BIOCHEMICAL FUNCTIONS AND CLINICAL IMPORTANCE OF UNCONJUGATED PTERIDINES

Gordana Bjelaković1, Tatjana Jevtović-Stoimenov1, Bojko Bjelaković2, Ivana Stojanović1

1Institute of Biochemistry, Faculty of Medicine, University of Niš
2Clinic of Pediatrics, Faculty of Medicine, University of Niš, Serbia and Montenegro

Summary. Tetrahydrobiopterine (BH4) and its relatives are classified as unconjugated pteridines or pterins distinguishing them from the folates. BH4 is not a vitamin for mammals, since they can synthesize it. GTP is the major precursor of atoms in the pterin nucleus. The initial step in this pathway is conversion of GTP to D-erythro-7, 8-dihydronoopterin triphosphate, a reaction catalyzed by the enzyme GTP-cyclohydrolase I (EC 3.5.4.16; GTP-CH). There are many important metabolic functions of BH4: it is a crucial cofactor in hydroxylation reactions of phenylalanine, tyrosine and tryptophane. The finding of BH4 participation in monoaminergic neurotransmitter metabolism regulation contributed to the knowledge of atipic neurological symptoms in some kinds of "phenylketonuric" children. As a cofactor of nitric oxide syntheses BH4 is a crucial metabolite involved in physiological function of cardiovascular system. The literature data confirm that the BH4 depletion is crucial in the control of both NO and superoxide generation (OH2O2), synthesized by endothelial NOS isoforms, and consequently the formation of cell toxic peroxynitrite (ONOO-). Relationships between biosynthesis of BH4 and guanine nucleotide regulatory proteins (G proteins) or GTP-binding proteins taking part in protein synthesis has to be explained. The idea that all oxidases using molecular oxygen and producing H2O2 need BH4 appears rather relevant and it may explain more successfully the polyamine oxidase activity (PAO) in the regulation of polyamine metabolism.

Key words: Unconjugated pteridines, BH4, metabolic function, GTP binding proteins, polyamines, clinical disorders

Introduction

For a long time the investigation of pteridines has been focused on obtaining knowledge about biochemical functions of folic acid and its conjugates, composing the group of conjugated pteridines, the compounds with the key role in the nucleic acid (RNA and DNA) synthesis. In humans, tetrahydrobiopterin (BH4), the most important nonconjugated pteridin, differs in de novo biosynthesis contrary to folic acid as vitamin (1). The study of biosynthesis and biochemical roles of nonconjugated pteridines has been started in 1959, when BH4 has been recognized as cofactor of aromatic amino acids hydroxylases (1,2). Its involvement in hydroxylation reactions of phenylalanine, tyrosine and tryptophane, as the cofactor of phenylalanine hydroxylase and other specific hydroxylases of the mentioned amino acids, has become the sphere of interest in the last decade when BH4 biosynthesis and degradation pathways were recognized. The participation of BH4 in monoaminergic neurotransmitter metabolism regulation has given the rise to the knowledge of atipic neurological symptoms in some kinds of "phenylketonuric" children (2,3).

Beside this knowledge, since 1979 a number of publications has documented the anomalies in nonconjugated pteridines metabolism in patients suffering from malignant diseases, in which there was a characteristi- cally elevated urinary excretion of neopterin, intermediary product of BH4 synthesis. These reports have potentiated the role of neopterin as the marker of immune system stimulation (2). All these articles point out the importance of human "biopterines" metabolism research.

Metabolism of unconjugated pteridines

Biosynthesis

In 1963 it was postulated that BH4 is synthesized de novo in organism. Up to now it has not been revealed if all human tissues could or could not produce the necessary tetrahydrobiopterin quantities in situ. Guanosin triphosphate (GTP) has been identified as the precursor in tetrahydrobiopterin synthesis (Fig.1). The initial and key enzyme in this synthesis, GTP cyclohydrolase I (EC 3.5.4.16), was purified and characterized in 1987 by Japanese scientist Takikawa and his collaborators (3). In the first reaction GTP accepts a water molecule and with loosing formic acid is transformed into dihydronoopterin-triphosphate. Then it looses phosphate being transformed into 6-pyruvoyl-tetrahydropterin under the influence of 6-pyruvoyl-tetrahydropterine synthase (6-PTS). By the action of 6-pyruvoyl-tetrahydropterin reductase (PTHR) this compound is reduced into 6-lactoyl-tetrahydropterin, which produces tetra-
hydrobioperin (BH₄) under the influence of sepiapterin reductase, (Figure 1) (2-5).

![Biochemical pathway image]

The role of tetrahydrobioperin as the cofactor in hydroxylation reactions of cyclic amino acids is the best described as the part of phenylalanine hydroxylase system (6). PAH system is composed of three essential components - enzyme phenylalanine hydroxylase (PAH), dihydropteridine reductase (DHPR) and nonconjugated pteridine, tetrahydrobioperin (BH₄). Physiological function of BH₄ is linked to its ability to reduce molecular oxygen; BH₄ provides electrons and as a consequence it is transformed into 4-α-hydroxytetrahydrobioperin. This compound is dehydrated into quinonoid dihydrobioperin - qBH₂ under the influence of the enzyme carbinolamine dehydratase (pterin-4a-carbinolamine dehydratase -PCD; EC 4.2.1.96). BH₄ is regenerated through the reduction of qBH₂ by the action of the enzyme dihydropterin reductase (DHPR; EC 1.6.99.7) with NADH or NADPH as coenzyme; this coenzyme cycles between the tetrahydro and the qBH₂ derivative during the hydroxylation reaction (Fig. 2). Dihydropteridine reductase is an essential enzyme in the hydroxylating system for phenylalanine, tyrosine, and tryptophan. In contrast to PAH, DHPR is widely distributed in tissues (7). Whereas its occurrence in brain and adrenal medulla is not surprising in view of its role in the tyrosine hydroxylation system in these tissues, and in the tryptophane hydroxylation system in brain, DHPR should be found in tissues such as heart, kidney and lungs which have little or no aromatic amino acid hydroxylation activity. Its wide distribution, together with BH₄, hints undiscovered roles for both BH₄ and DHPR. In addition to its role in regenerating BH₄ it has been proposed that DHPR (together with dihydrofolate reductase) plays role in keeping folate in the tetrahydro form in brain (7).

The newest data concerned to enzymes involved in metabolism of BH₄ revealed that the primary structure of PCD is identical with that of protein of the cell nucleus, named dimerization cofactor (DCoH) of hepatocyte nuclear factor 1α (HNF-1α). The tertiary structures of GTP-CH and PTP S revealed in both enzymes a central pore for which the function is still unknown. There is similarity between these pterin-binding enzymes and small GTP-binding proteins in its subsequent dehydration and reduction (4).

![Regeneration of BH₄ image]
Catabolism

There are only few, relatively old, reports attempting to explain the possible catabolic pathways of unconjugated pteridines; the difficulties arise from instability of BH₄.

The main metabolites are neopterin and bioterpın. Neopterin is formed by dephosphorylation of dihydro-neopterin triphosphate, the compound produced from GTP by the action of GTP cyclohydrolase. Biopterin is synthesized by BH₄ oxidation. The both metabolites are mostly excreted by urine in reduced or oxidized form (1,3).

Up to now, the researches of biosynthesis and metabolic functions of unconjugated pteridines have not discovered any transport protein for BH₄, inspite of folic acid whose specific transport protein has been found in blood plasma and cell membrane-folate binding protein (FBP)(8,9). Beside, a number of data point out feeble permeability of cell membrane for BH₄; blood-brain barrier seems to be relatively impermeable for BH₄ which has been documented in animal experimental models and by mild efficacy in supplementation treatment with BH₄ in patients deficient in this compound.

Alimentary income seems negligible because intestinal absorption of BH₄ is low in children (12% in average), compared to folic acid absorption-conjugated pteridine (80%) pointing out the side chain of pteridine ring as chemically important component for membrane permeability (1,7).

Distribution of unconjugated pteridines in biological fluids

A number of unconjugated pteridines has been identified in human biological fluids. Pteridine urinary excretion depends on age; in the first months of life neopterin and bioterpın (N/B) are excreted in 4.2 ratio and 0.53 on adults. The examination of amniotic fluid suggests that this maturation debited from the fetal period. In serum the variations are less pronounced, although the level in cord blood is significantly higher than in children blood. Mother's milk contains significant amounts of bioterpın, 90 times higher than in serum, pointing out that mammary glands produce BH₄, whose physiological role in newborn nutrition is left to be proven (1).

Metabolic roles of unconjugated pteridines

BH₄ is an essential cofactor of at least five enzymes: phenylalanine hydroxylase, which catalyses the conversion of phenylalanine to tyrosine, tyrosine hydroxylase, necessary for the metabolism of tyrosine to DOPA (a precursor of dopamine, noradrenaline and adrenaline, as well as melanin pigments), tryptophan hydroxylase, needed for the synthesis of serotonin and melatonin, all three isoforms of nitric oxide synthase, and alkylglycerol monooxygenase, which influences ether lipid metabolism. BH₄ is a unique cofactor in that its intracellular level, which is determined by its synthesizing enzymes, regulates the activity of all BH₄ requiring enzymes, and therefore the levels of important hormones and neurotransmitters. The cofactor function of BH₄ in the hydroxylation reactions of aromatic amino acids is related to its ability to reduce molecular oxygen; BH₄ provides electrons and in turn is oxidized to qBH₂(7).

The other roles of BH₄ have been noticed: hydroxylation of proline, methemoglobin reduction and the role in mitochondrial oxidoreduction. It has been also suggested that it is possible that DHPR could partly provide folate reduction into tetrahydrofolate (THF) in brain, where dihydrofolate reductase (DHFR) activity is rather low. Renal tissue is bigger producer of tetrahydrobioterpın compared to liver tissue. However, no precise role of bioterpın has been proved in kidney with possibility that bioterpın takes part in diuresis (1). High concentration of pteridines in renal tissue may aggravate the anemia of inflammation (10).

The role of tetrahydrobiopterin (BH₄) in nitric oxide synthase (NOS)

Structure and function

In the tissues three different genes for three isoforms of NOS exist: neuronal or NOS I, macrophagal or inducible (NOS-II) and endothelial or NOS-III. By the action of nitric oxide synthase, in the presence of molecular oxygen (O₂) and NADPH L-arginine converts to L-citrulline and nitric oxide (NO). Tetrahydrobiopterin (BH₄) serve as a NOS cofactor for all three isoforms of NOS acting as a redox switch in the catalytic mechanism of the enzyme and allowing electron transfer from the prosthetic heme to L-arginine. The oxygen atoms incorporated into both L-citrulline and NO are derived from atmospheric oxygen (11-13).

The literature data refer that the BH₄ depletion is crucial in the control of both NO and superoxide generation (H₂O₂) by endothelial NOS isoforms, and the consequently on the formation of cell toxic peroxynitrite (ONOO⁻) (14).

Today, there is a growing scientific data referred to the significance of BH₄ for NOS functions, H₂O₂ and peroxynitritre production, respectively (15-17).

A deficiency of BH₄ results in eNOS uncoupling, which is associated with, increased superoxide and decreased NO production. BH₄ has been suggested to be a target for oxidation by peroxynitrite (ONOO⁻) and ascorbate has been shown to preserve BH₄ levels and enhance NO production; mechanisms underlying these processes remain poorly defined. The immediate product of the reaction between ONOO⁻ and BH₄ was trihydrobiopterin radical (BH₃) which may be reduced back to BH₄ by ascorbate (18).

Biosynthesis of BH₄ and the immune response

There is an interesting correlation between the cellular response to infections and pterin metabolism.
Neopterin is produced and secreted by interferon-gamma-stimulated monocytic cells (1). Activated macrophages generate and release pteridines. T lymphocyte, but not macrophages, produces BH₄ presumably because only the former cells do not have an intact pathway for biosynthesis of BH₄. Activation of the human cellular immune system is associated with greatly increased formation of the pteridines, neopterin and 7,8-dihydropterin and increased neopterin excretion. The increase of neopterin during cell immune response stimulation seems to be nonspecific and secondary phenomenon. On the other hand the investigations have not proved immunomodulatory roles of dihydroneopterin triphosphate (1,7). Also, no immune deficit in children with GTP-cyclohydrolase deficit has not been proven.

High urinary neopterin concentrations are found in patients with viral infections, allograft rejection episodes, and some malignant diseases. In various tumor types high urinary neopterin concentrations are associated with a worse prognosis (19).

Biochemical disorders of tetrahydrobiopterin (BH₄) metabolism

The disorders in BH₄ metabolism impair hydroxylation of phenylalanine causing tetrahydrobiopterin-deficient forms of hyperphenylalaninemia.

There are two forms of hyperphenylalaninemia, classic phenylketonuria (typically associated with mental retardation if amino acid phenylalanine is present in the intaken food and nonphenylketonuric hyperphenylalaninemia, non responsible to restriction of phenylalanine from the diet; this other form is responsive to BH₄ (2,3).

BH₄ deficiency can be caused by mutation in genes encoding the enzyme involved in its biosynthesis. These inherited enzyme defects are identified through screening program due to existence of evident hyperphenylalaninemia. The mutations are all inherited in an autosomal recessive manner. It is a heterogeneous group of diseases accompanied by the progressive neurological symptoms a cause a deficit of neurotransmitters dopamine and 5-hydroxytryptamine, serotonin (20-25). In the present literature 4 congenital defects of enzymes have been described. The most frequent is the congenital deficit of 6-pyruvoyltetrahydrobiopterin synthase (6-PTS) followed by less frequent GTP-cyclohydrolase (GTP-CH). The deficit of carbaminolamine dehydratase (PCD), as well as dihydropteridine reductase (DHPR) has been also established. Clinical manifestations of these enzymes deficits, found in children, are very similar and don't differ from those in classical phenylketonuria (PKU) (7).

The diseases affect either all organs, including the central nervous system, or only the peripheral hepatic phenylalanine hydroxylasing system. The disturbance of brain function such as loss of head control, hypertonia, drooling, swallowing difficulties and myoclonic convulsions develop during three months and is unresponsive to a low-phenylalanine. The exception is in the case of carbamin dehydratase deficiency; there are no mentioned clinical manifestations except mild hyperphenylalaninemia. Moreover, two forms of BH₄ deficiency may occur without hyperphenylalaninemia in infancy; the autosomal dominantly inherited and compound heterozygote form GTP-CH deficiency (Dopa-responsive distonia or Segawa disease) together with an apparent central nervous system - localized form of DHPR deficiency (7).

6-Pyruvoyltetrahydropterin synthase (6-PTS) deficiency  caracterises with high level of plasma phenylalanine promptly falling and remains normal for 2 days after infusion of BH₄ (2.5 mg /kg). A block in biosynthesis of BH₄ causes high neopterin levels and no detectable biotin in urine. The diagnosis of BH₄ defect or inefficacy of corresponding enzymes could be established by measuring of neopterin and biotin in body fluids, especially urine. Neopterin is oxidative product of dihydronopterin triphosphate, while biotin is oxidative product of dihydro and tetrahydrobiopterin/.

Fig. 3. Biochemical roles of BH₄
GTP-CH deficiency blocks the beginning of the BH₄ synthesis pathway. Neopterin and biotin-dependent enzymes are both low, and neopterin/biotin ratio is normal in urine, plasma, and CSF; the high levels of phenylalanine and low levels of neurotransmitter derivatives in body fluids return to the normal values with BH₄ replacement (7).

The patients with carbinolamine dehydratase deficit (PCD) excrete 7-biotinorn by urine.

Dihydropteridine reductase (DHPR) deficiency was the first described disorder in BH₄ homeostasis. This enzyme deficit has a profound effects on metabolism (Mikaeloff Epileptic disorders, 2000). The patients develop convulsions and other signs of neurological disturbances, starting from the third week of life, inspite of good dietary control of phenylalanine concentration. The patients are deficient in neurotransmitters (dopamine, noradrenalin and serotonin), which synthesis depends on DHPR-dependent tyrosine and tryptophan hydroxylases. They have low level of urinary and cerebrospinal 3-methoxy-4-hydroxy phenylglycol (MHPG) and homovanillic acid (HVA), major metabolites of norepinephrine; homovanillic acid (HVA), major metabolites of dopamine and 5-hydroxyindolacetic acid (5-HIAA)-the metabolite of serotonin. The level of hepatic phenylalanine hydroxylase and BH₄ were adequate, but there was no activity of DHPR. It has been proven that the enzyme DHPR keep folates in active tetrahydro form (THF), which is necessary for DNA and RNA synthesis. The other consequence of DHPR inactivity is folate deficit connected to diminish synthesis of proteins (7).

BH₄ and other metabolic disease - perspectives

BH₄ is a cofactor which regulates the function of three hydroxylases (for phenylalanine, tryptophan and tyrosine) and therefore the levels of important hormones and neurotransmitters - catecholamines, melanine, se-rotonine, melatonin. As a cofactor for all three iso-enzymes of nitric oxide synthase (NOS), BH₄ participates in production not only of nitric oxide (NO), more over hydrogen peroxide (H₂O₂) and peroxy-nitrite radical (ONOO-). There are many relationships between BH₄ and diabetes mellitus and hypertension (26,27). In patients with the depigmentation disorder vitiligo accumulated hydrogen peroxide is accompanied by high concentration of 6- and 7- biotinorn in their epidermis. The investigators of this event suggest that H₂O₂ derived from various sources could be a general mechanism in the regulation of all 6BH₄-dependent processes (28).

The investigation of relationship between polyamine oxidase (PAO), the key regulatory enzyme of polyamine metabolism, generating H₂O₂ and BH₄ may be of interest for explanation of biochemical function of polyamines; on the other hand the biosynthesis of polyamines, spermine, spermidine and putrescine depend on the amino acids arginine and methionine (29) as S-adenosyl methionine (SAM)(29,30). All these facts suggest possibility of connection (participation of BH₄ in polyamine metabolism) between polyamines and BH₄ metabolism.

The fact that BH₄ synthesis requires the depletion of GTP, nucleoside triphosphate important both in many signaling pathways through Guanine nucleotide regulatory proteins, (G proteins) or GTP-binding proteins in translation process during the protein synthesis points out many possibilities of BH₄ in the mentioned metabolic event in the cells.

Forthcoming investigations will explain the exact biochemical roles of BH₄, the chief natural molecule for the future time.

References

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Biohemijske funkcije i klinički značaj nekonjugovanih pteridina

Gordana Bjelaković1, Tatjana Jevtović-Stoimenov1, Bojko Bjelaković2, Ivana Stojanović2

1Biohemijski institut, Medicinski fakultet, Univerzitet u Nišu
2Pedijatrijska klinika, Medicinski fakultet, Univerzitet u Nišu

Kratak sadržaj: Tetrahydrobiopterin (BH₄) i njegovi metabolitii klasiﬁkuju se kao nekonjugovani pteridini ili pterini za razliku od foline kiseline i njenih derivata koji čine grupu konjugovanih pteridina. BH₄ nije vitamin za sisare, koji mogu da ga sintetiziru. GTP je glavni prekursor atoma u molekulu pterina. Glavni stupanj na putu prevodjenja GTP-a u BH₄ je reakcija katalizovana od strane enzima GTP-ciklohidrolaze I (EC 4.2.1.35). Postoje brojne metaboličke značajne funkcije BH₄: on je ključni kofaktor u reakcijama hidroksilacije fenilalaniна, trozina i triptofana. Upoznavanje učesca BH₄ u regulaciji metabolizma monoaminoergičkih neurotransmitera dovelo je do boljeg upoznavanja atipičnih neuroloških simptoma u nekim oblicima "fenilketonurije" dece. Kao kofaktor azot oksid sintaze BH₄ je ključni metabolit zar fiziološke funkcije kardiovaskularnog sistema. Posjeduju literaturu ukazuje da deplecija BH₄ je ključna u kontrolu produkcije NO i vodonik peroksida (H₂O₂) pomoću endotelijalne NOS izforme, i posledičnog formiranja celularno toksičnog peroxinitrita (ONOO⁻). Međuodnosi u biosinteti BH₄ i guanin nukleotid regulatornih proteina (G proteina) ili GTP-vezućih proteina koji su uključeni u proces biosinteze proteina potrebno je tek razjasniti. Ideja da sve oksidaze koje razliku od foline kiseline i njenih derivata koji

Ključne reči: Nekonjugovani pteridini, BH₄, GTP-vezući proteini, poliamini, klinički poremećaji