Theoretical Stereostructure of the Neutral Form of Natural Tetrahydrobiopterin

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Summary

The absolute conformation and the reactivity of atoms of the neutral form of natural tetrahydrobiopterin [(6R)-6-(L-*erythro*-1',2'-dihydroxypropyl)-5,6,7,8-tetrahydropterin] was determined by the Molecular-Orbital method within the MINDO/3 framework. Two different conformations (Form A and Form B) of the alkyl side chain relative to the pterin ring were obtained as the optimized form for the lowest energy conformation ($\Delta H = -174$ kcal/mol) of this molecular constitution with the same molecular width of 11.2 Å. Form A and B were distinguishable in the dihedral [O1'-C1'-C6-H6] of -88° and 30° , respectively. Net charges of atoms of Form A and B suggested that the predominant reactivity of the atoms of N1, C2, C4, O4, C4a, and C8a of the pterin ring and C1', C2', O1' and O2' of the side chain. It was also suggested that the position 2' is more active than the position 1', in the alkyl side chain of Form A and B, toward NADP⁺ in the reverse reaction of sepiapterin reductase.

Key words: 6(R)-tetrahydrobiopterin, Neutral form, Absolute conformation, Molecular-orbital method, Atomic net charge

Introduction

Tetrahydrobiopterin [6-(1',2'-dihydroxypropyl)-5,6,7,8-tetrahydropterin; BH₄] (Fig. 1) is an important naturally occurring substance controlling the level of phenylalanine in the liver or biogenic monoamines in neural tissues (1). In addition to this established role, several other new physiological activities of BH₄ have recently been proposed. These are, for example, as a factor required for the synthesis of nitrogen oxide, the endothelium-derived relaxing factor (EDRF) in the conversion of arginine to citrulline in macrophages (2,3), and as a factor involved in the mechanism of dopamine release in the brain through a specific receptor (4).

Of the total of eight configurational isomers of the BH₄ molecule (Fig. 1), naturally-occurring BH₄ cofactor in mammals is the 6R-L-*erythro* form (idiomatically referred to as 6R-BH₄). The stereochemistry

of the ring structure and the alkyl side chain of the natural BH₄ supplies specific regulatory function to the molecule. Enzymes or receptors associated with BH₄ activity may have specific domains fitted to the stereostructure of 6R-BH₄.

The stereochemistry of 6R-BH₄ molecule was previously studied by NMR and CD analyses (5-7) at acidic pH and its absolute conformation was determined by X-ray analysis (5) with dihydrochloride crystals. In these studies, the atoms of N5 or N1, and N5 of the BH₄ molecule were protonated. The neutral form of 6R-BH₄ at physiological pH, however, is quite labile under aerobic conditions, and its exact stereostructure is unclear. In the present study, we determined the absolute conformation and the reactivity of the constituting atoms of the neutral form of 6R-BH₄ by the Molecular-Orbital Method to know the physiological conformation of it.

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Methods

The formal chemical structure of 6R-BH₄ is (6R, 1'R, 2'S)-6-(1', 2'-dihydroxypropyl)-5,6,7,8-tetrahydropterin (Fig. 1). For optimisation of the conformation of the 6R-BH₄ molecule, the ring structure of tetrahydropterin in half-chair conformation and the R configuration of equatorial substitution of the alkyl side chain at position C6 on the ring were set up in accordance with the results of previous physical analyses of 6R-BH₄ structure (5-7) and the determination of theoretical structure of 6-pyruvoyl tetrahydropterin (8), a metabolic precursor of 6R-BH₄. The two hydroxyl groups at 1' and 2' positions were set up in the *trans* form around the C1'-C2' bond as the most stable conformation. The first optimisation was performed for the conformation in which the bonds of O1'-C1' and C6-H6 were in the *trans* position. The alkyl side chain, in the optimized form, was rotated relative to the pterin ring at intervals of 30°, and the geometry was optimized at each point. The dihedral O1'-C1'-C6-H6 was the reaction coordinate. The lowest energy conformation of the results was applied to the final optimisation.

Calculations were performed with the MOPAC on an FACOM M-360/MSP computer. The geometries were optimized within the MINDO/3 framework (9).

Figure 1. Tetrahydrobiopterin: (a) Natural type (6R-L-erythro form), (b) R & S enantiomers, and (c) Four epimers of the alkyl group.

Results and Discussion

The MO-calculation of the BH₄ molecule is quite complicated since the molecule has a total of three chiral centers at C6, C1', and C2' (Fig. 1). There are R and S enantiomers for the attachment of the alkyl side chain to the pterin ring at C6. The second and third chiral centers, C1' and C2', introduce four diastereoisomers of the side chain (*L-erythro*, *L-threo*, *D-threo*, and *D-erythro* forms) for each of the R and S enantiomers.

Molecular-Orbital calculations within the MINDO/3 framework of the neutral molecule of 6R-BH₄ (the (6R)-*L-erythro* epimer) optimized two different conformations (Form A and Form B) as the lowest energy conformer ($\Delta H = -174$ kcal/mol) (Table 1). Fig. 2a and b show their optimized geometries and stereostructures by computer generating (10), respectively. The side chain attached to C6 in Form A and Form B took the equatorial conformation with a different dihedral of the O1'-C1' bond around the C1'-C6 bond [O1'-C1'-C6-H6] of -88° and 30° , respectively (Table 1). These conformations were different from that of 6R-BH₄ dihydrochloride of which analytical value of that dihedral was not mentioned but apparently suspected to be almost 180° from its perspective drawing structure (5). The planar structure of the pterin ring except C6 ($\Theta[C4-C4a-N5-C6] = -157.8^\circ$ and -159.4° in Form A and B, respectively) of these forms was quite similar to those structures of 6-pyruvoyl tetrahydropterin (8) and 6-methyl tetrahydropterin (11) analyzed by the MO-method, and 6R-BH₄ dihydrochloride determined by X-ray diffraction (5). The conformation around the C1'-C2' bond of each form was *trans* ($\Theta[C3'-C2'-C1'-C6] = -168^\circ$ and 169° in Forms A and B, respectively) (Table 1) as in 6R-BH₄ dihydrochloride ($\Theta = 174^\circ$) (5). Two hydroxyl groups in the

Table 1. Optimized geometry of 6R-L-erythro-tetrahydrobiopterin.

	Form A	Form B
$\Theta[=O1'-C1'-C6-H6]$	-88.28°	30.42°
$\Theta[=O2'-C2'-C1'-O1']$	-164.82°	176.23°
$\Theta[=C3'-C2'-C1'-C6]$	-167.95°	168.86°
Molecular width[H2a-H3'b]	11.236 Å	11.254 Å
Formation energy:		
ΔH (kcal/mol)	-174.21358	-174.45627
(kJ/mol)	-729.95484	-730.97168
Electronic energy (eV)	-19717.31579	-19773.28895
Core-core Repulsion (eV)	16455.36527	16511.32790
Total energy: E (eV)	-3261.95052	-3261.96105
Ionization potential	7.20370	7.10501

Figure 2. Perspective views of the optimized molecules of the neutral form of 6R-L-*erythro* tetrahydrobiopterin. (a) Optimized geometries (Bond lengths, Å and angles in small letters, deg.), (b) Computer-drawings, and (c) CPK-Modelings.

side chain of Form A and B were in *trans* around the C1'-C2' bond ($\Theta = -164^\circ$ and 176° , respectively) and the molecular width (H2a-H3'b) of the Form A and B was the same, 11.2 Å. (Table 1).

Form A and B were structured by the CPK-Model (Fig. 2c). Two oxygen atoms in the side chain of Form A apparently located on the same side of the pterin ring, while those of Form B were on the back side of the ring.

These structures were compared with the theoretical structures of tetrahydropterins involved in the biosynthesis of 6R-BH₄. Since 6R-BH₄ is formed from 6-pyruvoyl tetrahydropterin by the function of

sepiapterin reductase with NADPH (12-15) in the biosynthesis from GTP, besides 6-pyruvoyl tetrahydropterin in the previous study (8), the lowest energy conformations of 6-lactoyl tetrahydropterin ($\Delta H = -165.23$ kcal/mol) and 6-hydroxyacetyl tetrahydropterin ($\Delta H = -162.22$ kcal/mol), which are hydroxy-keto intermediates observed in the conversion of 6-pyruvoyl tetrahydropterin into 6R-BH₄ by the enzyme (15), have been determined by the same framework (conformational data were not shown). The result implies that Form A is a more favorable conformation of a natural 6R-BH₄ than Form B.

Net charges of atoms of Form A and B molecules

Table 2. Net charges of atoms constituting the neutral form of 6R-L-*erythro*-tetrahydrobiopterin.

Atoms	Form A	Form B	Atoms	Form A	Form B
N1	-0.40	-0.40	H7b	-0.06	-0.06
C2	+0.44	+0.43	N8	-0.13	-0.13
N2	-0.19	-0.19	H8	+0.07	+0.07
H2a	+0.10	+0.09	C8a	+0.34	+0.34
H2b	+0.11	+0.11	C1'	+0.33	+0.33
N3	-0.25	-0.25	H1'	-0.04	-0.06
H3	+0.08	+0.08	O1'	-0.49	-0.48
C4	+0.66	+0.66	(O1')H1'	+0.24	+0.25
O4	-0.58	-0.59	C2'	+0.36	+0.36
C4a	-0.32	-0.31	H2'	-0.12	-0.11
N5	-0.08	-0.10	O2'	-0.48	-0.49
H5	+0.04	+0.04	(O2')H2'	+0.25	+0.25
C6	+0.10	+0.11	C3'	+0.01	+0.01
H6	-0.07	-0.05	H3'a	-0.01	-0.02
C7	+0.18	+0.17	H3'b	-0.01	-0.01
H7a	-0.06	-0.06	H3'c	-0.01	-0.01

were calculated (Table 2). The results indicate that N1, C2, C4, O4, C4a, and C8a, which are atoms comprising the pyrimidine ring, and C1', C2', O1' and O2', which are in the side chain, were predominantly reactive than those of the other atoms of the molecule in Form A and B, as was seen in these corresponding atoms in 6-pyruvoyl tetrahydropterin (8). Atomic charges (absolute values) of C & H at position 2' were predominant than those at position 1' of Form A and Form B (Table 2). This suggests that H2' is more active than H1' toward NADP⁺, and that 6-hydroxyacetyl tetrahydropterin (katoh pterin, the 2'-keto type) may thus reasonably be the direct product from 6R-BH₄ as actually observed (14) in the reverse reaction (dehydrogenase reaction) of sepiapterin reductase.

Theoretical conformation of the 6R-BH₄ molecule in its neutral form was also predicted by other workers (16,17) who used different tools than we did. As already described in Methods, the present study was performed by a semiempirical MO-method (MOPAC, MINDO/3 framework), and we surveyed the lowest energy conformation from the structure in solution that is in accord with experimental findings (6,7,18) and general chemical considerations (19). Ayling *et al.* (16) might do as well but employed the Molecular Mechanics method for calculations. While the study by Ziegler *et al.* (17) was based on a combination of Molecular Mechanics (CHARMm) and semiempirical MO-(AMPAC, AM 1 framework) methods, and they surveyed the global minimum conformation from many families of possible gas-phase structures. The perspective structure of the 6R-BH₄ having the lowest energy conformation was

illustrated in their reports (16,17). In the former report (16), it was apparently similar to that of Form A (Fig. 2b). While, in the latter one (17), it was quite different from both of Form A and B obtained in our study (Fig. 2b & 2c) since it has an axial C6 side chain.

Ayling *et al.* (16) indicated that the conformation of 6R-BH₄ has a hydrogen bond between N5-H and the C2'-O. In Form A and B in our study interatomic distances between N5 and O2' (and O1') were 2.99 Å (3.66 Å) and 3.35 Å (3.17 Å), respectively. Thus it is difficult to find any certain hydrogen bond in these forms. Ziegler *et al.* (17) did not find it either in the 6R-BH₄ having the lowest energy conformation as calculated by them. On the other hand, the result of net charge calculations for Form A and B (Table 2) suggest the possibility of hydrogen bonding between 1'-OH, 2'-OH, or N1 and some atom contained in the immediate environment such as the BH₄-fitting domain of enzymes. Both these groups (16,17), however, did not mention the detailed geometry and other theoretical properties about atoms constituting the optimized molecule of 6R-BH₄.

In the physiological role of natural BH₄, the conformation of the 6(R)-L-*erythro*-dihydroxypropyl side chain, besides that of the tetrahydropteridine ring, is critical for many aspects of enzyme regulation (20-22). 6R-BH₄ is now a hopeful therapeutic agent for certain neurological diseases such as atypical phenylketonuria (PKU), Parkinson's disease, Alzheimer's disease, and autism. It is important to know the stereostructure and the reactivity of neutral form of 6R-BH₄ as the physiological form.

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