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BIOSYNTHESIS OF TETRAHYDROBIOPTERIN: INCORPORATION OF LABEL FROM REDUCED PYRIDINE NUCLEOTIDE DURING FORMATION FROM DIHYDRONEOPTERIN TRIPHOSPHATE

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Introduction

The biosynthetic conversion of dihydroneopterin triphosphate (NH_2P_3) to tetrahydrobiopterin (BH_4) is still unclear in its details. While earlier proposals favoured the involvement of sepiapterin as an intermediate (1,2,3), more recent work from several laboratories suggests that this is not the case, and that biosynthesis proceeds via tetrahydropterin intermediates (4-8). The conversion of NH_2P_3 to BH_4 requires reduction with 4 electrons or 2 hydride equivalents (9); the latter are likely to originate from reduced pyridine nucleotides (7). Earlier studies from Krivi and Brown (10) dealt with the incorporation of label from (4S-^{3}H)NADPH into sepiapterin. As also discussed elsewhere (9), the lack of stereospecificity in the incorporation of label into sepiapterin, and the probable noninvolvement of the latter in the biosynthesis (4-8), question the relevance of these (10) results.

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Studies on the origin of the hydrogens at the C(2'), and C(3') positions of the side chain of BH_4, and on the mode of transfer of the reducing equivalents from NAD(P)H might help elucidate the mechanism of biosynthesis, and in particular might provide information on the intermediate occurrence and structure of tetrahydropterins. In this report we summarize results of experiments involving the use of specifically labelled (4R- or S-^{2}H)NAD(P)H in the biosynthesis of BH_4 in normal as well as in deuterated solvent. The details of these studies will be published elsewhere (8).

Results

The analysis of the incorporation experiments was carried out by gas chromatography-mass spectrometry by recording the molecular ion of BH_4 and 7,8-dihydrobiopterin (BH_2), as well as (whenever possible) the fragments resulting from rupture of the C(1')-C(2') bond (in the case of BH_2) and C(6)-C(1') bond (in the case of BH_4) (8). The reduction of 7,8-dihydrobiopterin to BH_4 with NADPH catalyzed by dihydrofolate reductase proceeds via transfer of the 4-pro R hydrogen of NAD(P)H to the C(6) position of the dihydropterin to yield the C(6)-R configuration of the side chain. This stereochemistry parallels that found during the reduction of the normal substrate of this enzyme, 7,8-dihydrofolate (11).

Sepiapterin reductase catalyzes the reduction of either sepiapterin to BH_2 or dihydrosepiapterin (6-lactoyl-tetrahydropterin) to BH_4. We found that during these reactions, the 4-pro-S hydrogen of NADPH was transferred specifically to the C(1') position of the substrates.

The results of the studies involving the use of partially purified human liver extracts, starting with NH_2P_3 as a substrate, are summarized in Table 1.

Table 1 Incorporation of deuterium into BH_4 from solvent and from NADP(P)H using human liver extracts and NH_2P_3 as the substrate

Pyridine nucleotide	Solvent	Inhibition of exchange by diaphorases	Number of experiments	Number of ^2H in BH_4	Number of ^2H in BH_2
(4,4'-$^2H_2$)NADH	H_2O	No	1	0	-
(4R-^2H)NADH	H_2O	No	1	0	-
(4R-^2H)NADPH	H_2O	No	1	0	0
(4R-^2H)NADPH	H_2O	Yes[a]	1	0	0
(4S-^2H)NADPH	H_2O	No	3	0	-
(4S-^2H)NADPH	H_2O	Yes[b]	1	-	2
NADPH	^2H_2O	No	3	4	3
NADPH	^2H_2O	Yes[c]	2	2	1

(-) not measured

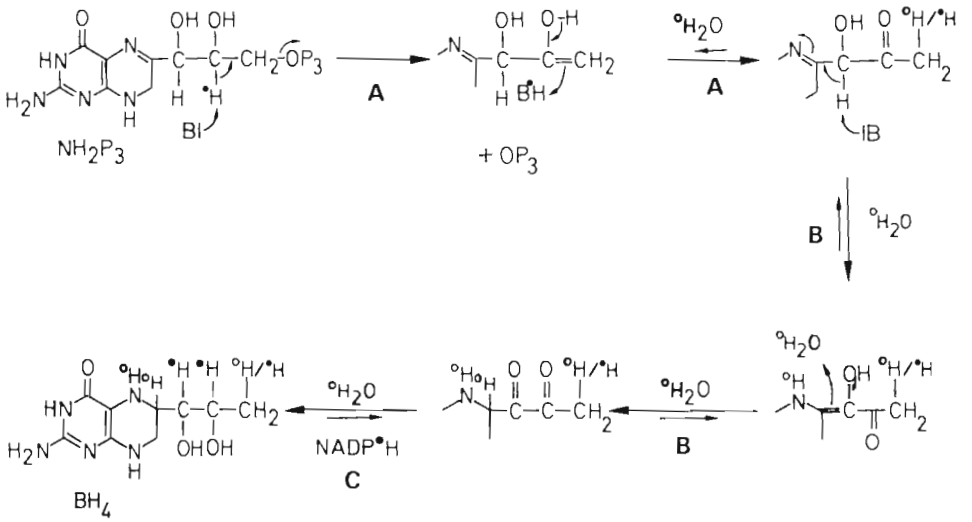
(a-c) Exchange of label in NADPH was determined at the end of the incubation as a control

A striking result was obtained when the incubation was carried out in ^2H_2O using unlabelled-NADPH (Table 1); under these conditions approx. 4 equivalents of label were incorporated into BH_4, and most probably one each at the positions C(6), C(1'), C(2'), and C(3'). In accordance with this, no label was incorporated when the experiments were conducted in H_2O using either (4R- or 4S-^2H)NAD(P)H (Table 1). This unexpected result was due to a relatively rapid exchange of the label in the pyridine nucleotide, which was catalyzed most probably by a diaphorase activity in the extracts. In fact, upon addition of the diaphorase inhibitor, arsenite (12), the exchange was inhibited considerably, although it could not be suppressed completely (Table 1). Thus in the presence of $NaAsO_2$, approx. 2 equivalents of label from (4S-^2H)NADPH were incorporated into BH_2.

This, and the analysis of the MS-fragments clearly indicate that the 4-pro-S hydrogen of NADPH was transferred to BH_4, and that one label was introduced into the position C(1'), and one into C(2'). In the complementary experiment carried out in ^2H_2O using unlabelled NADPH, approx. one equivalent of label was found in BH_2, most probably at the position C(3'), and two equivalents in BH_4 at the positions C(6) and C(3').
The incubation of human liver extracts with sepiapterin and (4S-^{2}H)NADPH in ^2H_2O resulted in the incorporation of label into BH_2. However, under the same conditions using labelled NADH, no label was transferred. This indicates that the exchange of the 4-hydrogens with solvent is much faster with NADH, as compared to NADPH under our experimental conditions.

Discussion

Dihydrofolate reductase was shown to catalyze the transfer of the 4R-label of NADPH to the position C(6) of BH_2 to yield 6(R)BH_4. Since there was no transfer of label to this position during the formation of BH_4 from NH_2P_3 by human liver extracts, this appears to rule out dihydrofolate reductase as an enzyme involved in the conversion of NH_2P_3 to BH_4. This is in agreement with the conclusions of others (13). The finding of incorporation of solvent hydrogen into position C(6) of BH_4 is of obvious significance. Clearly the N(5)-C(6) double bond is not being reduced directly by NAD(P)H. An alternate pathway for the generation of a 5,6-dihydro function has been put forward by our groups (7,9), as well as by others (4-6) and involves an internal transfer of a formal hydride from C(1')-H to the N(5)-C(6) double bond. This mechanism is similar to the well known internal redox reactions observed in amino-sugar chemistry, which is thermodynamically feasible (9) and has counterparts in some pterin model reactions studied by Pfleiderer (15).
The state of the art knowledge on the mechanisms of biosynthesis of BH_4 from NH_2P_3 can be summarized by the sequences of Scheme 1.



Scheme 1

Mechanism and sequences of reactions leading to formation of tetrahydrobiopterin (BH_4) from NH_2P_3. Note that the order of steps (A), and (B) can be inverted without affecting the conclusions. (Adapted from Ref. 8).

In this scheme an active center base might initiate catalysis by attacking C(2')-H, and thus promoting elimination of triphosphate (step A). In fact, triphosphate has recently been shown to be eliminated from NH_2P_3 when the latter is incubated with purified "phosphate-eliminating enzyme" from human liver (16). The proton abstracted by this base can be redonated (partially) to the enolic double bond at C(2')-C(3') and thus incorporated into the final product, BH_4. The base involved could partially exchange with solvent, or it might be a bi- or trifunctional one which does not exchange. This in turn would explain the partial incorporation of label from the solvent into position C(3') of BH_4 (8). It is conceivable that the same base also functions in the abstraction of a proton at C(1')-H (Scheme 1, step B), thus initiating the transfer of electrons to the pterin nucleus.

In accordance with this, the same fraction of phosphate-eliminating enzyme from human liver, which catalyzes the elimination of $-OP_3$ from NH_2P_3, also catalyzes the formation of the tetrahydropterin chromophore, i.e. its conversion to a 5,6-dihydro,C(1')-keto-pterin (17). It should be pointed out that the presence of a second enzyme in the fraction catalyzing this event cannot yet be excluded, however, it appears that processes A and B are early events in the NH_2P_3 conversion to BH_4 (17). On the other hand, the sequence of steps A and B (Scheme 1) could be reversed without altering the general conclusions.

The direct reduction of the diketo-tetrahydropterin by NADPH would then terminate the sequence (Scheme 1, step C) and ensue in BH_4. It has been shown that sepiapterin reductase can catalyze the oxidoreduction at both the C(1'), and the C(2') functions of biopterins (17). This suggests that its function might indeed consist in these last two reductions steps, the involvement of a different enzyme, however, cannot be excluded at present.

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