The conformational flexibility of 5,6,7,8-tetrahydrobiopterin and 5,6,7,8-tetrahydroneopterin: a molecular dynamical simulation

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Abstract 5,6,7,8-Tetrahydrobiopterin is an essential cofactor of diverse enzymes. Of the eight possible stereoisomers, only the 6*R*,1′*R*,2′*S*-configuration is biologically active. Other stereoisomers, as well as other reduced pterins such as, e.g. 5,6,7,8-tetrahydroneopterin, fail to exhibit significant cofactor activity. Different theoretical models (molecular mechanics, semi-empirical quantum chemical calculations) investigating the stereostructure of tetrahydrobiopterin have yielded diverging answers. It has been claimed on the basis of semi-empirical quantum chemical calculations that conformational properties, and thus particular features in overall shape, might be responsible for the unique biological properties of natural tetrahydrobiopterin in contrast, e.g. to 6*R*,1′*S*,2′*R*-5,6,7,8-tetrahydroneopterin. Molecular dynamical simulations of both molecules at realistic temperatures demonstrate, however, that they possess sufficient conformational flexibility as to render questionable any biological significance of mere conformational properties.

Key words: Pterin cofactor; Molecular structure; Molecular mechanics; Molecular dynamics

1. Introduction

The metabolic roles of 5,6,7,8-tetrahydrobiopterin in mammals include cofactor activity in phenylalanine-4-, tyrosine-3- and tryptophan-5-monooxygenases [1,2]. In addition, glyceryl ether cleavage in rat liver [3,4] and nitric oxide formation by conversion of arginine to citrulline require tetrahydrobiopterin [5–7].

Tetrahydrobiopterin contains three asymmetric carbon atoms and, thus, there are eight possible stereoisomers. The stereochemistry of the naturally active cofactor is dictated by the biosynthetic pathway; the configuration found in mammals is nearly exclusively (6*R*,1′*R*,2′*S*)-5,6,7,8-tetrahydrobiopterin [8]. This was proved by nuclear magnetic resonance and circular dichroism analyses in acidic solutions [9,10] and by X-ray analysis of dihydrochloride crystals [10]. The preferred conformations of the neutral form of the molecule are, however, unknown. Several authors applied computational techniques, such as molecular mechanics [11], and semi-empirical quantum-chemical methods, such as AM1 [12] and MINDO/3 [13], to neutral tetrahydrobiopterin, attempting to explore the conformational characteristics of the molecule.

One of these earlier studies compared tetrahydrobiopterin with 6*R*,1′*S*,2′*R*-5,6,7,8-tetrahydroneopterin [12]. On the basis of a combination of molecular mechanical and semi-empirical quantum chemical calculations, the authors concluded that significant conformational differences exist between both compounds: importantly, the side chain at carbon atom C6, according to their results, was predicted to be in axial orientation in tetrahydrobiopterin but in equatorial orientation in tetrahydroneopterin. They argued that the resulting significant difference in the molecular overall shape between the two molecules might be responsible for the significant differences in their observed cofactor activities.

While being an intriguing hypothesis, their study suffers from two methodological weaknesses: first, as others have indicated, the possibility of intramolecular hydrogen bonds in the molecules under consideration cannot be ruled out [11] with certainty, and secondly, all the studies cited above have investigated the structural features of the molecules in vacuo at zero temperature, i.e. only the energetic ground state, neglecting the possibility of internal rotations and vibrations which are to be expected at realistic temperatures of, say, 310 K and in an aqueous environment.

Using molecular dynamics, we have performed, separately for tetrahydrobiopterin and tetrahydroneopterin in their above-mentioned natural configurations, simulation runs over a time period of 20 ps. Even during this relatively short time interval, both molecules show sufficient conformational flexibility to undergo significant variations of molecular shape, particularly conversions of the reduced pyrazine part of the pterin ring system. Our results therefore render questionable any speculations regarding biological activity which are based merely on conformational arguments.

2. Materials and methods

2.1. Systematic generation of reasonable geometries for tetrahydrobiopterin and tetrahydroneopterin and molecular mechanics calculations

The chemical structure of both molecules was translated into a SMILES string. SMILES (simplified molecular input line entry system) is a chemical notation system allowing rigorous structure specification (notably including specification of stereochemistry) by use of a very small and natural grammar, which is well suited for high speed machine processing. The SMILES strings were processed by the program COBRA (Oxford Molecular Ltd., Oxford, England) which systematically generates, starting with a SMILES string, reasonable conformations of a flexible molecule. The conformations generated by COBRA were subjected, one after another, to the molecular modelling program PIMMS (Oxford Molecular) in order to perform, for each conformation, a geometry optimization within the framework of molecular mechanics. In PIMMS, the COSMIC force field [14] was used. Parametrization of this force field allows the investigation of hydrogen bonding by reducing the van der Waals radius of hydrogen atoms attached to electronegative atoms to 0.05 nm when such a hydrogen interacts with a hydrogen acceptor atom; the well depth is increased to 1.0 kcal/mol. These parameters guarantee similar potential functions as those used

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in the well-known MM2 force field [15]. Moreover, comparison with ab initio quantum chemical calculations has shown that the molecular mechanical technique described yields results which are excellently comparable with those of the much more sophisticated ab initio approach (Reibnegger et al., submitted). Thus, the most stable conformations for both molecules were identified among the many conformations tested.

2.2. Molecular dynamics simulation

Molecular dynamical calculations were performed using the program HyperChem Release 3 for Windows (Autodesk Inc., Sausalito, CA) running on an IBM compatible PC with an 80486 compatible CPU. The previously obtained optimum geometries were used as a starting point. The simulation was performed for 20 ps at a simulation temperature of 310 K, with a step length of 1.0 fs. Throughout the simulation the conformational characteristics of the molecules were studied by recording the torsional angle (C_{4a}-N_5-C_6-C_7) since positive values of this angle indicate an axial orientation of the side chain and negative values indicate an equatorial side chain orientation. Thus, this parameter represents major conformational changes of the molecule.

3. Results

3.1. Systematic generation of reasonable geometries for tetrahydrobiopterin and tetrahydroneopterin

The chemical constitutions of (6*R*,1′*R*,2′*S*)-5,6,7,8-tetrahydrobiopterin and (6*R*,1′*S*,2′*R*)-5,6,7,8-tetrahydroneopterin are shown in Fig. 1. In both molecules pseudoaxial (A′) and pseudoequatorial (E′) orientations are possible for the hydrogen atom at N_5, and axial (A) as well as equatorial (E) orientations are possible for the side chain at C_6. For each molecule, 40 chemically plausible low-energy conformations were generated by COBRA. Of the conformations generated for tetrahydrobiopterin, 20 had the orientations A′A (at N_5 and C_6), 14 had E′A, 3 had A′E and 1 had the E′E orientation. For tetrahydroneopterin, 18 conformations had the orientations A′E, 11 had A′A, 9 had E′E, and 2 had E′A.

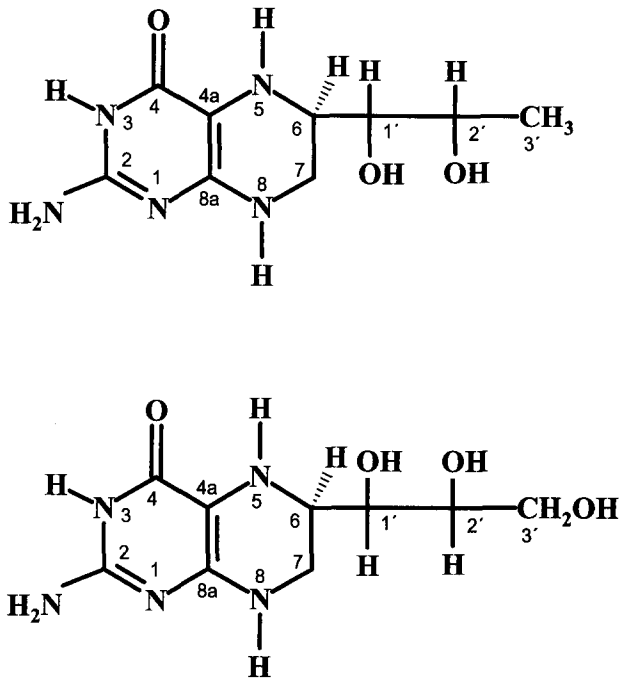

Fig. 1. Molecular structure and conventional atomic numbering system of (6*R*,1′*R*,2′*S*)-5,6,7,8-tetrahydrobiopterin (upper panel) and (6*R*,1′*S*,2′*R*)-5,6,7,8-tetrahydroneopterin (lower panel).

3.2. Molecular mechanical calculations

When subjecting the 40 conformers of tetrahydrobiopterin

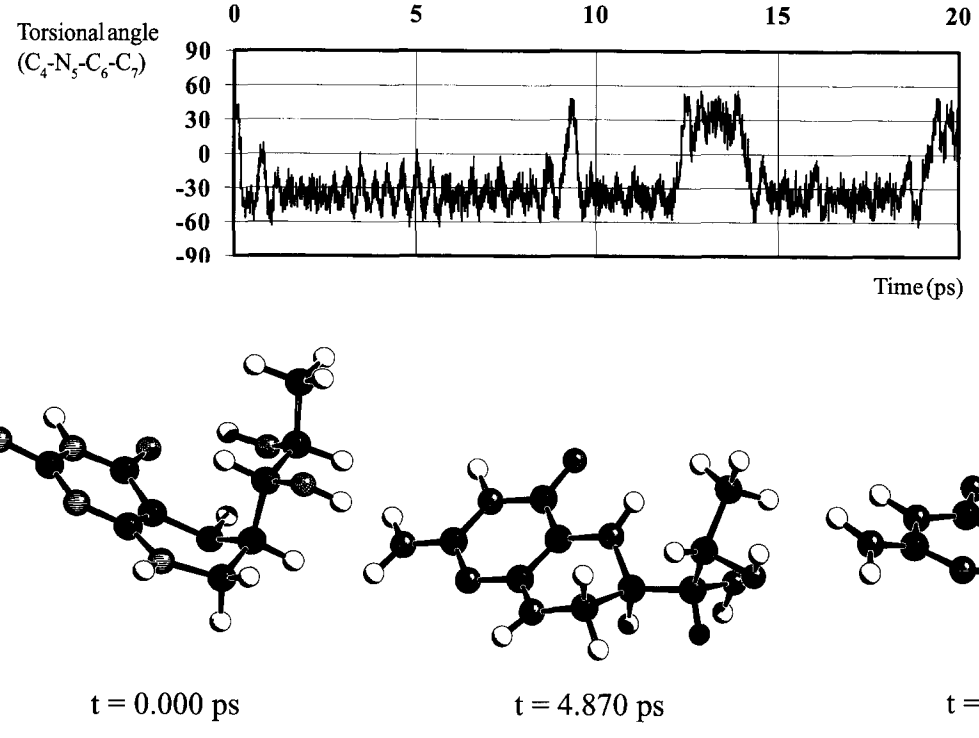

Fig. 2. Molecular dynamic simulation of tetrahydrobiopterin in vacuo. (Upper panel) Temporal behaviour of the torsional angle C_{4a}-N_5-C_6-C_7. Positive values of this torsional angle indicate an axial orientation of the side chain, negative values denote an equatorial side chain orientation. (Lower panel) Snapshots of the molecule at specified times during the simulation.

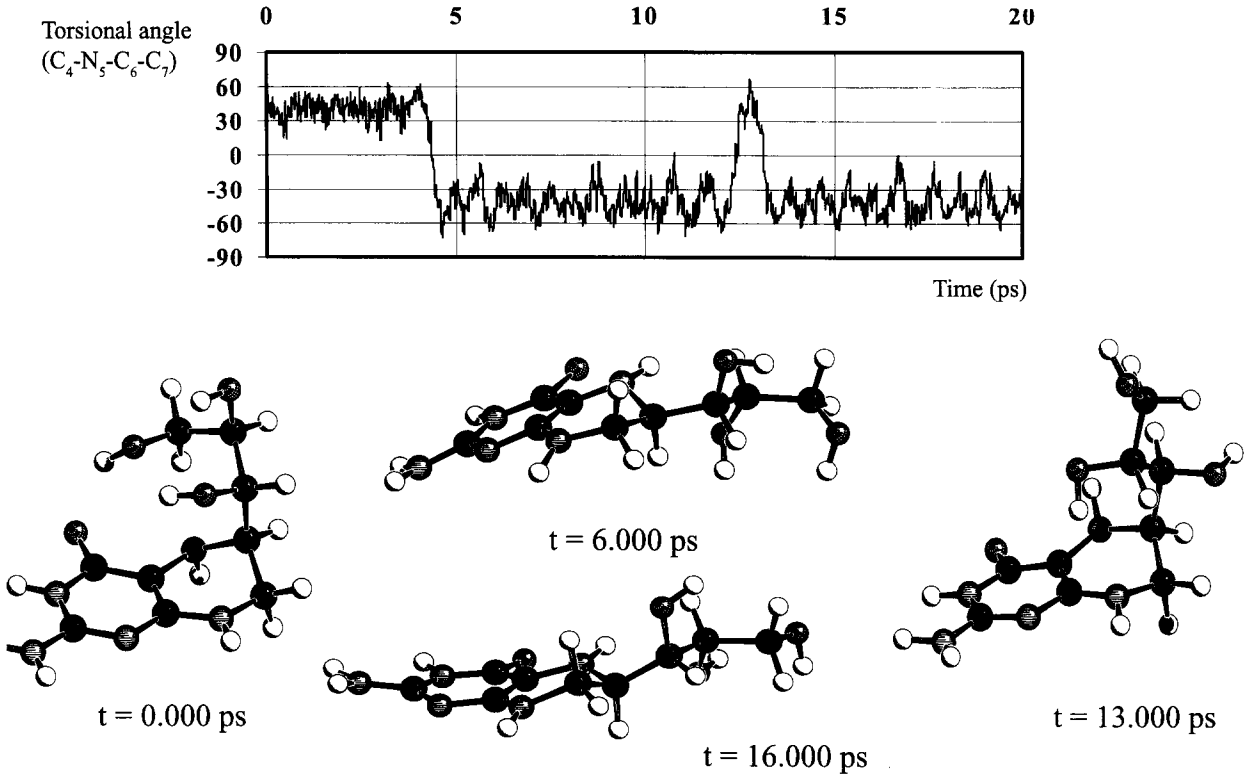

Fig. 3. Molecular dynamic simulation of tetrahydroneopterin in vacuo. (Upper panel) Temporal behaviour of the torsional angle C_{4a}-N_5-C_6-C_7. Positive values of this torsional angle indicate an axial orientation of the side chain, negative values denote an equatorial side chain orientation. (Lower panel) Snapshots of the molecule at specified times during the simulation.

to molecular mechanical geometry optimization, the most stable structures showed the E′A orientations; on average, they were more stable than E′E by about 2.5 kcal/mol, and more stable than A′A and A′E orientations by about 3.5 kcal/mol. The finding of a more stable axial side chain orientation at C_6 agrees well with results by others using the AM1 hamiltonian [12] but disagrees with MINDO/3 results [13] predicting equatorial orientation. The difference between mean energies of E′E, E′A and A′A conformations of tetrahydroneopterin was negligible (less than 1 kcal/mol); A′E conformations were less stable by about 2.0 kcal/mol. The three most stable geometries, however, all had the orientation A′A, which means that, in contrast to the AM1 calculations [12], the side chain is in an axial orientation, as in tetrahydrobiopterin; the hydrogen at N_5, however, is in an axial position.

Importantly, all conformations of both molecules with particularly low energies exhibited intramolecular hydrogen bond-like structures. For example, in low energy conformations of tetrahydrobiopterin the oxygen at $C_{2'}$ is in close contact (about 0.190 nm) with the hydrogen atom at N_5. This molecular feature agrees well with the results of other force field computations [11] but is in contrast to the conclusions drawn from semi-empirical quantum chemical calculations [12,13]. The most stable of the 40 conformations (E′A orientation), in addition, had a second hydrogen bond-like interaction between the hydrogen atom bonded to the oxygen at $C_{2'}$ and the oxygen atom at C_4 of the pterin ring (interatomic distance 0.196 nm). Similarly, the three most stable (iso-energetic) conformations of tetrahydroneopterin (A′A orientation) had such a hydrogen bond-like interaction between the hydrogen atom bonded to the oxygen at $C_{3'}$ and the oxygen atom at C_4 of the pterin ring. This particular feature seems to provide an extra stabilization of the resulting geometries.

3.3. *Molecular dynamics simulation*

Fig. 2 shows the results of a 20 ps simulation of tetrahydrobiopterin in vacuo. The temporal profile of the torsional angle C_{4a}-N_5-C_6-C_7 indicates that the molecule is able to undergo major conformational changes at a temperature of 310 K: the oscillation of the torsional angle between positive and negative values demonstrates that the side chain changes rapidly (in the ps range) between an axial and equatorial orientation. Fig. 2 also shows some snapshots of the molecule during the simulation.

Fig. 3 shows the equivalent simulation of tetrahydroneopterin. As indicated by the temporal profile of the torsional angle C_{4a}-N_5-C_6-C_7, this molecule undergoes equally major conformational changes under the conditions studied: similarly as for tetrahydrobiopterin, the simulation results suggest a slight predominance of the equatorial side chain orientation, but axial structures occur as well.

4. Discussion

This study on conformational properties of the natural cofactor (6*R*,1′*R*,2′*S*)-5,6,7,8-tetrahydrobiopterin and the chemically similar but biologically inactive (6*R*,1′*S*,2′*R*)-5,6,7,8-tetrahydroneopterin was provoked by the suggestion of others based on semi-empirical quantum chemical calculations that conformational properties might explain the unique biological activity

of the former compound [12]. Our computations using force-field calculations show, however, that both molecules show an axial side chain orientation in the most stable conformations; this certainly does not seem to justify a major role of differences in molecular shape when an explanation for the biological activity of the natural cofactor is sought.

Moreover, and even more convincingly, the molecular dynamical simulations at realistic temperatures do not lend any support to the idea of conformational characteristics as a plausible reason for differences in biological activities of reduced pterins: both tetrahydrobiopterin and tetrahydroneopterin show considerable conformational flexibility, and ring conversion appears to occur on the very short time scale of a few ps. This, however, seems to indicate that one has to be very cautious in the interpretation of 'static' geometry minimizations at zero temperature (which of course is always done when one only uses energy minimization by force field or quantum chemical techniques).

One might ask whether the force-field computations used here for geometry optimization as well as for molecular dynamics allow reliable prediction of the behaviour of the molecules studied. We have observed earlier that, at least in the pteridine series, molecular geometries predicted by simple molecular mechanical approaches are in better agreement with reliable but enormously time- and storage-consuming ab initio quantum chemical approaches than semi-empirical techniques; in particular, if properly parametrized, the force field approaches are better suited for the purpose of detecting even weak intramolecular hydrogen bonds than semi-empirical quantum chemical techniques (Reibnegger et al., submitted). This advantage of molecular mechanics compared to semi-empirical quantum chemical methods has been well known among theoretical chemists for some time [16], but appears to be less known among theoretically oriented biochemists. Semi-empirical quantum chemical calculations are much more rapid and inexpensive in comparison with ab initio techniques, but these advantages may be more than outweighed by possibly erroneous structural conclusions drawn from them. The severe limitation of semi-empirical methods in correctly describing weak intramolecular hydrogen bonds has also been noted earlier in studies of other pteridine derivatives [17].

References

[1] Nichol, C.A., Smith, G.K. and Duch, D.S. (1985) Annu. Rev. Biochem. 54, 729–764.
[2] Kaufman, S. (1986) in: Chemistry and Biology of Pteridines (Cooper, B.A. and Whitehead, V.M., eds.) pp. 183–200, Walter de Gruyter, Berlin.
[3] Tietz, A., Lindberg, M. and Kennedy, E.P. (1964) J. Biol. Chem. 239, 4081–4090.
[4] Kaufman, S., Pollock, R.J., Summer, G.K., Das, A.K. and Hajra, A.K. (1990) Biochim. Biophys. Acta 1040, 19–27.
[5] Tayeh, M.A. and Marletta, M.A. (1989) J. Biol. Chem. 264, 19654–19658.
[6] Kwon, N.S., Nathan, C.F. and Stuehr, D.J. (1989) J. Biol. Chem. 264, 20496–20501.
[7] Werner-Felmayer, G., Werner, E.R., Fuchs, D., Hausen, A., Reibnegger, G. and Wachter, H. (1990) J. Exp. Med. 172, 1599–1607.
[8] Kaufman, S. (1963) Proc. Natl. Acad. Sci. USA 50, 1085–1093.
[9] Armarego, W.L.F., Randles, D. and Taguchi, H. (1983) Eur. J. Biochem. 135, 93–403.
[10] Matsuura, S., Sugimoto, T., Murata, S., Sugawara, Y. and Iwasaki, H. (1985) J. Biochem. 98, 1341–1348.
[11] Ayling, J.E., Dillard, S.B. and Bailey, S.W. (1991) in: Pterins and Biogenic Amines (Blau, N., Curtius, H.-C., Levine, R.A. and Cotton, R.G.H., eds.) pp. 269–282, Lakeshore, Grosse Pointe.
[12] Ziegler, I., Borchert, M., Heaney, F., Davis, A.P. and Boyle, P.H. (1992) Biochim. Biophys. Acta 1135, 330–334.
[13] Katoh, S., Sueoka, T. and Kurihara, T. (1993) Pteridines 4, 27–31.
[14] Abraham, R.J. and Smith, P.E. (1988) J. Comput. Chem. 9, 288–297.
[15] Allinger, N.L., Kok, R.A. and Imam, M.R. (1988) J. Comput. Chem. 9, 591–595.
[16] Levine, I.N. (1991) Quantum Chemistry, 4th ed., pp. 594, Prentice Hall, Englewood Cliffs, NJ.
[17] Reibnegger, G., Denny, B.J. and Wachter, H. (1993) Pteridines 4, 23–26.