EVIDENCE FOR THE ENZYMATIC SYNTHESIS OF N-(5'-PHOSPHORIBOSYL) ANTHRANILIC ACID, A NEW INTERMEDIATE IN TRYPTOPHAN BIOSYNTHESIS[1]

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Cell-free extracts of Escherichia coli can convert anthranilic acid and PRPP[3] to IGP[3]. Accumulation studies and work with extracts (Doy and Gibson, 1959; Smith and Yanofsky, 1960) have established 1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate[3] as an intermediate in this sequence. However, similar studies (Doy, 1960a and unpublished observations; Smith and Yanofsky, 1960) have failed to detect the formation of N-(5'-phosphoribosyl) anthranilic acid[3], which has been postulated (Yanofsky, 1956) as an earlier intermediate. These failures have been ascribed to the lability of anthranilic acid glycosylamines (Doy and Gibson, 1959; Doy, 1960a, b), which might lead a mutant blocked after PRA to accumulate anthranilate rather than RA. The present communication provides evidence for the formation of PRA by extracts of such mutants.

Extracts of various strains of E. coli, Aerobacter aerogenes, and Salmonella typhimurium were prepared as described by Srinivasan (1959). The disappearance of anthranilic acid was measured in an Aminco Bowman Spectrofluorometer with 310 mμ for activation and 409 mμ for emission.

Several observations suggested that the conversion of anthranilic acid to

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3 The following abbreviations have been used: PRA, N-(5'-phosphoribosyl) anthranilic acid (elsewhere called N-o-carboxyphenylribosylamine 5-phosphate); CDRP, 1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate; IGP, indole 3-glycerol-phosphate; ATP, adenosine triphosphate; PRPP, 5-phosphoribosyl 1-pyrophosphate; RA, N-ribosyl anthranilic acid; DPN^+, diphosphopyridine nucleotide.

PRA would result in the lowering of fluorescence. Thus, N-ribosyl anthranilic acid (i.e., dephosphorylated PRA) is considerably less fluorescent than anthranilic acid itself (Doy, unpublished). Furthermore, the similar compound CDRP is less fluorescent under ultraviolet light than its dephosphorylated form (Dr. F. Gibson, personal communication, and Doy, unpublished). Accordingly, extracts of various mutant strains which accumulate anthranilate were used to search for the conversion of this compound to a labile, less fluorescent product.

With some extracts addition of PRPP, or a PRPP-generating system[4,5], resulted in an initial lowering of fluorescence followed by a gradual restoration to the original level (Fig. 1). The restored fluorescence had the same spectrum as anthranilate. Regeneration of anthranilic acid was further indicated in that the fluorescence could again be lowered by the addition of fresh PRPP (addition A in Fig. 1).

These findings suggest competition between two processes: the enzymatic formation of a derivative of anthranilic acid, and the spontaneous breakdown of that derivative to again yield anthranilic acid. Full fluorescence is restored when further enzymatic formation is prevented by exhaustion of PRPP. The rapidity of this exhaustion is accounted for by the observation that the extracts readily destroy PRPP by reactions not requiring anthranilate.

The labile derivative of anthranilic acid may be provisionally identified as PRA on the following grounds. (1) Of the compounds or mixtures used to promote its formation, PRPP was the most rapid to initiate reaction and then the most efficient to maintain the lowered level of fluorescence. (2) It can be converted enzymatically (see below) to IGP, a later intermediate in tryptophan biosynthesis, presumably via CDRP (Smith and Yanofsky, 1960), the Amadori rearrangement product

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4 From the work of Moyed (1958) it would be expected that such extracts would convert ribose 5-P to PRPP.

5 Because Lingens et al. (1958) have suggested that N-hexose derivatives of anthranilic acid might participate in tryptophan biosynthesis (see discussion by Doy, 1960a; Smith and Yanofsky, 1960) it is of particular interest that glucose 6-P, or glucose 6-P + ATP required the addition of TPN^+ or DPN^+ (Fig. 1); that is, conditions favoring the formation of PRPP.

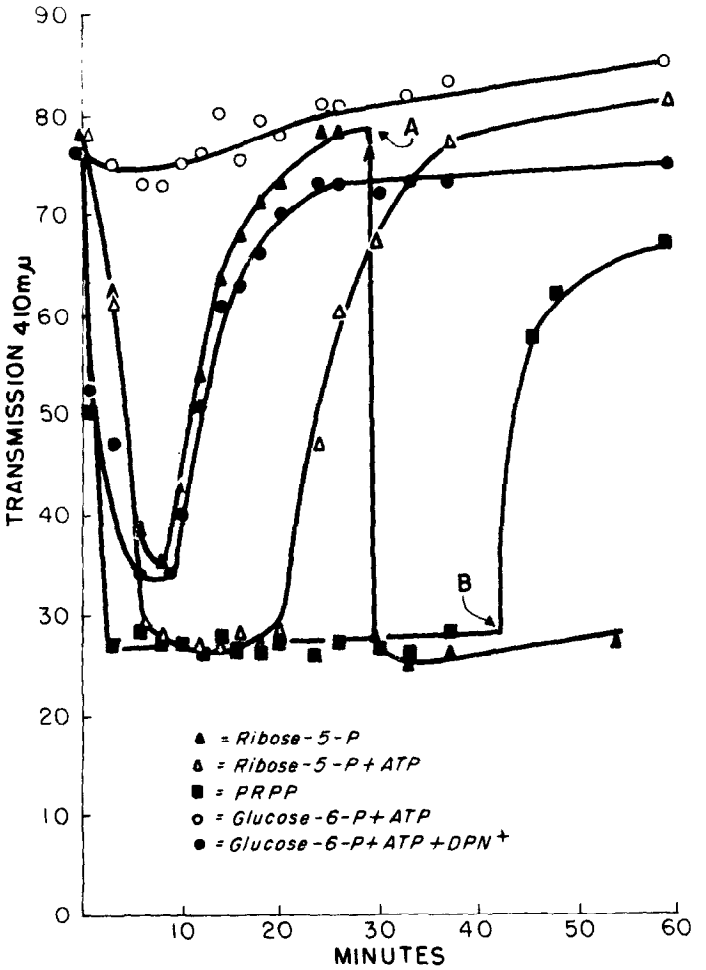

Fig. 1. The conversion of anthranilic acid + PRPP to a less fluorescent, and acid-labile compound. The reaction mixture was 0.2 ml extract (8.4 mg protein per ml, from tryptophan auxotroph E. coli M19-2); 0.015 μmole anthranilic acid; 50 μmole tris (hydroxymethyl) aminomethane, pH 8.0; 5 μmole $MgCl_2$; with the addition of 0.5 μmole amounts of ribose 5-P, glucose 6-P, ATP and DPN^+; 1 μmole PRPP, as indicated. Total volume 1 ml; reaction at pH 7.8 and 23°. Addition A: 1 μmole PRPP. Addition B: the reaction mixture was adjusted to pH 6.0 with 1N HCl.

of PRA (in which the ribosyl group is replaced by 1-deoxyribulose). (3) Its lability resembles that of N-ribosyl anthranilic acid (Doy, 1960a, b) in being increased by acid and decreased by alkali. Thus, when the reaction mixture, at the stage of minimum fluorescence, was adjusted to pH 6 (addition B in Fig. 1) the fluorescence rapidly returned to the original level. In contrast, increased stability was observed at pH 10. Thus far the lability of PRA has prevented its isolation

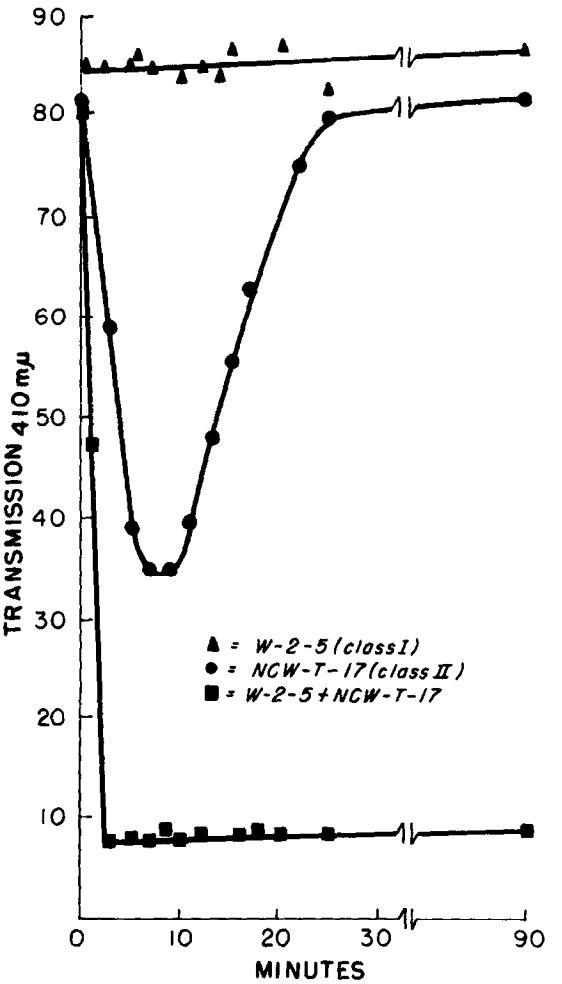

Fig. 2. Results with Group I (E. coli W-2-5) and Group II (A. aerogenes NCW-T-17) extracts, singly and when mixed. The reaction mixture was 0.015 μmole anthranilic acid; 5 μmole $MgCl_2$; 50 μmole tris (hydroxymethyl) aminomethane, pH 8.0; 0.5 μmole ribose 5-P; 0.5 μmole ATP and 0.2 ml extracts as indicated. Total volume 1 ml, reaction at pH 7.8 and 23°.

and chemical synthesis.

On the basis of similar studies, the available mutants blocked between anthranilate and IGP were found to fall into three groups.

Group I: blocked between anthranilate and PRA. These mutants accumulate anthranilic acid and their extracts do not decrease the fluorescence of that compound in the presence of PRPP (Fig. 2).

Group II: blocked between PRA and CDRP. These mutants accumulate large quantities of anthranilate but their extracts catalyze the interaction of anthran-

ilic acid and PRPP described above.

Group III: blocked between CDRP and IGP. These mutants accumulate the non-phorphorylated form of CDRP (and a smaller amount of anthranilic acid). Their extracts were found to convert anthranilic acid and PRPP to a less fluorescent compound which, unlike PRA, is acid-stable. This stability would be expected for the Amadori compound (Doy and Gibson, 1959; Smith and Yanofsky, 1960).

The biosynthetic role of the acid-labile compound was established by its further conversion to a known intermediate in tryptophan biosynthesis. Thus, extracts of some mutants[6] of Group I, when mixed with extracts of Group II, convert anthranilate + PRPP to indole and small amounts of IGP[7]. Similar complementation was obtained with a mixture of Group I and Group III extracts. Indole (and/or tryptophan) was estimated microbiologically with a mutant blocked between anthranilate and IGP; and the presence of indole and IGP was confirmed with the Ehrlich and the $FeCl_3$ tests (Yanofsky, 1955, 1956).

The evidence presented establishes an acid-labile compound, provisionally identified as PRA, as an intermediate in the biosynthesis of tryptophan. Furthermore, these findings provide information on the role of anthranilate. Previously available evidence was consistent with the suggestion (Doy and Gibson, 1959; Doy, 1960a, b) that all, or some, mutant accumulations of anthranilate might be artefacts resulting from breakdown of PRA or its non-phosphorylated form. Group II confirms this prediction for some mutants; but the enzymatic defect in the Group I mutants strengthens the view that anthranilate is the immediate precursor of PRA and hence is an essential intermediate in tryptophan biosynthesis. However, the role of this compound must remain in some doubt until the preceding reaction is determined.

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6 Some Group I mutants may be deletions, since they cannot complement Group II.

7 Conversion to tryptophan is limited by not adding serine; however, conversion of IGP to indole is still possible (Yanofsky, 1960).

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