FURTHER EVIDENCE ON THE NATURE OF PREFOLIC A

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In a previous publication (Donaldson and Keresztesy, 1959), the conversion of prefolic A (one of the naturally-occurring forms of folic acid) to tetrahydrofolic acid was described. The reaction was postulated to be an oxidative one, however, we were unable to determine the site of reduction.

The recent observation by Larrabee and Buchanan (Federation Proc. 20, 9, 1961) and the discussion which followed the presentation of "The Interconversion of Prefolic A and Tetrahydrofolic Acid", (Donaldson and Keresztesy, 1961) suggested the possibility that prefolic A may be a methyl tetrahydrofolic acid.

These authors have synthesized a new folic acid intermediate of methionine biosynthesis and showed that this compound contains 1 mole of methyl group per mole of compound.

Further investigation into the nature of prefolic A revealed that during its oxidation to tetrahydrofolic acid, formaldehyde is formed. In addition the reaction may be reversed by reducing methylene tetrahydrofolic acid with DPNH as the electron donor.

The enzyme was prepared from hog liver as previously described (Donaldson and Keresztesy, 1959). Prefolic A was isolated as its barium salt from a hot aqueous ascorbate extract of commercial frozen horse liver, as described by Donaldson and Keresztesy (1959), and Keresztesy and Donaldson (1961). The purified prefolic A is better than 90% pure based on the free acid.

The requirements for the conversion of prefolic A to tetrahydrofolic acid are shown in Table 1. The reaction is completely dependent on all components added to the system. The activity observed, when menadione or FAD is omitted, is due to the presence of endogenous FAD on the enzyme. These values are lowered substantially by removing the bound FAD with ammonium sulfate at acid pH essentially as described by Horecker (1950).

Table 1

Requirements for conversion of prefolic A to Folate-H_4

System	Folate-H_4 formed μg
Complete	3.08
Omit prefolic A	$<$.02
" menadione	.78
" FAD	.77
" enzyme	.14

The reaction was carried out in an atmosphere of helium at 37° for 1 hr. The reaction mixture contained .5 ml. M/3 phosphate buffer pH 6.6, 10 mg. sodium ascorbate pH 6.5, and where indicated 10 μg FAD, 15 μg menadione, 20 μg Ba-prefolic A and 0.24 mg. enzyme protein, in a total volume of 2.0 ml.

Evidence for the formation of formaldehyde during the oxidation of prefolic A to tetrahydrofolic acid was obtained by using prefolic A-C^{14}-synthesized from tetrahydrofolic acid and C^{14}-labeled formaldehyde as described below.

The tetrahydrofolic acid formed from C^{14}-prefolic A was formylated to CF with formylglutamate and transformylase (Silverman et al., 1957), since it has been reported that tetrahydrofolic acid will bind formaldehyde (Kisliuk, 1957). Then 2 mg. of unlabeled formaldehyde was added as carrier, and the dimedon derivative isolated and counted. The dimedon contained 8650 CPM which represented approximately 80% of the radioactivity added to the reaction mixture as prefolic A-C^{14}.

The requirements for the reduction of methylene tetrahydrofolic acid to prefolic A are shown in Table II. The reaction is completely dependent upon all components added.

Table II

Requirements for the reduction of methylene tetrahydrofolic acid to prefolic A

System	Prefolic A formed μg
Complete	124.0
Omit DPNH	15.0
" FAD	57.0
" HCHO	12.0
" Folate-H_4	3.5
" Enzyme	0

The reaction was carried out in an atmosphere of helium at 37° for 30 minutes. The reaction mixture contained .5 ml. M/3 phosphate buffer pH 6.6, 10 mg. sodium ascorbate pH 6.5, 10 μg FAD, 408 μg dl tetrahydrofolic acid, 100 μg HCHO, 2 mg. DPNH and 4.8 mg. of enzyme protein, in a total volume of 2.0 ml.

Evidence for the incorporation of formaldehyde into prefolic A, when synthesized from tetrahydrofolic acid was obtained by using C^{14}-labeled formaldehyde. After the reaction was complete, the entire reaction mixture was chromatographed on DEAE cellulose (Keresztesy and Donaldson, 1961), and the fractions, checked for radioactivity and for biological activity. The radioactivity (CPM) in each fraction paralleled the microbiological activity in every case.

The synthesized prefolic A is identical with the material isolated from horse liver with respect to ultra violet absorption and microbiological activity.

The location of the one carbon moiety on prefolic A is unknown at this time. However, it is not unlikely that this entity is located on the 5 rather than the 10 nitrogen of tetrahydrofolic acid in the form of a methyl group. In support of this postulate is the greater stability

of prefolic A when compared with tetrahydrofolic acid. The possibility that this entity is located at some position other than the N^5 or N^{10} is unlikely, since prefolic A will not bind formaldehyde.

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