TRYPTOPHAN-MEDIATED SUBSTRATE INHIBITION OF ANTHRANILATE-5-PHOSPHORIBOSYLPYROPHOSPHATE PHOSPHORIBOSYLTRANSFERASE[1]

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Received February 27, 1969

Summary

A previously unrecognized type of endproduct inhibition is described. Anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (PR transferase) activity from Salmonella typhimurium when aggregated to active or inactive anthranilate synthetase, is subject to endproduct-mediated substrate inhibition. Inhibition of PR transferase activity by the substrates, 5-phosphoribosyl-1-pyrophosphate (PRPP) and anthranilate, is observed in the presence of tryptophan. Substrates for the aggregated anthranilate synthetase enzyme antagonize the tryptophan-mediated substrate inhibition of PR transferase activity.

Tryptophan synthesis in Salmonella typhimurium (1), Escherichia coli (2,3) and Aerobacter aerogenes (4) is regulated, in part, by endproduct inhibition of anthranilate synthetase and anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (PR transferase) activities. These two enzymes which catalyze the first two reactions of tryptophan synthesis are aggregated in wild-type cells. Unaggregated anthranilate synthetase and PR transferase activities can be obtained from appropriate mutant strains. Whereas PR transferase is subject to inhibition by tryptophan when aggregated to anthranilate synthetase, the enzyme is insensitive to endproduct inhibition when dissociated from anthranilate synthetase. The reactions catalyzed by anthranilate

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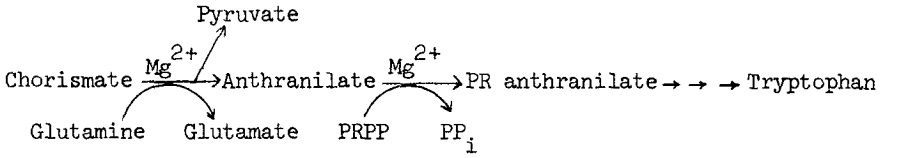
[1]Journal Paper No. 3633 Purdue Agricultural Experiment Station. Supported in part by Grant GB-5841 from the National Science Foundation.

[2]National Science Foundation predoctoral fellow.

synthetase and PR transferase are shown below.



The purpose of this paper is to describe experiments which elucidate, in part, the mechanism of endproduct inhibition of PR transferase by tryptophan. It is found that tryptophan mediates substrate inhibition of PR transferase activity.

METHODS

Two types of PR transferase were studied. PR transferase, obtained from S. typhimurium strain trp E2, is aggregated to catalytically active anthranilate synthetase. The enzyme obtained from S. typhimurium strain trp A8 is aggregated to inactive anthranilate synthetase. Both strains were grown under conditions that allowed derepression of the tryptophan operon. The native enzyme aggregate from strain trp E2 was purified 68-fold to a specific activity of 2540 mμ moles anthranilate utilized/min./mg protein by $(NH_4)_2SO_4$ precipitation (0 - 38% saturation), DEAE cellulose chromatography and Sephadex G-200 gel filtration. The enzyme aggregate from strain trp A8 was purified 19-fold to a specific activity of 635 mμ moles anthranilate utilized/min./mg protein by ammonium sulfate precipitation (0 - 40% saturation) and DEAE cellulose chromatography. Both enzyme aggregates have a molecular weight of approximately 230,000 ($s_{20,w}$ = 10.7S) as determined by sucrose gradient centrifugation (5), using yeast alcohol dehydrogenase (6.7S) as a marker. PR transferase activity was determined at room temperature (22°) by spectrofluorometric assay of the rate of anthranilate utilization. Reaction mixtures contained 0.05 M triethanolamine-HCl pH 7.5, 20 μM 5-phosphoribosyl-1-pyrophosphate (PRPP), 20 μM anthranilate, 2 mM $MgCl_2$, 1 mM dithiothreitol and a

limiting amount of enzyme. Deviations from this procedure are specified where applicable.

RESULTS AND DISCUSSION

As reported previously (2), PR transferase activity from S. typhimurium is inhibited by tryptophan. The upper curve in Figure 1 shows that at saturating concentrations of PRPP and anthranilate (20 μM each) inhibition by tryptophan approaches 70%. Half maximal inhibition is obtained at less than 1.0 μM tryptophan. In order

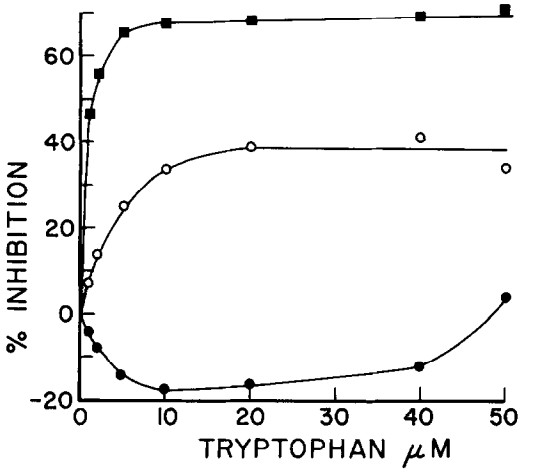


Figure 1. Effect of tryptophan concentration on inhibition of PR transferase activity at saturating and limiting concentration of substrate. The reaction mixture is as described in Methods except as noted. The symbols are ■—■, 20 μM anthranilate and PRPP; o--o, 2 μM anthranilate and 20 μM PRPP; ●—●, 20 μM anthranilate and 1 μM PRPP Enzyme is from strain trp A8. Negative values for inhibition denote activation.

to determine the type of inhibition exerted by tryptophan, the effect of varying the concentration of one substrate at fixed concentrations of the second substrate was studied. In Figure 2A a plot of 1/v against 1/PRPP is shown. In the absence of tryptophan the usual linear double reciprocal plot is obtained. However, 2 μM or 20 μM tryptophan cause substrate inhibition, as shown by upward curvature at high PRPP concentrations and apparent convergence at low PRPP

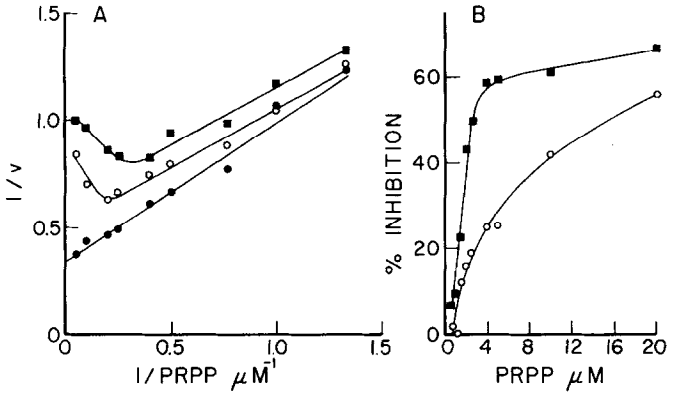


Figure 2. Effect of PRPP concentration on tryptophan-mediated inhibition. (A) Double reciprocal plot. (B) Same data plotted as % inhibition against PRPP concentration. The symbols are ●——●, no tryptophan; o——o, 2 μM tryptophan; ■——■, 20 μM tryptophan. Enzyme is from strain trp A8.

concentrations. The dependence on PRPP for tryptophan-mediated inhibition is shown more clearly in Figure 2B where data from Figure 2A have been replotted in the form of % inhibition against PRPP concentration. Inhibition of PR transferase activity by tryptophan is clearly a function of the PRPP concentration. The data in Figures 3A and 3B show the same qualitative relationships for saturation by anthranilate. In the presence of 2 μM or 20 μM tryptophan, inhibition of PR transferase activity by anthranilate is observed. Convergence of the curves at low concentrations of anthranilate is variable and was not observed in this experiment.

Since tryptophan causes substrate inhibition of PR transferase activity, inhibition should be reduced at low substrate concentrations. The data in Figure 1 show reduced inhibition by tryptophan when the concentration of anthranilate is lowered from 20 μM to 2 μM. When the PRPP concentration is lowered from 20 μM to 1 μM, tryptophan no longer inhibits, rather slight activation is observed. Activation by tryptophan at low PRPP concentrations is variable.

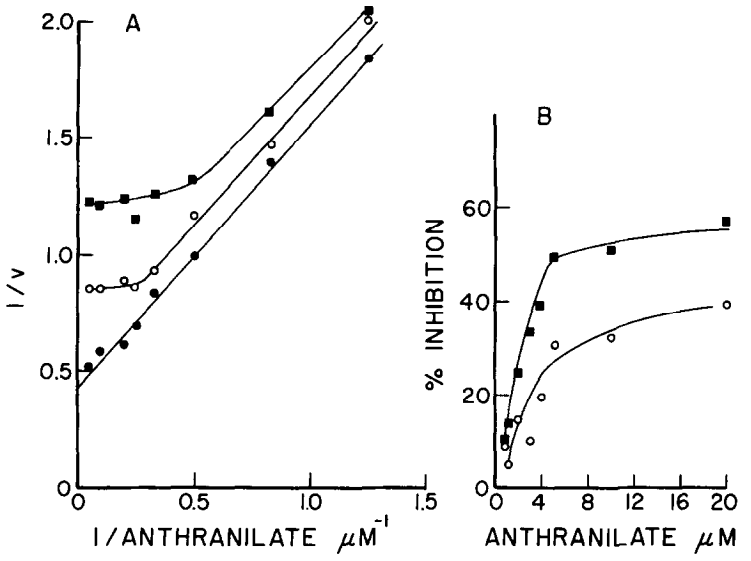


Figure 3. Effect of anthranilate concentration on tryptophan-mediated inhibition. (A) Double reciprocal plot. (B) Same data plotted as % inhibition against PRPP concentration. The symbols are given in Figure 2. Enzyme is from strain trp A8.

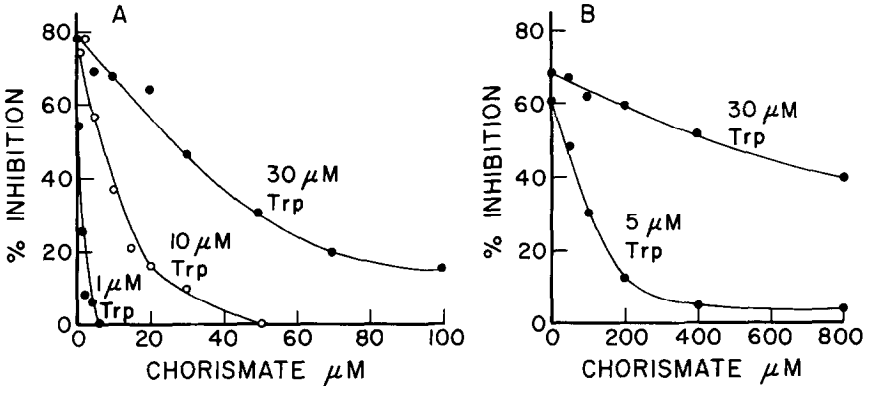


Figure 4. (A) Effect of chorismate on tryptophan-mediated substrate inhibition with wild-type enzyme. (B) Effect of chorismate plus glutamine on tryptophan-mediated substrate inhibition with enzyme from strain trp A8. The glutamine concentration is 5.0 mM. The abbreviation for tryptophan is trp.

Although the data in Figures 1-3 only show results for PR transferase from strain trp A8 (inactive anthranilate synthetase) qualitatively similar data are obtained for PR transferase from strain trp

E2 (active anthranilate synthetase).

Tryptophan-mediated substrate inhibition of PR transferase activity is antagonized by the substrates for the aggregated anthranilate synthetase enzyme. Data in Figure 4A show the antagonistic effect of chorismate on the native enzyme and in Figure 4B the antagonistic effect of chorismate plus glutamine on the enzyme from strain trp A8. Glutamine enhances the antagonistic effect of chorismate. Chorismate exerts a stronger effect on the aggregate that contains an active anthranilate synthetase compared to the aggregate with an inactive anthranilate synthetase. We suggest two explanations to account for the antagonistic effect of chorismate on tryptophan-mediated substrate inhibition of PR transferase activity. (a) The only tryptophan binding site(s) is on the anthranilate synthetase enzyme. Inhibition of anthranilate synthetase activity by tryptophan is competitive with chorismate (3). (b) Substrates for anthranilate synthetase induce a conformational change which renders PR transferase insensitive to endproduct-mediated substrate inhibition.

The potential physiological significance of endproduct-mediated substrate inhibition of PR transferase activity is presently uncertain. Further experiments are in progress.

It is of interest that in the tyrosine pathway in A. aerogenes, inhibition of prephenate dehydrogenase by tyrosine is dependent upon the NAD concentration (6). Inhibition by tyrosine is strongest at high concentrations of NAD and is minimal at low concentrations of NAD. These data suggest endproduct-mediated coenzyme inhibition. Prephenate dehydrogenase activity is associated with the preceeding enzyme activity in the pathway, chorismate mutase (6,7).

ACKNOWLEDGEMENT

Expert technical assistance was provided by Mrs. Lian-Hua Hwang. Discussions with Stanley W. Artz were instrumental to this work.

We are grateful to Dr. R. Bauerle for supplying S. typhimurium strain trp A8.

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