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Theoretical studies of biliverdin: energetics of the reduction pathways to bilirubin

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Abstract Geometries and energies of formation of bilirubin formed by reduction of biliverdin via three meso carbon sites, the β, γ and δ positions, have been calculated using semiempirical methods. It has been shown that γ-bilirubin with a ridge-tile conformation forms six intramolecular hydrogen bonds and is the most stable of the three above mentioned positions by at least 22 kcal mol^{-1}. Reduction pathways for β-, γ- and δ-bilirubin formations from biliverdin are studied in detail. The roles of loss of conjugation and hydrogen bond formations in stability of different conformers have been discussed. γ-Bilirubin was fully optimized by using ab initio methods. Fine refinements of calculated results show excellent agreement with experimental results. Electronic supplementary material to this paper can be obtained by using the Springer LINK server located at http://dx.doi.org/10.1007/s00894-002-0078-9.

Keywords Semiempirical calculations · Ab initio calculations · Natural biliverdin · Reduction · Bilirubin

Introduction

Biliverdin IX-α is a specific product of heme catabolism in the presence of heme oxygenase. Biliverdin IX-α converts to γ-bilirubin with biliverdin reductase in natural systems. [1, 2, 3, 4] For decades the reaction pathways and properties of biliverdin have been under investigation, but recently more attention has been paid to studies of heme catabolism in enzymatic systems and modeled coupled oxidation reactions. [5, 6, 7, 8, 9, 10, 11, 12, 13, 14] The chemistry of bilirubin has been the subject of numerous reviews and monographs. [15] High plasma levels of bilirubin resulting in jaundice may produce central nervous system toxicity. Brodersen [16] studied the solubility of bilirubin and showed that the solubility and other biological properties of bilirubin can be explained based on its structure, so examination of the structure requires more attention. However, reaction pathways of conversion of biliverdin to bilirubin in nature or in chemical systems have not been studied in detail. It is well known that the product of biliverdin conversion to bilirubin is stereospecific formation of γ-bilirubin in nature but, as far as we know, there is no experimental or theoretical evidence to analyze the product of reduction of biliverdin in the absence of the enzymes. In our previous theoretical study, [17] we calculated several tautomeric forms of biliverdin and showed that the keto–keto tautomer is the most stable form, in accordance with experiment. In our subsequent investigation [18] on the dynamics and energetics of the self-association process of biliverdin to biliverdin oxide it was shown that formation of intramolecular hydrogen bonds is possible. In the present work the reduction process of biliverdin to bilirubin via three meso carbon sites, the beta, gamma and delta methine positions, have been studied. The stabilities of different proposed forms of β-, γ- and δ-bilirubin and the roles of hydrogen bond formation versus loss of conjugation have been investigated. Comparison of the energies of various pathways as well as determination of transition states and saddle point geometries along the reaction coordinate have been carried out.

Computational scheme

A personal computer was employed for our calculations. The MOPAC version 6.0 [19] was used with the data input in the Z-matrix form. In order to visualize and preoptimize geometries, the HyperChem program version 4.0 was employed. For optimization of various structures, AM1, PM3, PRECISE, MMOK, EF and GNORM were used as keywords.

Throughout the calculations the convergence limit for the energy gradients was 0.01 kcal mol^{-1}. After optimi-

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zation of all geometries, in order to locate the transition state, the keywords SADDLE along with GEO-OK and XYZ were used. Transition states were refined by the NLLSQ algorithm and checked by diagonalizing the force constant (Hessian) matrix using FORCE calculation. Complementary ab initio studies using the Gaussian 98-W program and three standard basis sets STO-3G, 3-21G and 6-31G were carried out to obtain more accurate geometrical information.

Result and discussion

Geometric optimization of the precursor and proposed reduction products

The keto–keto tautomer of natural biliverdin was chosen as our proper precursor molecule. [17] Three different proposed forms of bilirubin (β-, γ- and δ-bilirubin) were designed (Fig. 1) as possible reduction products. Two Hamiltonians AM1 and PM3 were used and each form was fully optimized to determine which is the most stable. The results for the heat of formation of each structure are summarized in Table 1. These data indicate that, regardless of the type of Hamiltonian used, the γ-form is the most stable isomer. According to AM1, the γ-form is 21 kcal mol^{-1} more stable than β-bilirubin and 21.8 kcal mol^{-1} more stable than δ-bilirubin. Figure 1 shows the reduction processes of natural biliverdin via the three methine positions beta, gamma and delta. Figure 2 indicates that the β and δ isomers have similar conformations but γ-bilirubin has a unique ridge-tile conformation with six intramolecular hydrogen bonds. A previously reported molecular orbital study on neutral bilirubin and the bilirubin dianion demonstrated that semiempirical molecular orbital calculations could correctly predict the formation of six and four intramolecular hydrogen bonds in neutral bilirubin and the bilirubin dianion respectively. [20, 21] Our calculated semiempirical results for γ-bilirubin geometrical parameters are consistent with the study on neutral bilirubin. [20]

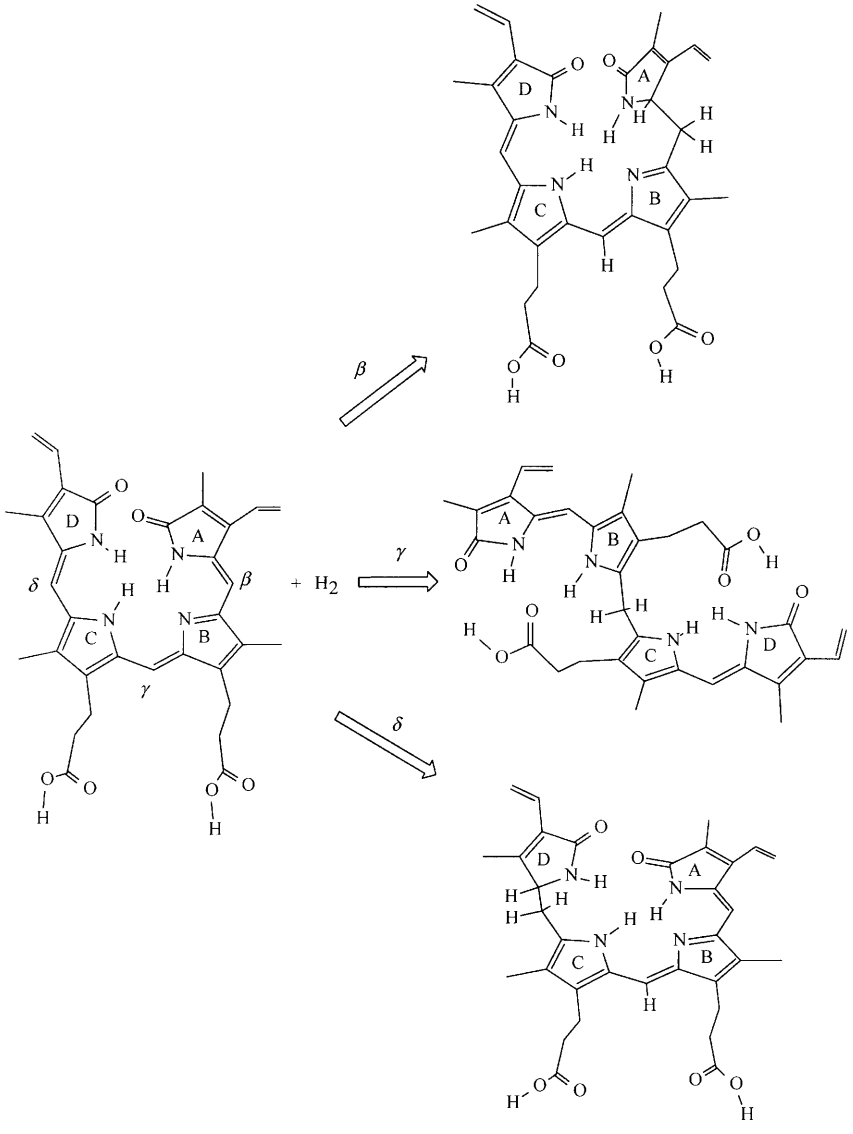

Fig. 1 Reduction processes of natural biliverdin via three methine positions β, γ and δ

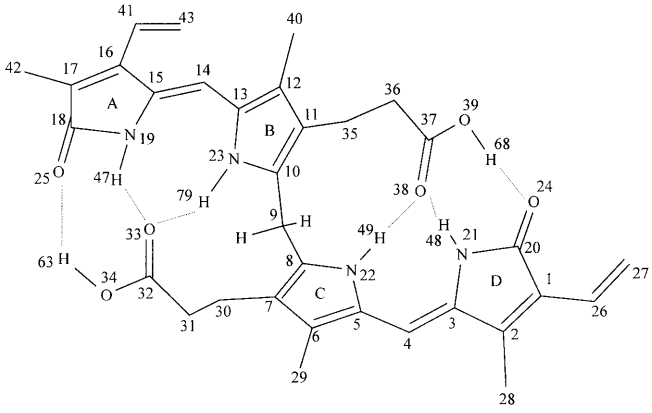

Fig. 2 Intramolecular hydrogen bonds in γ-bilirubin and numbering system used

Table 1 Comparison of ΔH_f of bilirubin isomers

Method	Isomer	ΔH_f (kcal mol^{-1})	Energy stability
AM1	β-Bilirubin	–95.13	21
	γ-Bilirubin	–116.13	0
	δ-Bilirubin	–94.28	21.85
PM3	β-Bilirubin	–150.2	17.3
	γ-Bilirubin	–167.5	0
	δ-Bilirubin	–151.41	16.09

Geometry optimization of γ-bilirubin using an ab initio method

As our semiempirical calculated geometry parameters of γ-bilirubin suggest, agreements of dihedral angles, intramolecular hydrogen bonds and heavy atom distances with X-ray results are poor. This has also been previously observed by Shelver et al. [20] Therefore, a more accurate ab initio investigation is desirable. In order to carry out such a study, γ-bilirubin was optimized using three basis sets STO-3G, 3-21G and 6-31G with only the result for the last basis set given below. Our results show that AM1 does a fairly good job in estimating bond distances and angles. This is illustrated by the root mean square deviation (RMSD) of the differences of bond distance and bond angles, which are 0.03 and 3.95, respectively. As has been previously reported [22, 23] the experimental values for hydrogen bond and heavy atom distances are shorter than those predicted by semiempirical calculation, indicating that the parameterization of these methods may need some improvement. Table 2 summarizes a comparison of such parameters for both methods and illustrates the differences in hydrogen bond and heavy atom distances between calculation and experiment.

Table 3 contains dihedral angles compared with X-ray data for ab initio and AM1. The ab initio method shows better estimation of dihedral angles compared with experimental data except for the vinyl tilt

Table 2 Comparison of calculated and observed values of hydrogen bond in γ-bilirubin

System	Atoms	X-ray	Exp – (6-31G)	Exp – AM1
Heavy atom distance	O24–O39	2.65	–0.05	–0.45
	N22–O38	2.84	–0.12	–0.16
	N21–O38	2.75	–0.11	–0.2
	O25–O34	2.55	–0.25	–0.65
	N19–O33	2.83	0.08	–0.23
	N23–O33	2.8	–0.16	–0.17
Hydrogen bond distance	H68–O24	1.58	–0.22	–0.52
	H48–O38	1.7	–0.22	–0.43
	H49–O38	1.81	–0.19	–0.34
	H63–O25	1.47	–0.21	–0.73
	H47–O33	1.79	–0.01	–0.42
	H79–O33	1.74	–0.22	–0.5

Table 3 Comparison of calculated and observed values of dihedral angles in γ-bilirubin

System	Dihedral angle	X-ray	Exp – (6-31G)	Exp – AM1
Side chain	C10–C11–C35–C36	–118.4	–3	5.2
	C11–C35–C36–C37	68.2	0.7	–20.4
	C35–C36–C37–O38	8.5	–1.2	–3.2
Hinge	C11–C10–C9–C8	117.3	–10.3	3.1
	C10–C9–C8–C7	116.4	–4.4	4
Ring N	C16–C15–N19–H47	–179.7	–11.8	–9.8
	C11–C10–N23–H79	178.1	4.4	1.4
	C6–C5–N22–H49	179.6	1.5	3.1
	C2–C3–N21–H48	174.3	1.5	–4.3
Pyrrol ring tilt	C15–C14–C13–C12	173.2	–0.9	25.2
	C6–C5–C4–C3	179.5	7.9	31.1
Vinyl tilt1	C17–C16–C41–C43	–173.8	35.8	37.4
	C2–C1–C26–C27	176	–3.7	–3.8

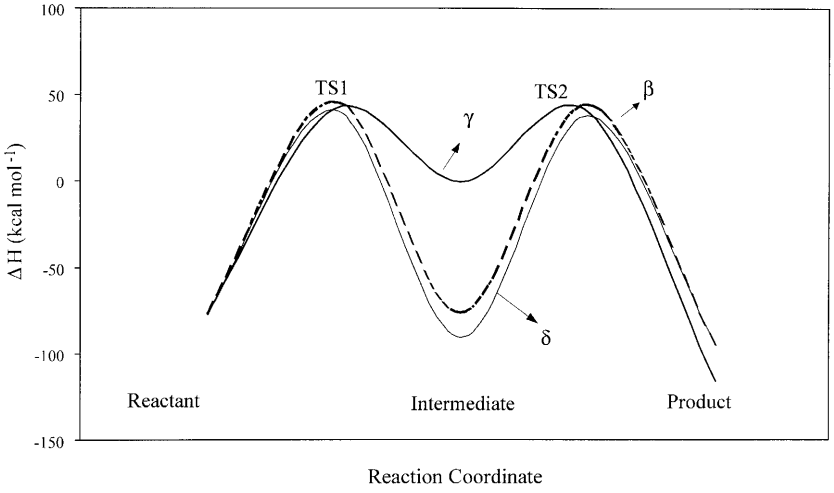

Fig. 3 Comparison of potential energy surfaces along the reaction coordinate from three methine positions β, γ and δ using AM1 method

Table 4 Comparison of calculated values for heat of formation ΔH_f and heat of reaction ΔH_r in the reduction process

Method	Route	Reactant	TS1	Intermediate	TS2	Product	ΔH_r
AM1	β-Position	–76.23	46.06	–75.5	44.34	–95.13	–18.9
	γ-Position	–76.23	41.7	–0.56	40.44	–116.13	–39.9
	δ-Position	–76.23	41.38	–90.6	38.4	–94.28	–18.05
PM3	β-Position	–144.74	–41.33	–159.13	–43.22	–150	–5.48
	γ-Position	–144.74	–77.42	–143.84	–77.9	–167.5	–22.76
	δ-Position	–144.74	–49.67	–150.2	–48.22	–151.4	–6.7

C17–C16–C41–C43 angle, for which both methods fail and which might mean an error in the X-ray report for this angle. [24, 25] As Korkin et al. have also suggested, the differences between experimental and theoretical values could be caused by the following: (a) the difference in the physical meaning of the crystallographic data and MO calculated equilibrium geometrical parameters; (b) the intramolecular interaction in the crystal. [26]

Potential energy changes along the reduction path for biliverdin to bilirubin

In order to obtain some information from the reduction of biliverdin to bilirubin, three methine β, γ and δ positions (Fig. 1) were chosen as potential sites for chemical reduction in these studies. Our goal was to gain some insight about the reduction energies and geometric changes along the reaction coordinate. Figure 3 illustrates the potential energy surfaces for reduction of natural biliverdin to the three β-, γ- and δ-bilirubin products using AM1. The same curves were obtained using PM3. Table 4 summarizes the energetic results for three routes. As the data in Table 4 and Fig. 3 indicate, γ-bilirubin is the most stable isomer, in accord with experiment. Although the intermediate along the γ route is less stable than those of the β and δ routes, which is shown by the depth of the potential wells, surmounting the transition state (TS2) requires much less energy for the former. Therefore, the γ route is the most convenient path for the reduction process. This conclusion is further justified by comparing the heat of reduction reactions via the three routes. The last column in Table 4 compares ΔH_r values. Both AM1 and PM3 confirm that the γ route is the least energy demanding path.

Geometries along the reaction coordinate

All of the transition states, reactants, products and intermediate geometries were refined and checked. The number of negative eigenvalues of the force constant matrix confirmed that all transition states have only one negative eigenvalue. Figure 4 illustrates the changes in both dihedral angle θ_D (C8–C9–C10–N23) and the heat of formation along the reaction coordinate. An interesting correlation between θ_D and ΔH_f is obvious from this figure, which emphasizes on the dihedral angle as an important factor during the course of reaction. The geometries of chemically interesting points for the γ route are also included in this figure. Planes drawn on each geometry are the calculated mean planes passing through the adjacent pyrrol pair rings. Because of the nature of double C9–C10 bond in natural biliverdin, these two planes make a very small angle to each other ($\theta_D \sim 2°$) to preserve conjugation in the π-system. For the transition state geometry, however, two free atomic hydrogens are situated near C9 and N23 while the γ-meso double bond is increased in length to some extent and weakened at the same time (Fig. 5). This causes θ_D to be increased to

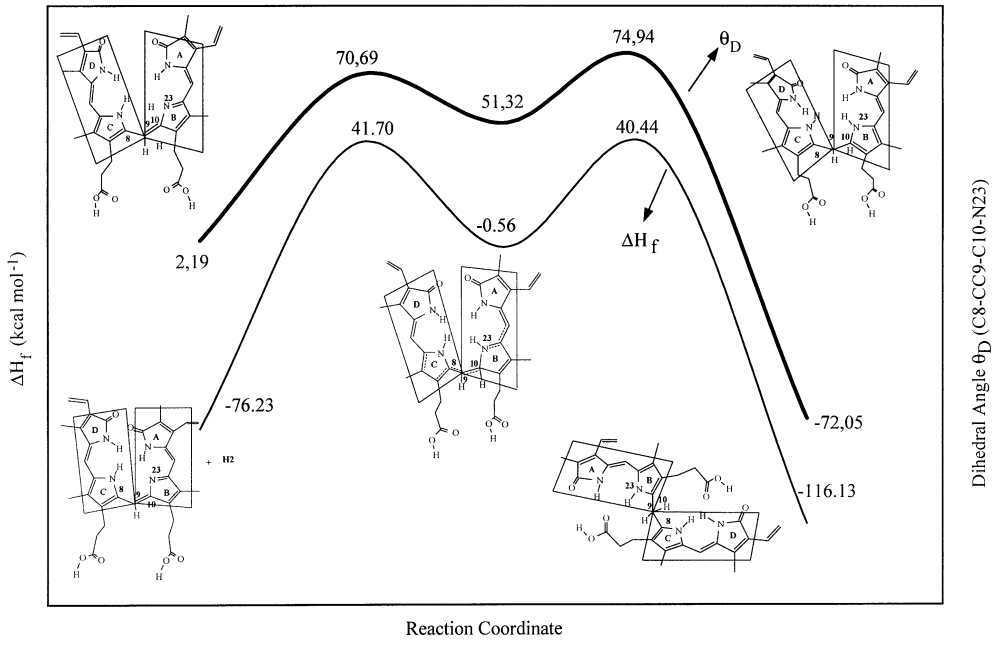

Fig. 4 Comparison of ΔH_f (*bottom*) and dihedral angle (*top*) changes for γ-position along the reaction coordinate using AM1 method. Geometries for reactant, product, transition states and intermediate are also shown

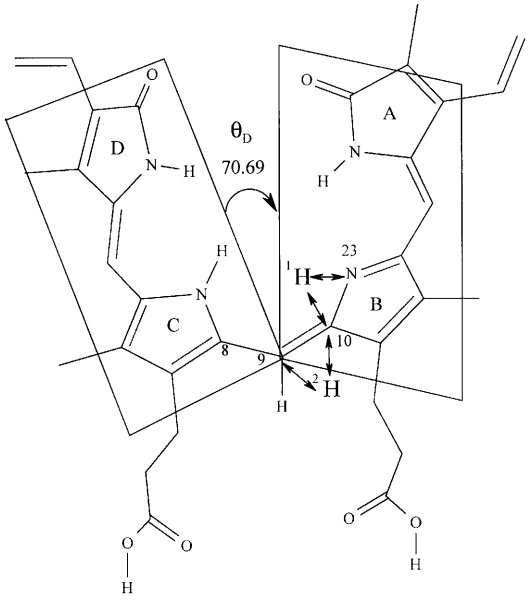

Fig. 5 Transition state geometry for γ reduction pathway. The angle between the two mean planes passing through pyrrole ring pairs is shown. Atom distances from the reduction center are mentioned in the text

about 70°, while ΔH_f is also increased due to an unstable geometry with two free hydrogen atoms. Figure 5 summarizes some of the important distances at the transition state configuration which are: ^{1}H–^{23}N (1.55 Å), ^{1}H–^{10}C (1.75 Å), ^{2}H–^{10}C (1.79 Å), ^{2}H–^{9}C (1.78 Å). At the intermediate stage both bonds of H's to C and N atoms make the γ-meso bond length decrease and the two planes less free to rotate. The result is a somewhat more stable geometry, which makes θ_D decrease at this stage. The same trend is observed until the TS2 barrier is reached. The γ-meso bond in bilirubin is converted to a single bond with formation of two new C–H, N–H bonds. Overall, ΔH_f is decreased on reaching the product with the two planes more free to rotate (θ_D~288°).

Comparison of energetics of proposed bilirubin molecules

Figure 1 shows the geometries of natural biliverdin and three β-, γ- and δ-bilirubin molecules. There are two counteracting factors in going from reactant to the three reduction products. Loss of conjugation favors the β- and δ-bilirubin routes, while formation of six hydrogen bonds in the ridge-tile natural bilirubin favors the γ route. Assuming ~8–10 kcal mol^{-1} stability per hydrogen bond, γ-bilirubin must be ~50 kcal mol^{-1} more stable than the other two. Table 1, however, suggests an energy stability of about 22 kcal mol^{-1}. The difference in the calculated stability (~22 kcal mol^{-1}) versus the estimated stability (~50 kcal mol^{-1}) of about 28 kcal mol^{-1} can be attributed to the loss of π-conjugation. A pyrrol ring resonance energy is known to be ~22–28 kcal mol^{-1}, [27] which nicely accounts for the difference mentioned above. Korkin et al. have also shown that the energy difference between Zs and Za conformers of pyrromethene could be explained based on their different ability to form intramolecular hydrogen bonds. [28]

Conclusions

The results obtained from our theoretical investigations suggest that the semiempirical methods employed are appropriate tools for studying large species on which experimental work is difficult. Using such theoretical tech-

niques, we showed that one can obtain valuable information about energies, geometries and the relative stabilities of various species along the reaction coordinates. For the reduction process of natural biliverdin to bilirubin via the three meso carbon sites β, γ and δ, two counteracting factors can affect the reduction process. Intramolecular hydrogen bond formation acts in favor of γ-methine reduction by providing ~50 kcal mol^{-1} stabilization, while loss of conjugation disfavors it by ~28 kcal mol^{-1}. Therefore a higher stability of about 22 kcal mol^{-1}, due to the six hydrogen bonds formed in γ-bilirubin, is proposed as the reduction driving force. Our results show that as a driving force hydrogen bond formation can ease the natural reduction process in living organisms. Furthermore, to our knowledge no detailed experimental analysis on the possible non-enzymic reduction products of biliverdin has been performed. This theoretical work predicts that, because of factors discussed in the text, the products of the non-enzymic reduction of biliverdin must also specifically be γ-bilirubin. Further investigations of this process by using a precursor such as octaethyl biliverdin would shed more light on the roles of hydrogen bond formation versus loss of conjugation, which is under consideration.

Supplementary material

All calculated structures are available as *xyz*-coordinates in the supplementary material.

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