redirected to the influence of pH on the toxicity of PQ^{2+}, when we noted that GSH prevented induction of the soxRS regulon in *E. coli* and then found that this was actually due to slight acidification of the medium by the GSH. The influence of uric acid on the effects of PQ^{2+} on *E. coli* K12 has therefore been reexamined. This has been done in terms of growth inhibition. Induction by PQ^{2+} of the soxRS regulon (4), as a function of pH, was also studied. Fumarase C (5) and glucose-6-phosphate dehydrogenase are members of this regulon and their inductions were used as a diagnostic for induction of the regulon. The protective effect of uric acid is due to its acidity and can be eliminated by neutralization.

MATERIALS AND METHODS

Materials. Uric acid was purchased from Aldrich; $MgCl_2$ from Mallinkrodt; Tris, PQ^{2+}, $NADP^+$, bovine serum albumin, and glucose 6-phosphate from Sigma; malate from ICN; and both yeast extract and bactotryptone from Difco.

Assays. Protein was determined as described by Lowry *et al.* (6) using bovine serum albumin as the standard. G-6-PD was measured as described by Kao and Hassan (7) and fumarase activity according to Hill and Bradshaw (8). The stable fumarase C was distinguished from the unstable fumarases A and B as previously described (5).

Culture conditions. *E. coli* AB1157 was obtained from Stuart Linn (9). They were cultured in L-broth when growth was to be monitored at $A_{600\,nm}$ and in LB medium when fumarase C and G-6-PD were to be assayed. Unless otherwise specified, overnight cultures were diluted 5-fold with fresh medium and grown for 1 h, at which point aliquots were diluted 100-fold into fresh medium ± PQ^{2+} ± uric acid. Where specified the pH of the medium to which uric acid had been added was readjusted to the initial pH with NaOH. All cultures were grown at 37°C while being shaken at 200 rpm.

RESULTS

Effect of uric acid on growth. The growth of *E. coli* in L-broth was inhibited by 0.5 mM paraquat. This is shown in Fig. 1 (lines 1 and 2). Addition of uric acid decreased the pH of the medium from 7.0 to 6.75 and this substantially relieved the growth inhibition imposed by paraquat (line 3). In contrast, when the pH was adjusted back to 7.0, urate did not protect against inhibition by paraquat (line 4). This indicates that the apparent protective effect of urate was actually due to its effect on the pH of the medium. It also appears that the ability of paraquat to

[1] This work was supported by research grants from the Council for Tobacco Research–U.S.A., Inc. and the Johnson and Johnson Focused Giving Program.

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[3] Abbreviations used: PQ^{2+}, paraquat; G-6-PD, glucose-6-phosphate dehydrogenase.

0003-9861/93 $5.00