Vol. 176, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1991 Pages 52-58

COMPUTER STUDIES ON THE STEREOSTRUCTURE AND QUANTUM CHEMICAL PROPERTIES OF 6-PYRUVOYL TETRAHYDROPTERIN, THE KEY INTERMEDIATE OF TETRAHYDROBIOPTERIN BIOSYNTHESIS

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Received February 24, 1991

Summary: The optimized geometry of the conformation of atoms constituting the 6-pyruvoyl tetrahydropterin molecule, the labile key intermediate of tetrahydrobiopterin biosynthesis, was obtained by molecular orbital calculations within the MINDO/3 framework. The stereostructure of the molecule showing the preferred mode for binding to sepiapterin reductase or pyruvoyl tetrahydropterin reductase was drawn in perspective. The resulting structure with the *equatorial* staggered configuration of the 6-1',2'-dioxopropyl (pyruvoyl) side chain indicated that O(1') and H(6) were located in the *trans* position around the C(6)-C(1') bond and that the two vicinal carbonyls in the side chain were fixed in the incomplete *trans* form. The calculation of atomic charges and LUMO coefficients of these carbonyls suggests that the C2'-carbonyl may be more reactive toward NADPH than the C1'-carbonyl in the enzymatic reaction.

6-Pyruvoyl tetrahydropterin (PPH_4) is the key intermediate in the biosynthetic pathway of tetrahydrobiopterin [(6R)-L-*erythro*-1',2'-dihydroxypropyl-5,6,7,8-tetrahydropterin; BH_4] [1,2] (Fig.1), the important cofactor of hydroxylases controlling the formation of monoamines [3]. PPH_4 is available enzymatically from dihydroneopterin triphosphate only in small amount and is quite labile under oxidative conditions. In spite of many difficulties in the study of its chemical and physical properties [4-8], the chemical structure of PPH_4 is now being accepted as 2-amino-6-(1',2'-

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Abbreviations: PPH_4, 6-pyruvoyl tetrahydropterin; BH_4, tetrahydrobiopterin; C1'-carbonyl PH_4, 6-1'-oxo-2'-hydroxypropyl tetrahydropterin; C2'-carbonyl PH_4, 6-1'-hydroxy-2'-oxopropyl tetrahydropterin; SPR, sepiapterin reductase; PPH_4-R, pyruvoyl tetrahydropterin reductase.

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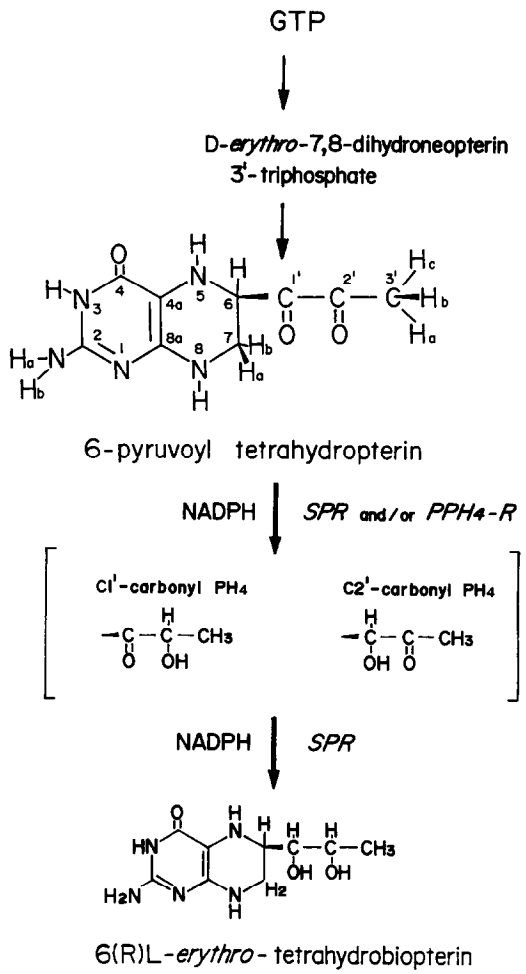

Fig. 1. Structure of 6-pyruvoyl tetrahydropterin and its reduction by sepiapterin reductase (SPR) and pyruvoyl tetrahydropterin reductase (PPH_4-R) in the biosynthesis of tetrahydrobiopterin.

dioxopropyl)-5,6,7,8-tetrahydropteridin-4(3H)one (Fig.1); however, its exact stereochemistry remains unknown.

Concerning the catalytic procedure on the reduction of the two carbonyl groups at positions 1' and 2' of this compound for the formation of BH_4 (Fig. 1), there have curiously been found a redundant activity carried out by two $NADP^+$ oxidoreductases, sepiapterin reductase (EC 1.1.1.153, SPR) and 6-pyruvoyl tetrahydropterin reductase (PPH_4-R), as shown in Fig. 1. SPR can reduce stepwise both carbonyl groups of PPH_4 to finally form BH_4 while PPH_4-R does only one of them. The final product of the PPH_4-R reaction was recognized as C1'-carbonyl PH_4, on the other hand, the monocarbonyl intermediate of the SPR reaction was observed in the usual experimental system as C2'-carbonyl PH_4. Recent studies on the amino acid

sequence and cDNA of these enzymes [9-12] has begun to throw light upon their protein structure and the mechanism for their different activities against the same substrate.

In this paper we have attempted to determine the absolute conformation of the PPH_4 molecule by a theoretical method in order to learn more clearly the properties of this important intermediate of BH_4 synthesis. From our findings we discuss the reactivity of the two carbonyls of PPH_4 in the enzyme reaction in relation to the reaction mechanism and the structure of the substrate binding site of the enzymes.

METHODS

All of the calculations were performed with the MOPAC on an FACOM M-360/MSP computer. The geometries were optimized with the MINDO/3 framework [13]. The stereostructure of the molecule was shown in perspective by ORTEP drawing [14]. Numbering of the atoms of the PPH_4 molecule is shown in Fig. 1.

The ring structure of tetrahydropterin and the configuration of the substitutes on the ring of PPH_4 molecule were basically set up in accordance with the structure of 6(R)-BH_4, a natural form of BH_4, which was indicated by solution ^{1}H-NMR [15,16] and crystal X-ray [17]. The ring structure of tetrahydropterin recently determined by Gready by a quantum chemical method was also taken into account [18-20]. The four carbon atoms at 6, 1', 2', and 3' positions were arranged on the same plane on the expectation that C(1') and C(2') were attached to each other by a sp^2-hybridized bond. The two carbonyl groups at 1' and 2' positions were set up on the same plane in the *trans* form with conjugate double bond as the most stable conformation.

Optimization was first performed for the moiety of the tetrahydropterin ring. The second optimization was done on the configuration of the pyruvoyl side chain by rotating it around C(6) at intervals of 30°, in the twist angle at H(6)-C(6)-C(1')-O(1'), from 180° to -180°. Geometries of the tetrahydropterin moiety were held fixed throughout the second optimization. The lowest energy conformation of the results was applied to the final optimization.

RESULTS AND DISCUSSION

Previous experience in the literature on geometry optimizations for the structure of tetrahydropterins have been limited to calculations on the pterins with the alkyl substituent of less than one-carbon [18-20]. PPH_4 consists of 28 atoms (17 heavy atoms and 11 hydrogens) in the whole molecule including 8 atoms (5 heavy atoms and 3 hydrogens) in the three-carbon alkyl side chain, as shown in Fig. 1. This comprehensive work has been calculated within the MINDO/3 framework rather than with the higher application of STO-3G and 3-21G methods for an economical reason.

Table I. Quantum chemical properties of the optimized molecule of 6-pyruvoyl tetrahydropterin

Molecular weight			237.218	
Formation energy (ΔH)			-637.64431 kJ/mol	
			(-152.18243 kcal/mol)	
Electronic energy			-18316.77102 eV	
Core-core repulsion			15114.83309 eV	
[Total energy, (E)]			[-3201.93793 eV]	
Net atomic charges	C(1')	0.4380	O(1')	-0.4646
	C(2')	0.4773	O(2')	-0.4723
LUMO coefficients		*2px*	*2py*	*2pz*
	C(1')	0.080	-0.074	-0.053
	C(2')	0.032	0.020	-0.115

The PPH_4 structure arranged as described above was geometrically optimized. The formation energy (ΔH) and the total energy (E) of the optimized molecule were calculated as -637.644 kJ/mol (-152.182 kcal/mol) and -3201.938 eV, respectively (Table I). The optimized geometry of the PPH_4 molecule was obtained as expressed in Fig. 2A. The stereostructure of the molecule is illustrated in perspective by ORTEP-drawing [14] in Fig. 2B to show the preferred mode of the molecule for binding with SPR or PPH_4-R. The size of the molecule was about 10.8 Å in width from the calculated interatomic distance between H(2a) and C(3'b). The results (Fig. 2A & 2B) show that PPH_4 has the flattened ring structure and the *equatorial* configuration of the side chain at C6, which were indicated in a concerted form by the molecular orbital calculation method [18-20] in tetrahydropterin and by ^{1}H-NMR [15] and X-ray [17] analyses in 6(R)-BH_4, respectively: (i)The C(7) environment was effectively planar and only C(6) deviated most markedly from the ring plane. (ii)The intrinsic tendency in tetrahydropterins for a shorter bond length for the C(8a)-N(8) bond than for the C(4a)-N(5) bond was present. (iii)A *trans-diaxial* attachment of H(6) and H(7a) on the ring plane, which resulted in the *equatorial* conformation of the pyruvoyl moiety, was effectively indicated. A feasible rotation of each carbonyl bond in the pyruvoyl side chain was anticipated; however, the initial structure was successively optimized. The two carbonyls were favourably fixed in the incomplete *trans* form with the twist angle of 104.3° (Fig. 2A) indicating that these are attached to each other with an incomplete conjugate double bond. The distance between O(1') and O(2') was 3.142 Å. O(1') and H(6) were located in the *trans* position around the C(6)-C(1') bond.

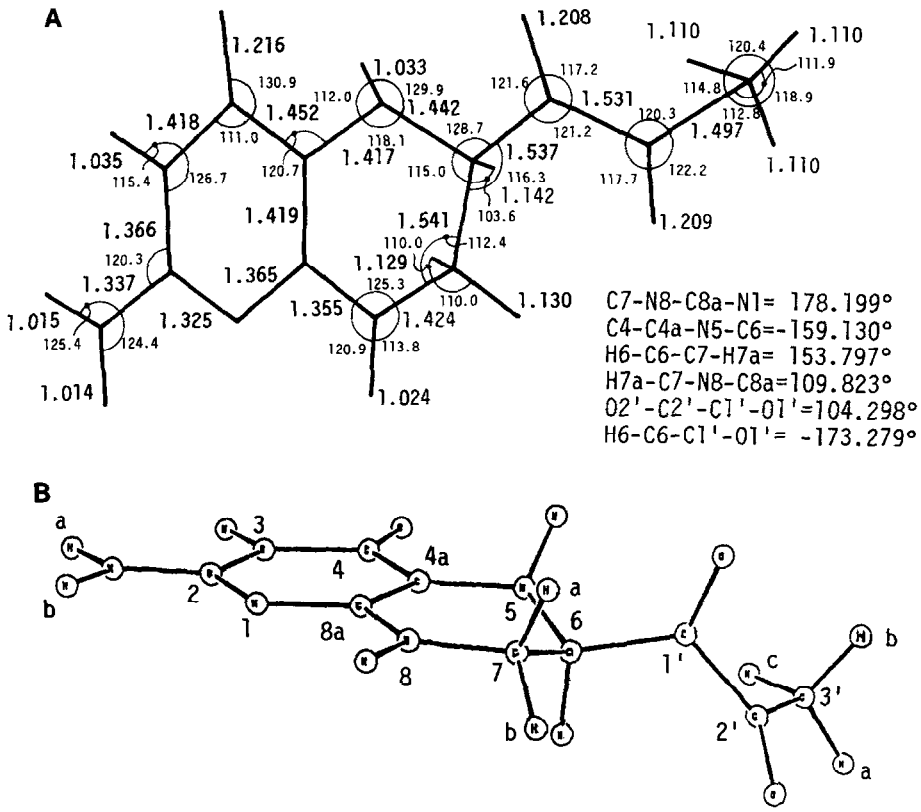

Fig. 2. Geometry and stereostructure of the 6-pyruvoyl tetrahydropterin molecule. (A)Optimized geometry. Bond lengths (large letters) are given in angstrom, and angles in deg. (B)Perspective structure by ORTEP-drawing method of low-energy conformation.

Quantum chemical properties were available to compare the level of reactivity of the two carbonyls with NADPH (Table I). Net atomic charges were predominant in the C2'-carbonyl (both C2' and O2' atoms) rather than in the C1'-carbonyl (C1' and O1'). LUMO coefficients of C atoms in the *pz* orbital were also predominant at the 2'-position rather than at the 1' (Table I) (HOMO-coefficients were mostly localized on the pteridine ring.). These results suggest that the C(2') is more reactive than the C(1') toward NADPH in terms of both a charge-controlled attack and a nucleophilic, frontier-controlled attack, showing superior binding and catalyzing of the C2'-carbonyl rather than the C1'-carbonyl by the enzyme.

If the C2'-carbonyl is more reactive than the C1'-carbonyl in the actual enzymatic reaction with NADPH, and only the C2'-carbonyl could specially be catalyzed by the enzyme in the initial reaction, the reasonable first product from PPH_4 might be expected to be C1'-carbonyl PH_4 (Fig. 1). In fact, it was recognized as the final product of PPH_4-R. For the complete

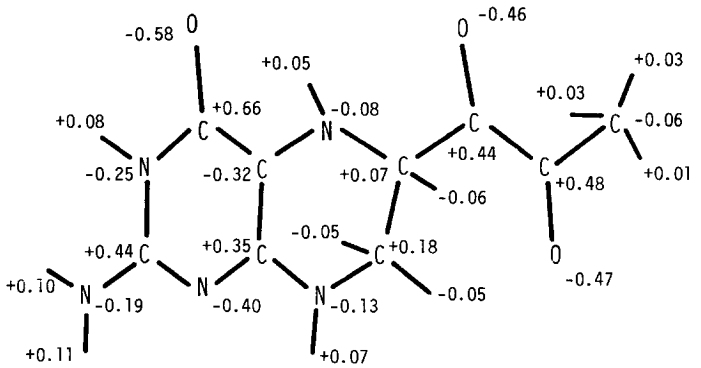

Fig. 3. Net charges of atoms constituting the 6-pyruvoyl tetrahydropterin molecule. Expression of H atoms was omitted.

reduction of both carbonyls through C2'-carbonyl PH_4, as generally observed in the SPR reaction, some additional mechanism, such as an isomerization of C1'-carbonyl PH_4 to C2'-carbonyl PH_4 [21], might be effective as proposed recently by Katoh *et. al.* [22,23].

Besides the two carbonyl atoms, N(1), C(2), C(4), O(4), C(4a), and C(8a) were also predominant in atomic charges (in absolute values) over the other atoms of the molecule, as shown in Fig. 3. These atoms might be important for the approaching of the substrate to the specific site of the enzyme for pteridine binding.

The present paper dealing with theoretical geometries is the first attempt useful for study of the properties of natural tetrahydropterins substituted with a three-carbon alkyl that are involved in the metabolism of biologically important BH_4.

ACKNOWLEDGMENTS

We are grateful to Dr. S. Matsuura of Nagoya University and Mr. T. Yamamoto of Josai University for their helpful advice for this work.

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