ENZYMATIC FORMATION OF DIHYDROPTEROIC ACID FROM 2-AMINO-4-HYDROXY-6-TRIHYDROXYPROPYL-7,8-DIHYDROPTERIDINE*

Theodore H. D. Jones, John J. Reynolds+ and Gene M. Brown

Division of Biochemistry, Department of Biology,
Massachusetts Institute of Technology, Cambridge, Massachusetts

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It has been established in previous publications from this laboratory that guanine nucleotides can be converted enzymatically into the pteridine portion of folic acid (Reynolds and Brown, 1962, 1964). A hypothetical biosynthetic pathway was proposed to account for the observations that had been made about the enzyme system (Reynolds and Brown, 1964). Included as an intermediate in this pathway is the phosphate ester of 2-amino-4-hydroxy-6-(D-erythro-1, 2, 3-trihydroxypropyl)-7, 8-dihydropteridine. Evidence that was offered in support of this compound as an intermediate was the observation that extracts of Escherichia coli could convert a compound labeled as 2-amino-4-hydroxy-6-trihydroxypropyldihydropteridine to dihydrofolic acid in relatively small yields. When this compound was first tested its configuration was not known, and also we later found that the preparation contained impurities of unknown chemical nature that

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+ Present address: Strangeways Research Laboratory, Cambridge, England.

inhibit the enzymatic synthesis of folic acid compounds. These uncertainties led us to examine more carefully the enzymatic transformation of this class of compounds to the pteridine component of folic acid.

2-Amino-4-hydroxy-6-(D-erythro-1, 2, 3-trihydroxypropyl) pteridine was synthesized from 2, 4, 5-triamino-6-hydroxypyrimidine and D-ribose (Rembold and Metzger, 1963). The compound was purified by chromatography on P-cellulose also according to the directions of Rembold and Metzger (1963). The purified material was identical in properties (chromatographically and spectrophotometrically) with the pure reference compound kindly supplied by Dr. Rembold. The corresponding L-erythro, D-threo, and L-threo isomers were synthesized by the same procedure by changing the carbohydrate component of the reactants to L-arabinose, D-xylose, and L-xylose, respectively. These compounds were also purified on P-cellulose columns.

Each of the above synthetic compounds was reduced to the corresponding 7, 8-dihydropteridine compound by treatment with dithionite (Futterman, 1957), and these dihydropteridines were then tested as substrates for the enzymatic formation of dihydropteroic acid as described by Reynolds and Brown (1964). The results summarized in Figure 1 show that, of the compounds tested, the D-erythro isomer was converted most effectively to dihydropteroic acid; the L-threo compound was used about half as well as the D-erythro isomer and the L-erythro and D-threo compounds were used about one-tenth as well. For purposes of comparison, the effectiveness with which 2-amino-4-hydroxy-6-hydroxymethyl-7, 8-dihydropteridine is converted to dihydropteroate is also shown in Figure 1. The latter compound has been established as the direct pteridine precursor of folic acid compounds (Shiota and Disraely, 1961; Weisman and Brown, 1964). The relative efficiency with which

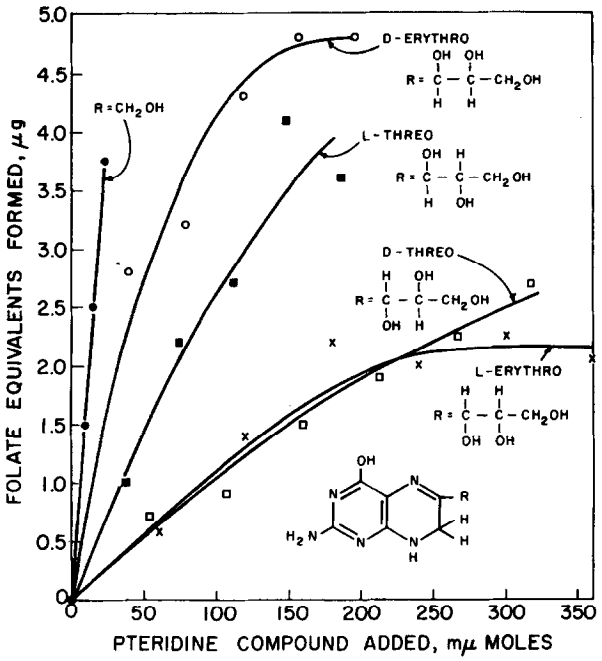

Fig. 1. The enzymatic formation of dihydropteroic acid from various pteridine compounds. Reaction mixtures contained per 1.2 ml: p-aminobenzoic acid, 0.08 mM; $MgCl_2$, 8 mM; ATP, 4 mM; tris-hydroxymethylaminomethane buffer (pH 8.6), 80 mM; enzyme preparation, 2 mg of protein; and pteridine compound as shown in the figure. The enzyme preparation was an extract of Escherichia coli that had been dialyzed and treated with charcoal as described by Reynolds and Brown (1964). Incubation was for 3 1/2 hours at 37° under nitrogen. Production of dihydropteroic acid was determined by microbiological assay with Streptococcus faecalis (ATCC 8043) as described by Brown, Weisman, and Molnar (1961). Since folic acid was used to prepare standard curves in these determinations, results are expressed as "folate equivalents formed".

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the D-erythro isomer is converted to dihydropteroate lends support to the view that this compound (or a phosphate ester of this compound) is an intermediate in the conversion of guanine nucleotides to the pteridine component of folic acid compounds. The D-erythro isomer would be the expected product formed in the enzymatic conversion of guanine nucleotides to a trihydroxypropylpteridine compound, since the ribose portion of naturally-occurring nucleotides is of the D-configuration.

The four trihydroxypropyldihydropteridine isomers presumably are converted by the enzyme system to 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine, the recognized precursor of folate compounds. The other expected product of such an enzymatic transformation would be a 2-carbon compound. Work is now under way in our laboratory to identify such a 2-carbon compound. It seems reasonable to think that the reaction may be of the aldolase type and, thus, that the product may be glycolaldehyde.

Since all four of the possible isomers of 2-amino-4-hydroxy-6-trihydroxypropyldihydropteridine are converted, albeit to varying extents, to dihydropteroate, it would appear that either a single enzyme with relatively broad specificity functions to convert these compounds to 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine, or that a multi-enzyme system operates for this purpose and that the extra enzymes are isomerases that convert the inactive isomers to the isomer that can then be attacked by the enzyme responsible for splitting off the 2-carbon compound. Additional information on the nature of the enzyme system must be obtained before one can decide between these two possibilities.

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