Occurrence of N^{10}-formyltetrahydrofolic acid and its general involvement in transformylation*

N^{10}-formylfolic acid** has been implicated in one-carbon transfer reactions[1,2]. This communication presents evidence that the actual compound involved is N^{10}-formyltetrahydrofolic acid or a closely related derivative[3,4] and that the formyl group is in equilibrium with one-carbon donors and acceptors and with the formyl group of citrovorum factor.

N^{10}-$CHOFAH_4$*** has been isolated and identified as a product of the reaction of formate, ATP and FAH_4 in pig liver extracts (tetrahydrofolate formylase) and of the conversion of serine to glycine after oxidation with DPN§. It has been isolated by paper chromatography under N_2 in the presence of EDTA and shown to be identical by paper chromatography and absorption spectrum with N^{10}-$CHOFAH_4$ synthesized by catalytic hydrogenation[5] of N^{10}-CHOFA. Heating of N^{10}-$CHOFAH_4$ with 0.1 *N* NaOH anaerobically should yield CF[6]. Treatment of acetone-deproteinized filtrates of the tetrahydrofolate formylase reaction in this manner yielded CF nearly quantitatively based on the formate incorporated. The CF was isolated by paper chromatography. In control reactions without FAH_4 no CF was detected. Interconversion of CF and N^{10}-$CHOFAH_4$ has been demonstrated by employing formyl-labeled CF, formyl-labeled N^{10}-$CHOFAH_4$, or ring-labeled folic acid and by isolating the folate compounds by chromatography§§. Table I shows that the ^{14}C-labeled β-carbon of serine is transferred to FAH_4 and that (after an oxidation step) it forms the CHO group of N^{10}-$CHOFAH_4$ prior to its appearance in CF.

When ^{14}C formate was employed as the formyl source, the label again appeared first in N^{10}-$CHOFAH_4$ and then in CF[4].

TABLE I

CONVERSION OF β-CARBON OF SERINE TO N^{10}-$CHOFAH_4$ AND TO CF*

Conditions: 10 μmoles serine-3-^{14}C, 7,300 cpm./μmole, 2 μmoles FAH_4, 1.5 μmoles ATP, 5 μmoles $MgCl_2$, 2 μmoles $MnSO_4$, 0.3 ml pig liver extract (1 to 1 homogenate of liver in 0.1 *M* K-PO_4, pH 7.4, and centrifuged at 27,000 × *g* for 30 min). Final vol. 1.0 ml, temp. 37°, gas N_2.

Time min	*Serine µmoles*	*N^{10}-$CHOFAH_4$ µmoles*	*CF µmoles*	*CF + N^{10}-$CHOFAH_4$ µmoles*
0	0	0	0	0
15	0.013	0.012	0	0.012
30	0.037	0.022	0.006	0.028
45	0.065	0.027	0.049	0.076
60	0.103	0.019	0.073	0.092

* No ^{14}C formate was found.

TABLE II

TRANSFORMYLATION OF THE FORMYL GROUP OF N^{10}-CHOFA TO SERINE, PURINE AND HISTIDINE

Conditions as in Table I, but plus 10 μmoles glycine, 10 μmoles glutamine, 17 μmoles glucose-6-phosphate, 0.8 μmoles DPN, 0.2 μmoles N^{10}-^{14}CHOFA, 6500 cpm./μmole. Time 60 min. In expt. II 2 μmoles of unlabeled HCOOH were added as a pool.

^{14}C-substrate	*^{14}C-compound isolated*	*I cpm**	*II cpm**
N^{10}-^{14}CHO-FA, 1300 cpm.	Serine**	283	246
	Purine***	57	
	Histidine	34	
	Ba-N^{10}-CHOFA	760	
	$BaCO_3$	31	
	HCOOH	6	
	Total	1171	

* Uncorrected for self-absorption.
** β-carbon counted as formdimedon.
*** Ag salts of purines in acetone filtrate.

* From a Habilitation Thesis submitted by L. JAENICKE, to the University of Marburg, June, 1954.

** Abbreviations: FAH_4, tetrahydrofolic acid; N^{10}-$CHOFAH_4$, N^{10}-formyltetrahydrofolic acid; N^{10}-CHOFA, N^{10}-formylfolic acid; CF, citrovorum factor (N^5-formyltetrahydrofolic acid); EDTA, ethylenediaminetetraacetate.

*** Recently other workers have isolated from bacterial sources compounds with some properties similar to those of N^{10}-$CHOFAH_4$[5,6,7].

§ Recent studies have shown that TPN is more active in this system.

§§ By ascending chromatography (Schleicher and Schüll 2043 B paper) with 12% Na_2HPO_4 containing 0.2% EDTA as a solvent, the R_F values of authentic compounds were: folic acid, 0.24, FAH_4, 0.48, N^{10}-CHOFA 0.78, N^{10}-$CHOFAH_4$, 0.54, CF, 0.62.

While N^{10}-CHOFAH$_4$ has been shown to occur in these reactions, it is conceivable that it is interconvertible with a more labile derivative. N^{10}-CHOFA is converted to N^{10}-CHOFAH$_4$ by a reducing system. In Table II ^{14}C-formyl-labeled N^{10}-CHOFA is shown to transformylate to glycine to form serine. A system forming reduced pyridine nucleotide is required. Transformylation for synthesis of histidine and purine derivatives also is shown.

These experiments have been repeated with labeled N^{10}-CHOFAH$_4$ and gave essentially similar results, but addition of DPNH was still necessary for serine synthesis. Beginning either with serine and FAH$_4$ or with N^{10}-CHOFAH$_4$ and DPNH a hydroxymethylfolic acid compound was formed*. The conversion to the hydroxymethyl level by reaction with reduced pyridine nucleotide can be coupled in this system with reduction of DPN to DPNH by a tetrahydrofolate compound. Thus in the presence of substrate amounts of formate, glycine, ATP and DPNH, serine synthesis occurred with catalytic quantities of FAH$_4$ (0.01 moles/mole serine). However when substrate amounts of FAH$_4$ were used, only catalytic concentrations of DPNH (0.05 moles/mole serine) were required. While it is clear that a stable cofactor acts in one-carbon transfer reactions[3, 7, 10-12], these studies provide evidence that N^{10}-CHOFAH$_4$ can function as a general transformylating agent, that its formyl group is in equilibrium by an oxidation-reduction system with the β-carbon of serine through a hydroxymethyl level derivative and that CF is not directly involved in this interconversion.

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* On isolation this compound was found to be oxidized to N^{10}-hydroxymethylfolic acid which was identified by comparison with the authentic compound synthesized by two different methods.

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On the occurrence of N^{10}-formyltetrahydrofolic acid by enzymic formylation of tetrahydrofolic acid and on the mechanism of this reaction

Pigeon[1,2] and pig[3] liver extracts catalyze an ATP*-dependent reaction between tetrahydrofolic acid and formate to yield a product which transformylates directly to 5-amino-4-imidazolecarboxamide-5'-phosphoriboside[1,4] to form inosine-5'-phosphate in the absence of ATP. During purification this compound was converted to a compound having the properties of N^{10}-formyldihydrofolic acid[2] and then to N^{10}-formylfolic acid[1,2,3,5,6,7]. This communication provides evidence that the overall reaction, catalyzed by an enzyme system which we propose to call tetrahydrofolate formylase, may be formulated as follows:

$$HCOOH + FAH_4 + ATP \longrightarrow CHOFAH_4 + ADP + H_3PO_4$$

The evidence that CHOFAH$_4$ is N^{10}-CHOFAH$_4$ or a closely related compound is: 1. synthetic N^{10}-CHOFAH$_4$, transformylates to imidazolecarboxamide ribotide in the absence of ATP, 2. CHOFAH$_4$ can be converted quantitatively to the N^5–N^{10}-*imidazolinium* derivative of formyltetrahydrofolic

* Abbreviations: FAH$_4$, tetrahydrofolic acid; CHOFAH$_4$, formyltetrahydrofolic acid; N^{10}-CHOFAH$_4$, N^{10}-formyltetrahydrofolic acid; N^{10}-CHOFAH$_2$, N^{10}-formyldihydrofolic acid; N^{10}-CHOFA, N^{10}-formylfolic acid; CF, citrovorum factor; ATP, adenosinetriphosphate; ACF, the N^5–N^{10}-imidazolinium derivative of formyltetrahydrofolic acid (anhydrocitrovorum factor, anhydroleucovorin); EDTA, ethylenediaminetetraacetate.