The Conformation of Bilin Chromophores in Biliproteins: Ramachandran-Type Calculations

Hugo Scheer, Helmut Formanek, and Wolfhart Rüdiger

Botanisches Institut, Universität München, Menzinger Straße 67, D-8000 München 19

Ramachandran-type calculations are performed for conformations of bilin chromophores present in the biliproteins phycocyanin, phycoerythrin and phytochrome. The atomic coordinates are taken from x-ray data of crystalline model compounds, namely biliverdin for pyrrole rings B, C, D and substituted succinimides for the hydrogenated ring A including a thioether containing β-substituent. Maxima and minima for steric hindrance are calculated for rotation of the thioether side chain, the rotation of pyrrole rings at single bonds (*syn-anti*-forms) and at double bonds (Z-E-isomers) of the methine bridges. Whereas quasi-planar structures are possible for all *syn*, Z-forms, only twisted structures are possible if *anti*, E-forms are considered. The relevance for the bilin conformations of native biliproteins and of the $P_r \rightleftharpoons P_{fr}$ phototransformation is discussed.
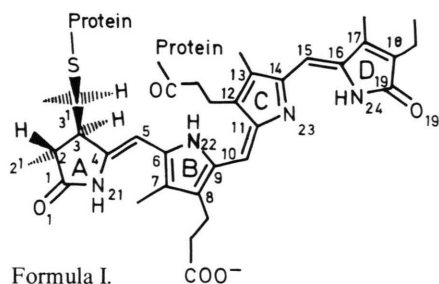
Bilins (contrary to most porphyrins) are flexible molecules. A great number of conformations can theoretically be drawn which arise from rotation around single bonds of methene bridges (Fig. 1). The shape of the molecule ranges from cyclic porphyrin-like to maximally opened (stretched) forms. Similar forms can be induced by *Z-E* isomerization of double bonds of methine bridges.

Porphyrin like forms (*i. e.* all-*Z* configuration, all-*syn* conformation) have been advanced for crystalline biliverdins [1, 2] and for biliverdins in solution [3, 4]. The only other bilindiones so far investigated are *E, Z, Z* bilindiones [5, 6] for which the *syn, syn, syn* conformation has been postulated on the basis of UV-visible spectral data [7].

The particular spectral properties of the bilindione chromophores of native biliproteins have been explained by extended chromophore conformations (viz. Formula **I**) induced by the protein. In the case of phytochrome, experimental spectral data were compared with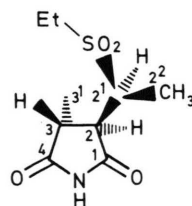 calculated spectral data [8]. In the case of phycocyanin, unfolding and refolding of the biliprotein proved the reversibility of the spectral changes which again were compared with spectral data from the literature calculated for various chromophore conformations [9]. Such calculations exist for a great number of assumed bilin conformations [10 – 13]. However, a great deal if not most of the assumed conformations are impossible in biliproteins because of steric hindrance of the β-pyrrolic side chains. This is especially true for planar structures [8, 12] but even where non-planar-structures were considered [7, 13] arbitrary torsional angles have been introduced for the purpose of fitting of spectral data without a systematic consideration of steric hindrance.

The elucidation of the stereochemistry of the side chain of ring A including the thioether linkage to the protein (Formula **I**) in phytochrome [14] and phycocyanin [15] together with x-ray crystallographic data of the model compounds **II** and **III** ([16], Lotter, Klein, Rüdiger in preparation) prompted us to calculate atomic distances and energy levels for conformations produced by rotations around the single and double bonds connecting the individual pyrrolic rings.
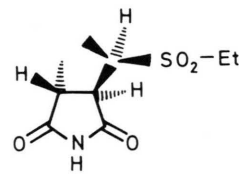

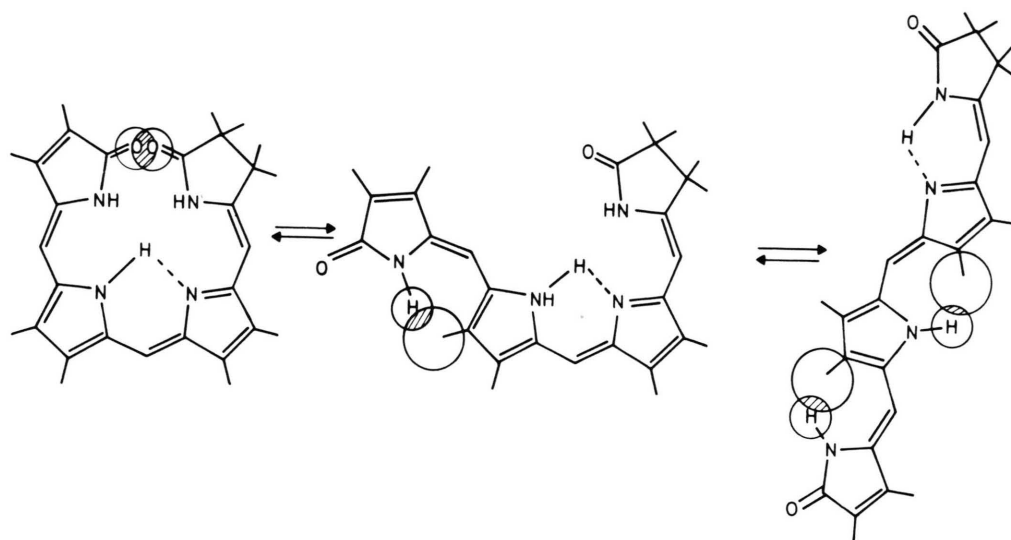Formula I.

Formula II.

Formula III.

Fig. 1. Schematic representation of different arrangements of the chromophores of **I** accessible by rotations around the C-10,11 and C-14,15 single bonds (from ref. [25]).
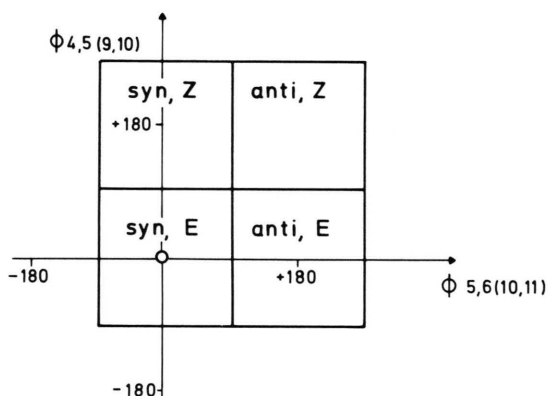

Fig. 2. Definition of the four basic forms of partial structures **IV** and **V**. Only the central part of each quadrant allows π-overlap sufficient for conjugation of the two rings. The planar structures for each form are shown in Figs 3 and 4.

Methods

The atomic coordinates for the saturated ring A of biliprotein chromophores have been taken from the succinimide **II** [16] and for the unsaturated rings from biliverdin-dimethylester [1]. They have been transformed into a rectangular coordinate system in such a way, that the N-atoms occupy the zero position, one C-atom lies in the X-axis and another C-atom in the X, Y-plane (Table I).

The atomic coordinates corresponding to conformations of different torsion angles have been calcu-

lated with a computer program using rotation matrices. The zero position of the φ-angles of partial structures **IV** and **V** are shown in Figs 3 and 4. The rotations have been performed counter clockwise viewing from C_5 or C_{10} to the rings. In the zero position of the angle τ (Fig. 5) of the thio-substituted side chain (Formula **I** – **III**), the atoms C_2, C_3, C_31 and S are in one plane and the S-atom has a *syn*-position to C_2. The rotations have been performed counter clockwise viewing from C_3 to C_31.

The energies corresponding to the different conformations have been calculated with the Kitaigorodskii function [17], where the interaction energy E between two non-bonded atoms is expressed as a function of a single variable z.
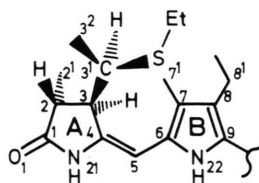
$$E = 3.5 \,(- 0.04/z^6 + 8.6 \times 10^3 \exp(- 13\,z))$$
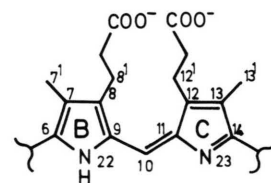$$z = r_{ij}/r_0$$
r_{ij} = distance between two non-bonded atoms
r_0 = van der Waals distance between non-bonded atoms.

The van der Waals distances are shown in Table II.

Formula IV.

Formula V.

Table I. Atomic coordinates for the Succinimide **II** and the four five-membered rings of biliverdin dimethylester. The data from refs. [16] and [1], respectively, are transformed as described in "methods".

Succinimide **II**		Biliverdin-dimethylester							
N_{21}	0.00 0.00 0.00	N_{21}	0.00 0.00 0.00	N_{22}	0.00 0.00 0.00	N_{23}	0.00 0.00 0.00	N_{24}	0.00 0.00 0.00
C_4	1.37 0.00 0.00	C_4	1.40 0.00 0.00	C_9	1.37 0.00 0.00	C_{11}	1.43 0.00 0.00	C_{16}	1.35 0.00 0.00
C_3	1.85 1.45 0.00	C_3	1.83 1.43 0.00	C_8	1.77 1.35 0.00	C_{12}	1.89 1.35 0.00	C_{17}	1.79 1.44 0.00
C_2	0.60 2.31 0.06	C_2	0.69 2.17 0.04	C_7	0.64 2.16 0.04	C_{13}	– – –	C_{18}	0.66 2.20 0.00
C_1	−0.53 1.26 0.06	C_1	−0.45 1.29 −0.03	C_6	−0.45 1.24 0.03	C_{14}	−0.34 1.28 0.04	C_{19}	−0.51 1.28 0.00
C_31	2.91 1.57 1.14	C_31	3.24 1.83 −0.01	C_81	3.23 1.86 0.00	$C_{12}1$	3.08 2.03 −0.14	$C_{17}1$	3.22 1.83 −0.04
C_21	0.45 3.31 −1.07	C_21	0.59 3.66 0.14	C_71	0.59 3.56 0.04	$C_{13}1$	0.76 3.60 0.10	$C_{18}1$	0.46 3.66 0.00
O_{onC_4}	2.08 −0.98 0.03	C_5	2.21 −1.09 0.10	C_{10}	2.15 −1.13 −0.01	C_{10}	2.17 −1.17 0.08	C_{15}	2.14 −1.09 −0.07
O_{onC_1}	−1.73 1.55 0.06	O_{onC_1}	−1.65 1.59 −0.10	C_5	−1.85 1.68 0.19	C_{15}	−1.69 1.72 0.09	$O_{onC_{19}}$	−1.68 1.60 0.07

Table II. Van der Waals distances used in the calculations.

	C	N	O	S
C	3.2	2.9	2.8	3.4
N		2.7	2.7	3.2
O			2.7	3.2

Results and Discussion

Biliproteins are characterized by a hydrogenated ring A linked via a thioester bond to the protein (Formula **I**) [18, 19]. The steric factors involved in the topology control of biliproteins involve, therefore, interactions between a hydrogenated and an unsaturated ring (partial structure **IV**) as well as interactions between unsaturated rings (partial structure **V**). The bond angles and lengths for the partial structure **V** have been taken from the central methine bridge of biliverdin dimethylester (rings B and C) as determined by the x-ray analysis of Sheldrick [1]. The bond lengths of the partial structure **IV** are derived from a composite of two structures: ring B is

again derived from biliverdin dimethylester [1], while ring A has been replaced by a dihydro-structure taken from the x-ray study of 2-(2'-sulfonylethyl) ethyl succinimide (**II**) [16]. The C_21-S distance of 1.83 Å in this sulfone is comparable to the C-S distance of 1.80 Å in the thioether, methionine [20].

To take into account the inverted stereochemistry at C_31 in biliproteins, two substituents have been interchanged at (the corresponding) C_21 in the succinimide **II**. The x-ray structure of the corresponding epimer **III** has been solved after completion of this work (Lotter, Klein, Rüdiger in preparation). There is a good agreement of the conformation of **III** in the crystal and the one predicted from these calculations.

We consider here only the sterical interaction of vicinal pyrrole rings because there is no interaction of rings A and C or B and D. Furthermore, interaction of rings A and D occurs only if the tetrapyrrole system is in a planar or nearly planar cyclic form. A small deviation from planarity (all φ's $= 10 - 20$ °) allows the system to avoid this sterical hindrance
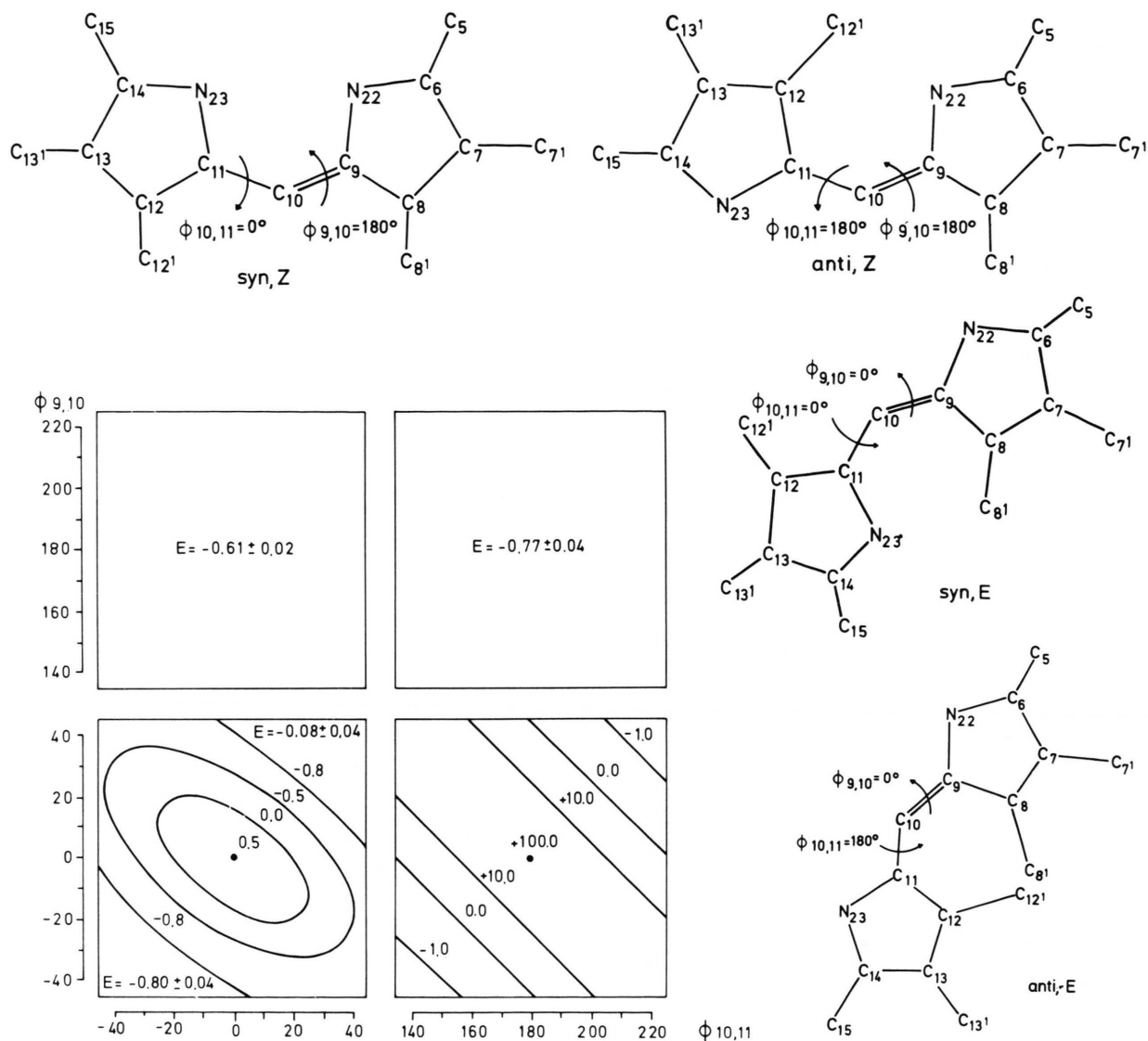
Fig. 3. Energy profiles (in kcal/mol) for the four basic forms of partial structure **V** representing the ring B, C junction in biliproteins. Only the central part of each quadrant with deviations from planarity ≤ 40 ° is shown, corresponding to conjugation between the two rings.

without a major loss of π-overlap. This gives the well-known helical geometry of biliverdins [1 − 4].

Ramachandran-type calculations take into account only steric interactions. They thus define sterically forbidden areas and are complementary to considerations regarding H-bonds and π-system interactions, which define allowed or favorable conformations or configurations. In the case of bile pigments, steric hindrance is also complementary in its effects to H-bonding and π-interactions, as the co-

planar structures favored by the latter are sterically unfavorable.

In the formalism used throughout, the two dimensional space defined by rotations about the bridging bonds C_4, C_5 and C_5, C_6 in **IV**, and C_9, C_{10} and C_{10}, C_{11} in **V**, respectively, is divided into four quadrants as defined in Fig. 2. The planar structures of the four forms are shown in Figs 3 and 4. Whereas coplanarity is possible for partial structure **V** in the Z configuration (Fig. 3, upper part), steric hindrance
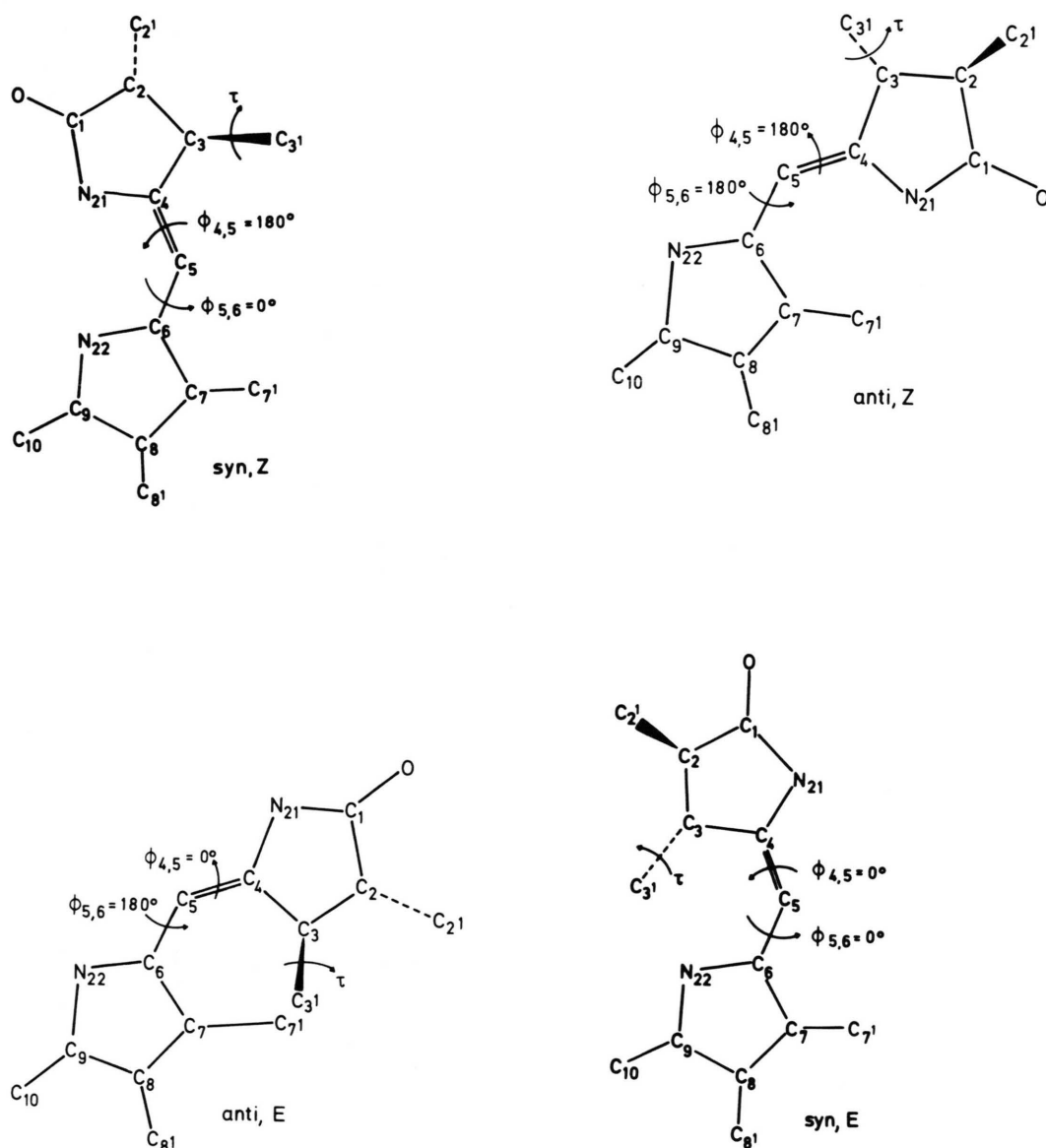
Fig. 4. Coplanar structures of the four basic forms of partial structure **IV**.

is observed in the *E* configurated system (Fig. 3, lower part). No planar structure is allowed in this case. If the C-9, 10 double bond is taken to be planar (*i. e.* $\varphi_{9,10} = 180$ °) the torsion angle $\varphi_{10,11}$ must be $+ 40$ ° or $- 40$ ° (*anti* conformation) or > 200 ° or < 160 ° (*syn* conformation). The angles could be somewhat reduced if the double bond is also twisted (see Fig. 3, lower part).

Such a strongly twisted structure has been found in the as yet only crystal structure of an anti, *Z-syn-Z, syn-Z* bilin [21] and is also proposed for the *E*-isomers of bilindiones [7].

Steric interactions are rather pronounced in the partial structure **IV** as a model for rings A and B in biliprotein chromophores (Fig. 5). In addition to rotations around the two bridging bonds (C_4, C_5 and
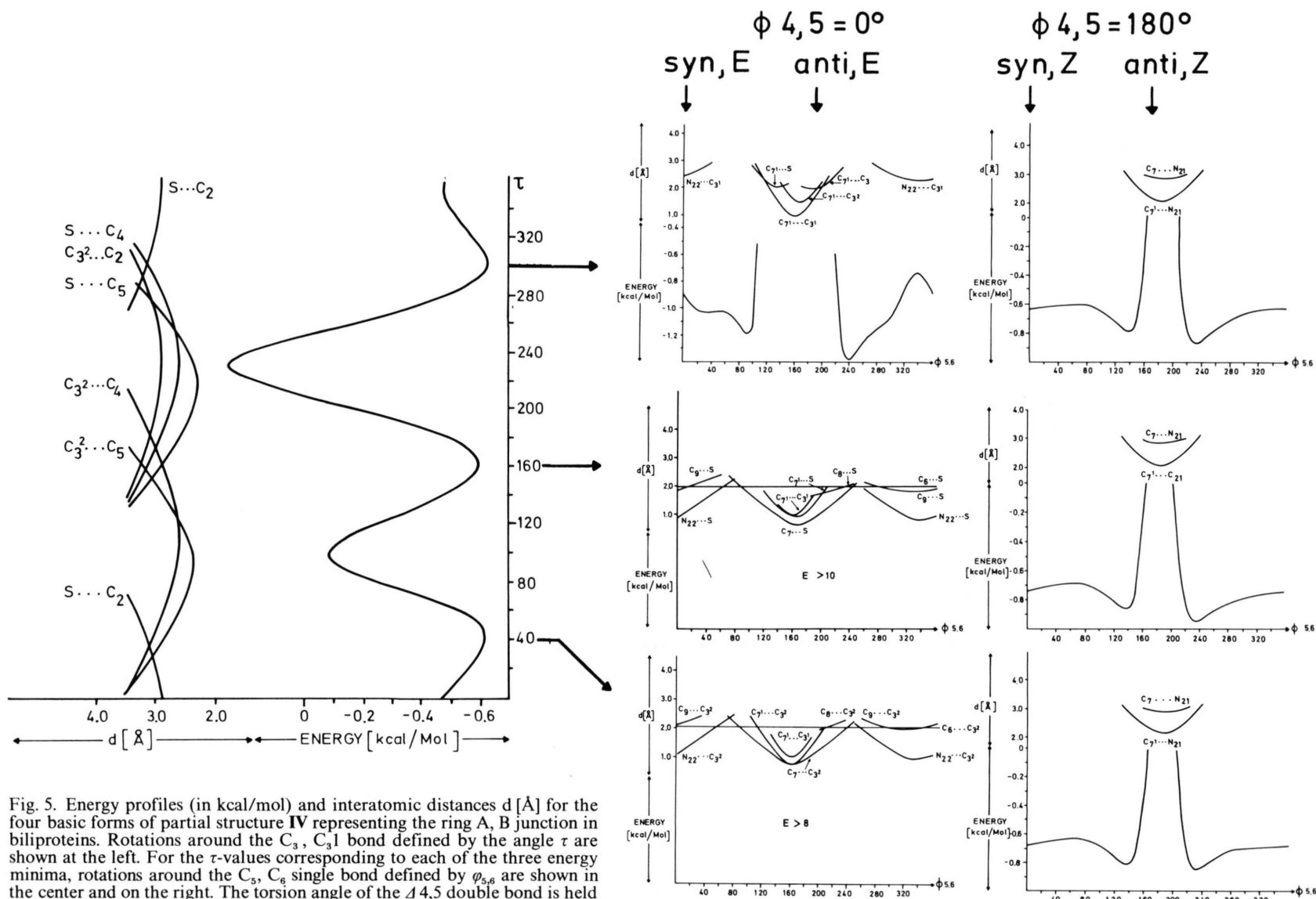
Fig. 5. Energy profiles (in kcal/mol) and interatomic distances d [Å] for the four basic forms of partial structure **IV** representing the ring A, B junction in biliproteins. Rotations around the C_3, C_31 bond defined by the angle τ are shown at the left. For the τ-values corresponding to each of the three energy minima, rotations around the C_5, C_6 single bond defined by $\varphi_{5,6}$ are shown in the center and on the right. The torsion angle of the $\Delta 4,5$ double bond is held constant at 0° (E-configurations, center column) and 180° (Z-configuration, right column).

C_5, C_6), rotations around the C_3, $C_3 1$ bond strongly influence the stability of **IV**. The latter rotation has three minima, defined by interactions of the C_3 substituents with C_2, C_4 and C_5, which are essentially independent of the conformations at the methine bridge (Fig. 5). These minima are separated by maxima, of which at least one ($\tau \approx 225$ °) is above the thermal energy at ambient temperature. E-configurations of the $\Delta 4,5$-double bond are restricted to only one of the minima ($\tau \approx 300$ °), which is, incidentally, the τ-value observed in the recent x-ray analysis of the $C_2 1$-epimer **III** of the sulfone **II** (Lotter, Klein, Rüdiger in preparation). Only an extremely twisted *anti, E* form is sterically allowed in this case ($\varphi_{5,6} \approx 220$ °), and the energy minimum for the *syn, E* form is at a twisted conformation, too ($\varphi_{5,6} \approx 30$ °).

For the *Z*-configuration at the $\Delta 4,5$-double bond, the restrictions imposed by τ are less stringent, but still pronounced. Coplanar conformations of the *anti, Z* form are forbidden for each τ-minimum, and the absolute minima are highly twisted *anti, Z* conformations in each case. These minima are close to out-of-plane angles of ± 40 ° (corresponding to 180 ± 40 ° in Fig. 5), the limiting angle for π-interactions. A balance between π-interactions and steric hindrance can, therfore, be expected.

The asymmetric C-atoms at ring A induce a pronounced asymmetry of the steric interactions. Not only are out-of-plane angles with minimum interactions different for the two different orientations, but the minima have also different energy. As an example, the minima for the *anti, E* form ($\tau = 300$ °) are at $\varphi_{5,6} = 90$ and 240 °, respectively, with an energy difference $\Delta H = 0.2 \, kcal/mol$ and for the *anti, Z* form at $\varphi_{5,6} = 140$ ° and 235 ° ($\Delta H = 0.1 \, kcal/mol$). These population differences may even be enhanced by electronic factors, *e. g.* only the minimum at $\varphi_{5,6} = 140$ ° would be sufficient for a conjugation of the $\Delta 4,5$ bond with the remaining π-system. Even disregarding the latter, these energy differences lead to preferential populations of one chiral conformer by a factor of 1.38 and 1.18, respectively, and similar population differences can be expected for other basic forms. In the helical topologies typical for bilindiones, all methine bridges are twisted in the same absolute sense, and hence the preference of one helicity should be in the same range. From the known $2R$, $3R$, $3^1 R$-configuration of phycocyanin (**I a**), allophycocyanin (**I b**), and P_r (**I c**), an M-helix is expect-

ed *. It should be noted, however, that in the phycoerythrin-chromophore the effect of the $16 R$ configuration [22, 23] would counteract the former effect. The cooperative twisting applies to the cyclic *all-syn, Z* forms of the chromophore only, but not to open forms. Here, the twist as the C_5 methine bridge does not necessarily propagate to the other two bridges. The CD-signals of the chromophores of native biliproteins containing extended chromophores is indicative, however, of a similar twist at all three methine bridges.

The stability of planar conformations of partial structure **V** decreases in the order *anti, Z* $\geq$ *syn, Z* > *syn, E* > *anti, E*. This difference in stability can be diminished however if the methine bridge is strongly twisted. The predominance of all-*syn, Z* conformations of bilindiones observed up to now is, therefore, due to the combined action of steric hindrance between neighborning rings and forces which drive the system towards coplanarity like π-interactions and H-bonds.

A noteworthy detail in **V** are the steric effects introduced into this partial structure by the alternating bonds in biliverdin. In the crystal, the distinction between single and double bonds decreases when going from the "outer" methine bridges (C_5 and C_{15}), to the inner one (C_{10}) [1]. Although this x-ray result is partly due to a disorder effect [2], the difference between rings B and C is still enough that rotation around C_9, C_{10} gives results different from the rotation around C_{10}, C_{11}. Thus, the *syn, E* form is sterically more hindered than the *anti-Z* form, its formal mirror image (Fig. 3). The latter is free of steric hindrance for all rotations, while no coplanar conformations are possible for the former.

If the atoms of the two pyrrole rings of **V** would have identical coordinates in Table I, both the *anti, Z* and the *syn, E* forms could be made identical by a rotation of 180 ° in the plane of the paper. It can, however, be taken from Table I, that a difference of $0.27 \, Å$ exists between the coordinates of $C_8 1$ and $C_{12} 1$. A further difference is between the angles $C_{10} C_9 N_{22}$ (124.7 °) and $C_{10} C_{11} N_{23}$ (122.3 °). These differences cause different distances of $C_8 1 \ldots N_{23}$ ($2.20 \, Å$) and $C_{12} 1 \ldots N_{22}$ ($2.64 \, Å$) and, therefore, different energy contents of the planar conformations of the *syn, E* and *anti, Z* forms, respectively.

* See note added in proof.

The bond lengths C_9-C_{10} (1.37 Å) and $C_{10}-C_{11}$ (1.39 Å) are nearly identical in biliverdin dimethylester, whereas $C_{15}-C_{16}$ (1.36 Å) has more double bond character than $C_{14}-C_{15}$ (1.48 Å). Provided a similar geometry at the C_{15} methine bridge of dihydrobilindiones, this increased asymmetry would destabilize the *E*-forms more strongly. The *anti, E* form proposed recently for the Δ 4-*E* isomers of bilindiones [6] indicates, however, that even this most hindered form is still accessible in a highly twisted conformation.

Steric interactions may play important roles during the $P_r \rightleftharpoons P_{fr}$ interconversions of phytochrome via intermediates [24] and for the conformational changes of chromophores associated with the denaturation and naturation of biliproteins [9, 25].

The chromophores of denatured (**I**) and probably as well of other biliproteins are predominantly in the cyclic-helical all-*syn, Z* form, while they have an extended configuration in the native state [19]. The driving forces for this conformational change are noncovalent interactions between the chromophores and the protein. The results presented allow an estimate of the energetics for rotations around the different methine bridges. Changes at the central methine bridge require only little energy. The steric interactions of the *Z*, as compared to the *E*-forms are small, but distinct, because the latter require a pronounced twist of the methine bridge (Fig. 3). Only the $N_{22}-$ H ... N_{23} hydrogen bond is broken when going from the *syn, Z* to any other form. At the C_5 methine bridge, steric interactions impose restrictions on all forms, but only the *E* form is hindered at the C_{15} methine bridge. The energy difference between the *Z* and *E* isomers is, therefore, higher in the latter case, and the ΔG ° $\approx$ 5 kcal/mol determined recently [26] for the *Z, E*-isomerization of bilindione can then be taken as an upper limit for transformations at *any* methine bridge. This energy would have to be provided by the protein, which may be a reason for the comparably low folding energy of phycocyanins ([27], for a discussion, see [25]).

With regard to the phytochrome interconversion, two transformations are currently known for bile pigments which qualify for model reactions. One is the photochemical attack of nucleophiles at C_5 [28], the other is a *Z, E*-isomerization similar to the one observed by Falk *et al.* [5, 6] in bilindiones. For the latter reaction, the calculations presented here allow an estimate on which of the methine bridges is ex-

pected to be involved in the isomerization, as far as steric hindrance is concerned. No stable *Z, E* isomers are expected for the B, C methine bridge, due to the low degree of double bond character and the absence of steric barriers. The defined double bonds at the outer bridges (Δ 4,5 and Δ 15,16, respectively), make stable isomers possible [5, 6]. The considerable energy difference ΔG ° $\approx$ 5 kcal/mol [26] for Δ 15,16 isomers is at least partly due to the pronounced twist imposed on the *E*-isomer by steric interactions. This energy difference is lowered at the A, B methine bridge, because the *Z*-forms are already strained in partial structure **IV**, which would at the same time decrease the activation barrier. This levelling effect may well be a reason for the well known increased reactivity of this position in 2,3-dihydrobilindiones (*cf.* [19]). The structural changes can further be restricted by the high rotational barriers between the three τ-minima. As only one τ-minimum is allowed for the *E*-configuration, this can be reached easily only from the corresponding *Z*-configuration. A cogwheel type rotation around C_3, C_31 and C_4, C_5 simultaneously, cannot decrease the barrier between the three τ-minima considerably, because the latter arise from interactions between C_5 or C_21 with the C_3 substituent. The strong twist expected for *E*-isomers would agree, too, with the short-wavelength shift of the P_{fr} chromophore in the denatured state [29].

In first order, the blue-shift of the long-wavelength band can be taken as a measure for the decrease in conjugation between *Z* and *E* isomers. For a fully unsaturated bilindione, $\Delta \tilde{v} = 1000$ cm^{-1} has been reported [5, 6]. Compared to the shift of 1580 cm^{-1} for denaturd P_r *vs.* denatured P_{fr} [29], this would indicate a rather strong distortion if the latter reaction were to correspond to a *Z, E* isomerization. Such a strong distortion for P_{fr} has also been concluded in a recent MO study [13].

The results are difficult to compare, however, because the data for phytochrome have been derived for the cationic forms, the ones for the *Z, E* isomers for the free bases. The chromophore of denatured P_{fr} is stable only as a cation, but reverts to the P_r chromophore above pH 5.25, the pK for the protonation of denatured P_r [29]. Unfortunately, data for the cations of *E*-isomers of bilindiones have not been published as yet, but they would be desirable for direct spectroscopic comparison between denatured P_{fr} and the *E*-isomer of bilindiones.

Note added in proof:

In biliverdin, a positive longwavelength Cotton effect has been calculated for the M-helical cyclic conformation [11]. This is in agreement with the CD-spectrum of denatured **pc** ($\Theta = +133\,000$/chromophore at 600 nm, H. Scheer and P. Bartholmes, unpublished).

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