enzyme could produce a tautomer that cannot be acted on by the next enzyme in the sequence. In this situation, the rate of anomerization may affect metabolic flux through the pathway.[35] For example, fructose-*1,6-P*$_2$, fructose-*6-P*, and ribose-*5-P* are involved in the photosynthetic carbon reduction cycle and are substrates for fructose-*1,6-P*$_2$ aldolase, fructose-*1,6-P*$_2$ phosphatase, transketolase, and ribose-5-P isomerase. Steady-state rates of photosynthesis in whole spinach leaves have been reported to be 20–60 nmol of CO_2 fixed·s^{-1}·mg^{-1} of chlorophyll at room temperature.[36] Ribose-*5-P* and fructose-*1,6-P*$_2$ are present in chloroplasts at levels of approximately 3.2 and 4 nmol·mg^{-1} of chlorophyll, respectively, under steady-state conditions.[37] From the measured values of the ring-opening rates, tautomeric proportions, and activation energies for tautomerization, one may use eq 8 to calculate rates of anomerization for these two compounds at pH 7.5 and 28 °C [$k_{\alpha\beta}$(ribose-*5-P*) ~20 s^{-1}; $k_{\alpha\beta}$(fructose-*1,6-P*$_2$) ~9.3 s^{-1}]. These rate constants correspond to in vivo rates of 23 and 4.8 nmol of β anomer produced·s^{-1}·mg^{-1} of chlorophyll, respectively, and are similar in magnitude to the observed metabolic rate. Therefore, under appropriate conditions, the anomerization reaction may affect the partitioning of substrates between different metabolic pathways. This conclusion is in accord with the analyses of possible effects of anomerization on the glycolytic and gluconeogenic pathways.[35] The presence of enzymes with anomerase activity could change the partitioning ratio, and if these anomerases were allosteric, they could represent important controls in the regulation of metabolic activity. Enzymes with anomerase activity have been characterized[38] and may be important in maintaining high metabolic fluxes, even for those pathways where rapidly anomerizing sugar phosphates are utilized.

Even without enzymes with anomerase activity, the phosphorylation of simple sugars provides a mechanism for increasing the rates of interconversion between different forms of the sugar. There are many cases where multiple forms of the sugar (or sugar phosphate) are present and the biologically active form is only a minor component (e.g., fructose-*1,6-P*$_2$, erythrose-*4-P*, glyceraldehyde-*3-P*). In these cases, rapid interconversion of the sugar species allows the particular enzymes to work at a faster rate than they would if they had to wait for a slow tautomerization to provide the catalytically active form of their substrate. For the pentoses, phosphorylation of the primary hydroxyl group has two functions: it restricts the resultant ring to an inherently less stable furanose configuration *and* catalyzes the rates of interconversion between the different forms of the sugar phosphates. For the tetroses, phosphorylation completely interferes with ring formation and immediately provides for an increased amount of the biologically active, free carbonyl form. For the trioses, the solution proportions of the various forms are not substantially altered upon phosphorylation, but rates of hydration and dehydration are substantially increased, thereby allowing for rapid production of the minor but biologically active free carbonyl form. In all these cases, sugar phosphorylation not only couples intermediary metabolism and oxidative phosphorylation (by way of ATP) but allows for immediately increased rates of metabolism.

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Registry No. α-D-Erythrofuranose, 72599-80-5; β-D-erythrofuranose, 72599-81-6; α-D-threofuranose, 80877-72-1; β-D-threofuranose, 80877-73-2; α-D-5-*o*-methylxylofuranose, 94707-49-0; β-D-5-*o*-methylxylofuranose, 94707-50-3; α-D-6-*o*-methylfructofuranose, 94707-51-4; β-D-6-*o*-methylfructofuranose, 94707-52-5; glucopyranose, 2280-44-6; galactopyranose, 10257-28-0; galactofuranose, 19217-07-3; α-D-ribofuranose 5-phosphate, 34980-65-9; β-D-ribofuranose 5-phosphate, 34980-66-0; α-D-arabinofuranose 5-phosphate, 69926-02-9; β-D-arabinofuranose 5-phosphate, 69881-35-2; α-D-xylofuranose 5-phosphate, 94798-99-9; β-D-xylofuranose 5-phosphate, 94799-00-5; α-D-lyxofuranose 5-phosphate, 94799-01-6; β-D-lyxofuranose 5-phosphate, 94799-02-7; α-D-fructofuranose 5-phosphate, 41452-28-2; β-D-fructofuranose 6-phosphate, 41452-29-3; α-D-6-*o*-methylfructofuranose 1-phosphate, 94707-53-6; β-D-6-*o*-methylfructofuranose 1-phosphate, 94707-54-7; α-D-fructofuranose 1,6-diphosphate, 34693-23-7; β-D-fructofuranose 1,6-diphosphate, 34693-15-7.

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Conformational Enantiomerism in Bilirubin. Selection by Cyclodextrins

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Abstract: Intramolecularly hydrogen-bonded, bichromophoric (4*Z*,15*Z*)-bilirubin IXα adopts either of two enantiomeric conformations that are in dynamic equilibrium in solution. Added α-, β-, or γ-cyclodextrin binds preferentially to one conformational enantiomer, and the complex exhibits a bisignate circular dichroism Cotton effect in the vicinity of the bilirubin long wavelength electronic transition. Analysis of these data, within the framework of exciton coupling models, indicates a preference for complexation of the left-handed (or negative) chirality enantiomer of bilirubin with cyclodextrin.

The constitutional structure of bichromophoric (4*Z*,15*Z*)-bilirubin IXα (**1**), the yellow-orange lipophilic and cytotoxic pigment of jaundice, was proved over 40 years ago;[2] however, its conformational structure has only recently been characterized,[3] and that

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largely by X-ray crystallography[4] and nuclear magnetic resonance spectroscopy.[5,6] One of the most interesting structural aspects of **1**, with important implications in biological function, is its ability and strong tendency to form intramolecular hydrogen bonds and thereby control its conformation and polarity.[1,7,8] The key structural elements that collectively govern the shape of **1** include the following: (a) *syn*-periplanar conformations of the two pyrromethenone chromophores, each with *Z*-configuration carbon–carbon double bonds at C_4 and C_{15}, (b) two propionic acid groups at C_8 and C_{12}, capable of forming *intra*molecular H bonds with the pyrromethenone lactam C=O/NH and pyrrole NH groups, and (c) an sp^3 carbon at C_{10} which keeps the two pyrromethenones (hence their long wavelength transition electric dipole moments[9]) ~109° apart. These features allow and even compel **1** to adopt either of the two enantiomeric conformations **1A** and **1B** (Figure 1), both of which are stabilized by six intramolecular hydrogen bonds.

The enantiomeric conformations have been found in crystalline bilirubin[4] and appear to be equally preferred in solutions of bilirubin in achiral organic solvents, where they interconvert rapidly at room temperature.[5,10] Remarkably, even when ionization of the propionic acid reduces the number of H bonds, but probably increases the strength of the remainder, conformers (**1C** and **1D**) like **1A** and **1B** persist in the crystal[11] and apparently in solution, too.[12] This marked preference for (enantiomeric) conformers in which polar groups are intramolecularly H bonded explains the lipophilic character of bilirubin, that property which prevents its ready excretion across the liver into bile.[8] It also explains why isomers of bilirubin with vinyl groups reduced to ethyl, e.g., mesobilirubins, or vinyl and methyl groups interchanged at C_2/C_3 or C_{17}/C_{18}, e.g., symmetrically substituted bilirubin IIIα and XIIIα, all exhibit similar solubility properties. However, isomers which have an E-configuration carbon–carbon double bond at C_4 or C_{15}, or isomers that do *not* have their propionic acid groups positioned at C_8 or C_{12}, e.g., mesobilirubin IVα (A, Figure 2), exhibit markedly different chemical and biological properties because they cannot fully achieve the intramolecular H bonding expressed in Figure 1.

The enantiomeric conformers (**1A** and **1B**) of Figure 1 are in dynamic equilibrium.[5,10] They interconvert by breaking and remaking all six H bonds, an enantiomeric equilibration process which has potential importance in biological reactions of bilirubin, e.g., enzymic glucuronidation, that probably involve stereoselective complexation.[7,8] Perturbation of the conformational equilibrium between the bilirubin enantiomers in favor of one enantiomer should lead to optical activity. This (displacement from a 1:1 conformational enantiomeric equilibrium) might be achieved in a chiral solvent or by crystallization from a chiral solvent or by selective interaction with a chiral solute. For example, aqueous solutions of bilirubin and albumin[13] or sodium deoxycholate[14] exhibit circular dichroism associated with the pigment chromophore, and induced circular dichroism has even been observed for bilirubin dimethyl ester in ethyl (*S*)-(−)-lactate and (*R*,*R*)-2,3-butanediol solutions.[15] Le Bas et al. first noted that aqueous alkaline solutions of bilirubin and cyclodextrin exhibited circular

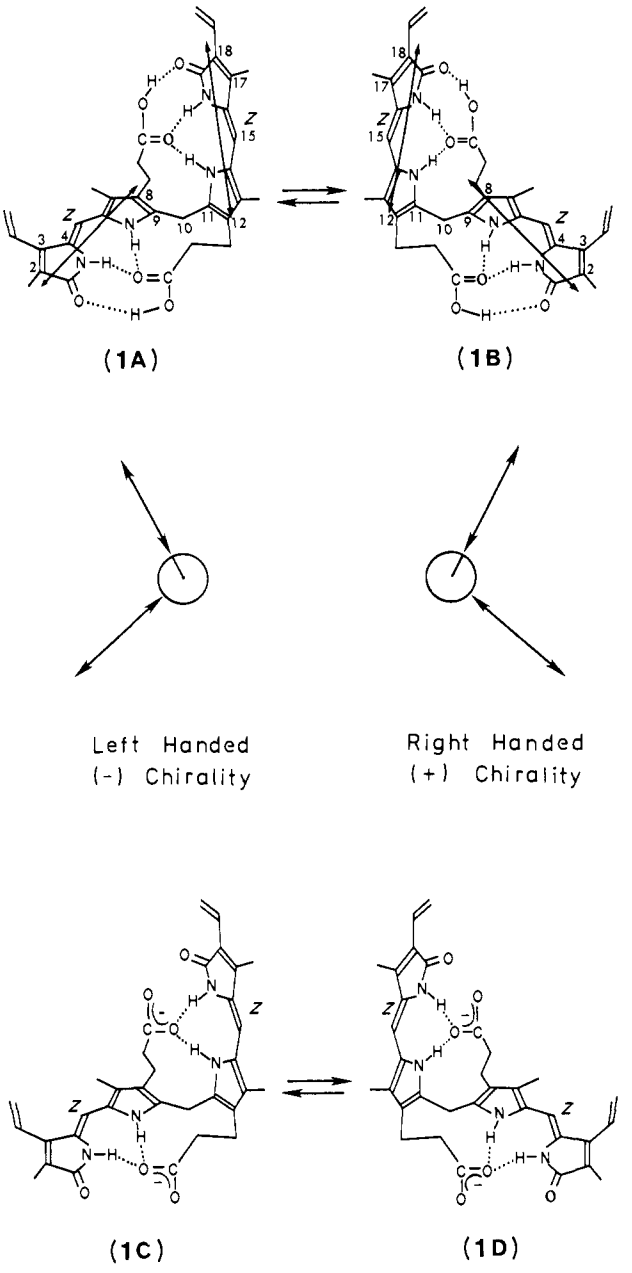

Figure 1. (Top) Interconverting enantiomeric, intramolecularly hydrogen-bonded conformers of bichromophoric (4*Z*,15*Z*)-bilirubin IXα. The double-headed arrows passing through each pyrromethenone chromophore represent the approximate computed direction of polarization of the dipole velocity transition moment for the long wavelength electronic transition (ref 9). (Middle) Pictorial representations for the electric dipole transition moments (as viewed at C_{10}) of the pyrromethenone chromophores of **1A**/**C** and **1B**/**C**. Conformers **1A** and **1C** exhibit a left-handed or negative chirality; conformers **1B** and **1D** exhibit a right-handed or positive chirality (ref 28). (Bottom) Interconverting enantiomeric, intramolecularly hydrogen-bonded conformers of bichromophoric (4*Z*,15*Z*)-bilirubin IXα bispropionate anion (ref 6 and 12).

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Table I. Circular Dichroism (CD) and Visible–Ultraviolet (UV) Spectral Data for 3–3.5 × 10^{-5} M Solutions of Bilirubin IXα (**1**) with 10^{-2} M α-Cyclodextrin[a]

solution 0.1 M Tris buffer	CD					UV[b] ε (λ^{max})
	Δε (λ$_1^{max}$)	λ$_2$ at Δε = 0	Δε (λ$_3^{max}$)	λ$_4$ at Δε = 0	Δε (λ$_5^{max}$)	
pH 10.0[c]			−3.0 (459)	429	+2.1 (408)	51 800 (436)
pH 9.0			−2.8 (459)	430	+2.2 (409)	49 800 (437)[d]
pH 8.0			−3.0 (460)	430	+2.4 (409)	50 000 (437)[e]
pH 7.0			−4.0 (498)	465	+3.0 (427)	34 300 (445)
(after 37 min)			−3.7 (502)	465	+1.5 (428)	30 400 (451) + sh (480)
H_2O/Me_2SO (1:1)	no Cotton	effects	detected			32 200 (450)
Me_2SO			+0.5 (464)			61 300 (455)[f]
Me_2SO–1% 0.02 N NaOH			+1.1 (465)	440	−0.6 (418)	66 800 (460)

[a] CD and UV measurements were determined at 25 °C within 3–5 min following preparation of the solutions. With 10^{-3} M cyclodextrin, no Cotton effects could be detected. [b] In the absence of cyclodextrin, the data are pH 10.0 (ε$_{436}^{max}$ 50 600), pH 9.0 (ε$_{437}^{max}$ 50 100), pH 8.0 (ε$_{436}^{max}$ 48 400), pH 7.0 (ε$_{438}^{max}$ 37 600) after 37 min (ε$_{438}^{max}$ 39 400 + sh 480–490 nm). [c] No significant changes in the CD spectrum after 35 min; UV, ε$_{436}^{max}$ 51 600. [d] After 30 min, ε$_{437}^{max}$ 48 000. [e] After 25 min, ε$_{437}^{max}$ 49 200. [f] After 29 min, ε$_{455}^{max}$ 61 100.

Table II. Circular Dichroism (CD) and Visible–Ultraviolet (UV) Spectral Data for 3–3.5 × 10^{-5} M Solutions of Bilirubin IXα (**1**) with 10^{-2} M β-Cyclodextrin[a]

solution 0.1 M Tris buffer	CD					UV[b] ε (λ^{max})
	Δε (λ$_1^{max}$)	λ$_2$ at Δε = 0	Δε (λ$_3^{max}$)	λ$_4$ at Δε = 0	Δε (λ$_5^{max}$)	
pH 10.0[c]	+0.4 (523)	499	−5.3 (458)	427	+3.3 (406)	50 400 (436)
pH 10.0 (10^{-3} M C7A)			−0.7 (455)	427	+0.4 (405)	49 800 (436)
pH 9.0	+0.35 (520)	500	−6.2 (458)	426	+3.3 (404)	49 900 (436)[d]
pH 8.0	+0.3 (520)	499	−6.5 (458)	426	+3.4 (405)	48 900 (436)[e]
pH 7.0			−1.6 (460)	430	+0.9 (410)	40 500 (438)
after 30 min			−0.9 (465)	435	+0.8 (410)	35 500 (442) + sh (480)
			−0.8 (500)			
after 110 min			sh (~470)	452	+1.0 (412)	29 200 (445) + sh (490)
			−1.2 (504)			
pH 6.0	no Cotton	effects	detected			37 700 (441) + sh (480)
H_2O/Me_2SO (1:1)	no Cotton	effects	detected			30 900 (450) + sh (480)
Me_2SO			≤+0.5 (~460)			63 000 (455)[f]
Me_2SO–1% 0.02 N NaOH			≤+0.5 (~460)			68 000 (460)

[a] CD and UV measurements were determined at 25 °C within 3–5 min following preparation of the solutions. [b] See footnote *b*, Table I. [c] No significant changes in the CD spectrum after 73 min; UV, ε$_{436}^{max}$ 49 700. [d] After 28 min, ε$_{436}^{max}$ 49 100. [e] After 32 min, ε$_{436}^{max}$ 47 400. [f] After 31 min, ε$_{455}^{max}$ 62 500.

dichroism.[16] In the present work, we report on our circular dichroism (CD) investigation of the selective complexation of bilirubin conformational enantiomers by (chiral) cyclodextrins.[17] On the basis of strong bisignate CD Cotton effects and exciton coupling theory, we demonstrate that the left-handed enantiomeric conformation (Figure 1) is selected preferentially. In contrast, bilirubin isomers which are incapable of intramolecular H bonding exhibit only vanishingly weak monosignate CD Cotton effects.

Experimental Methods

Bilirubin IXα was obtained from Sigma and purified by crystallization as previously described.[18] Xanthobilirubic acid and mesobilirubin XIIIα and IVα (Figure 2) were prepared by total synthesis.[6,19] Vinylneoxanthobilirubic acid (Figure 2) was prepared following reaction of bilirubin IXα dimethyl ester with molten resorcinol.[20] Buffer solutions were 0.1 M Tris (Trizma, from Sigma) (prepared as described previously),[21] and the α-, β-, and γ-cyclodextrins (purified) were all from Sigma. All circular dichroism (CD) measurements were carried out on a JASCO J-40A instrument equipped with a photoelastic modulator, and all UV–vis measurements were performed on a Cary 219 spectrophotometer.

Results and Discussion

Cyclodextrins, which are torus-shaped oligosaccharides, have an ability to slip organic molecules into their cavities by use of hydrophobic or generalized lyophobic forces.[17] The common cyclodextrins α-cyclodextrin (C6A, cyclohexaamylose), β-cyclodextrin (C7A, cycloheptaamylose), and γ-cyclodextrin (C8A, cyclooctaamylose) have toroid cavities with diameters of ~5.7, ~7.8, and ~9.5 Å, respectively, and can accommodate molecules the size of, e.g., cyclohexane (C6A, C7A, C8A), naphthalene (C7A, C8A), and anthracene (C8A).[17] However, guest molecules do not necessarily need to penetrate into the host's cavity in order to form association complexes, and the host–guest ratios, while typically 1:1, can be 2:1 (C7A with prostaglandins) or 1:2 (C8A with naphthalene).[17,22] All of the complexes are chiral, and the guest chromophore can be expected to exhibit an induced circular dichroism (ICD). In most of the reported examples of cyclodextrin ICD, the guest molecules have been achiral molecules, typically with rigid aromatic-type chromophores.[17,22] Surprisingly, rarely have cyclodextrins been used to resolve racemic mixtures, e.g., 2-methylcyclohexanol[23] and sulfoxides,[24] or to select dissymmetric conformations of achiral molecules, e.g., benzophenone[25] and benzil.[16]

Bilirubin, as a prospective guest, presents a somewhat different picture (Figure 1), one where conformational enantiomers **1A/C** and **1B/D** are in dynamic equilibrium.[5,10] If cyclodextrin associates[26] to differing extents with **1A/C** and **1B/D**, then the re-

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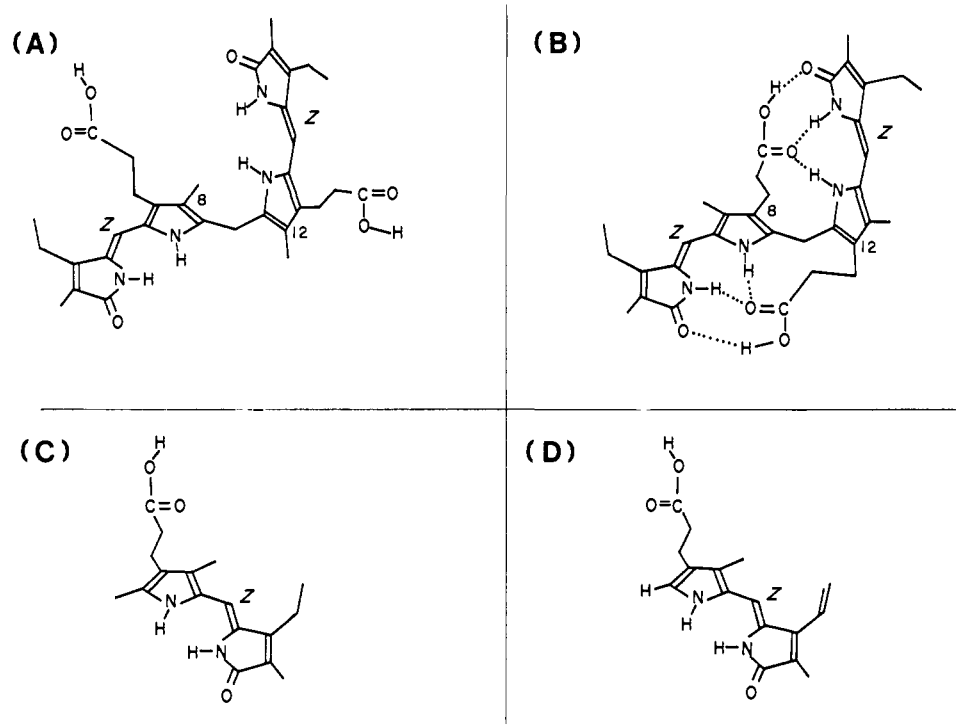

Figure 2. Structures of (A) (4*Z*,15*Z*)-mesobilirubin IVα, which has its propionic acid groups located at sites rendering sterically impossible their intramolecular H bonding to the pyrromethenone units, (B) (4*Z*,15*Z*)-mesobilirubin XIIIα, a structural isomer of the structure in part A, capable of the intramolecular H bonding shown in Figure 1, (C) (Z)-xanthobilirubic acid, the parent chromophore of the structure in part B, and (D) vinylneoxanthobilirubic acid, a parent chromophore in **1**.

Table III. Circular Dichroism (CD) and Visible–Ultraviolet (UV) Spectral Data for 3–3.5 × 10^{-5} M Solutions of Bilirubin IXα (**1**) with 10^{-2} M γ-Cyclodextrin[a]

solution 0.1 M Tris buffer	CD					UV[b] ϵ (λ^{max})
	$\Delta\epsilon$ (λ_1^{max})	λ_2 at $\Delta\epsilon = 0$	$\Delta\epsilon$ (λ_3^{max})	λ_4 at $\Delta\epsilon = 0$	$\Delta\epsilon$ (λ_5^{max})	
pH 10.0	+0.8 (523)	500	−8.6 (458)	424	+4.4 (405)	51 000 (437)[c]
pH 9.0	+0.7 (522)	501	−8.4 (457)	424	+4.4 (405)	50 400 (437)[d]
pH 8.0	+1.1 (521)	500	−9.4 (457)	425	+4.7 (403)	47 600 (437)[e]
pH 7.0			−2.4 (463)	433	+1.7 (411)	38 100 (440)
after 31 min			sh (~475)			
			−2.8 (502)	452	+1.5 (416)	32 100 (455) + sh (~475)
after 161 min			−4.2 (503)	461	+2.2 (419)	27 000 (450) + sh (480)
Me_2SO			+0.6 (460)			62 500 (455)
Me_2SO–1% 0.02 N NaOH			+1.5 (466)	430	−0.4 (410)	69 000 (460)

[a] CD and UV measurements were determined at 25 °C within 3–5 min following preparation of the solutions. With 10^{-3} M cyclodextrin, no Cotton effects could be detected. [b] See footnote *b*, Table I. [c] After 18 min, ϵ_{437}^{max} 50 800. [d] After 20 min, ϵ_{437}^{max} 50 000. [e] After 24 min, ϵ_{437}^{max} 47 000; after 106 min, ϵ_{436}^{max} 45 600.

sulting bilirubin–cyclodextrin complex should exhibit an ICD spectrum qualitatively characteristic of one enantiomer. Noncomplexed bilirubin (**1**) will remain net racemic and not contribute to the CD. We found that buffered (pH 7–10) aqueous solutions of **1** expectedly show no CD Cotton effects (CEs); however, when α-, β-, or γ-cyclodextrin is added to the solution (Tables I–III),[26] strong bisignate CEs [(-) CE near 460 nm, (+) CE near 405 nm] are readily seen (Figure 3), whose maxima flank but do not coincide with the UV–vis λ_{max} (~436 nm). These observations provide evidence for complexation of **1** with cyclodextrins and are reminiscent of the ICDs found for bilirubin complexed with albumins.[13] However, the complexation expressed here is not merely a matter of binding an achiral bilirubin to chiral cyclodextrin, thus leading to chiral induction in the chromophore, as in the ICD observed with, e.g., a naphthalene–C7A complex.[22] Nor is it simply the function of the host cyclodextrin to force the guest chromophore to assume a chiral conformation, as in the benzophenone–C7A complex[25] and the biliverdin–C7A complex.[16] For these examples all exhibit monosignate ICD CEs, in marked contrast to the bisignate ICD CEs observed with bilirubin–cyclodextrin solutions. The bisignate CEs of the latter implicate two proximal (electronically) interacting chromophores held in a chiral conformation. Such conformations might be adopted even in the absence of intramolecular H bonding, as in mesobilirubin IVα (Figure 2), which is incapable of achieving the enantiomeric intramolecularly H-bonded conformations expressed in Figure 1. However, in contrast to bilirubin IXα, mesobilirubin IVα shows only an extremely weak monosignate ICD CE ($\Delta\epsilon < 0.1$) in the presence of C7A. Moreover, even the parent pyrromethenones (Figure 2), e.g., xanthobilirubic and vinylneoxanthobilirubic acids (and even their more lipophilic decarboxylated analogues, e.g., kryptopyrromethenone[6]), show only a vanishingly small or no ICD with cyclodextrins (Table IV).[27] These potential guests either do not complex with cyclodextrins or, if they do associate, the complexes exhibit very weak ICDs. The cyclodextrins apparently do not induce a single chiral conformation in the pyrromethenone chromophore, nor do they complex in (dimeric) association, as does acridine orange with C8A.[22] These results are very significant because they demonstrate that complex formation can only be seen between unique chiral conformations of **1** with cyclodextrin.[28]

(27) Complexation of pyrromethenones with albumins, on the other hand, leads to significant ICD, see ref 20.

(28) An ICD has been reported for a pH 10.2 aqueous solution of **1** in the presence of β-cyclodextrin and ascribed to a "well-defined minimum energy chiral conformer" of the pigment (ref 16).

Table IV. Circular Dichroism (CD) and Visible–Ultraviolet (UV) Spectral Data for 3–3.5 × 10^{-5} M Solutions of Mesobilirubins and Xanthobilirubic Acid with 10^{-2} M β-Cyclodextrin in pH 8.0 Tris Buffer (0.1 M)[a]

	CD					
compound	$\Delta\epsilon$ (λ_1^{max})	λ_2 at $\Delta\epsilon = 0$	$\Delta\epsilon$ (λ_3^{max})	λ_4 at $\Delta\epsilon = 0$	$\Delta\epsilon$ (λ_5^{max})	UV[b] ϵ (λ^{max})
mesobilirubin XIIIα	very weak or no Cotton effects observed		−9.8 (435)	408	+6.6 (386)	49800 (416)[c]
mesobilirubin IVα					<+0.1 (~390)	34000 (390) + sh (420)
xanthobilirubic acid					≤+0.01 (~410)	32200 (410)[d]
xanthobilirubic acid[e]					≤+0.01 (~410)	30400 (410)[d]

[a] CD and UV measurements were determined at 25 °C within 3–5 min following preparation of the solutions. [b] See footnote *b*, Table I. [c] After 47 min, ϵ_{415}^{max} 45600. [d] No change after 15–20 min. [e] γ-Cyclodextrin in place of β-cyclodextrin.

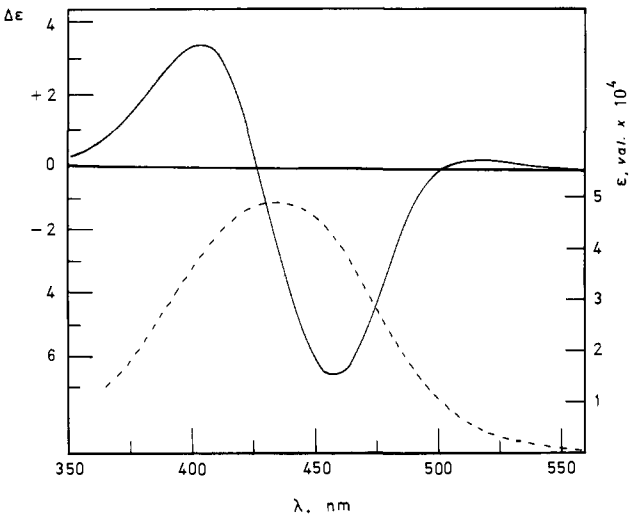

Figure 3. Circular dichroism (—) and UV–vis (- - -) spectra of 3.42 × 10^{-5} M **1** in pH 8.0 Tris buffer in the presence of 10^{-2} M α-cyclodextrin, all at 20 °C. The spectra were recorded 4–14 min after preparation of the solution and remain essentially invarient for hours at 20 °C. A CD spectrum of the same concentration of **1** without added cyclodextrin falls on the $\Delta\epsilon = 0$ line.

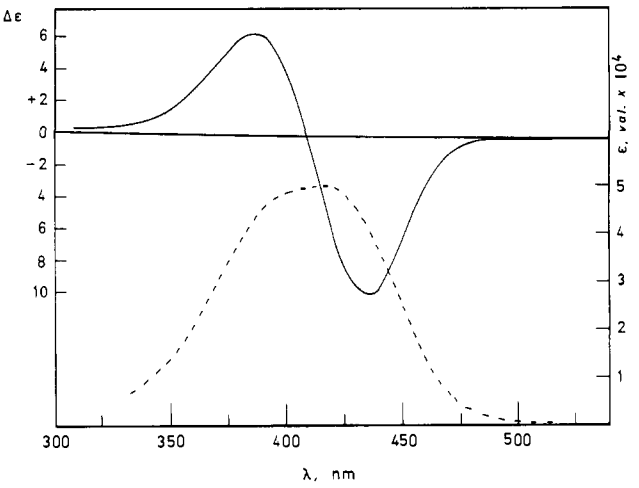

Figure 4. Circular dichroism (—) and UV–vis (- - -) spectra of 3.4 × 10^{-5} M mesobilirubin XIIIα (structure B, Figure 2) in pH 8.0 Tris buffer in the presence of 10^{-2} M β-cyclodextrin, all at 23 °C. The spectra were recorded 10–14 min after preparation of the solution and remained essentially invarient for hours at 23 °C. A CD spectrum of mesobilirubin XIIIα without added cyclodextrin falls on the $\Delta\epsilon = 0$ line as do the CD spectra of mesobilirubin IVα (structure A, Figure 2), xanthobilirubic acid (structure C, Figure 2), and vinylneoxanthobilirubic acid (structure D, Figure 2) in the presence of 10^{-2} M β-cyclodextrin in pH 8.0 Tris buffer.

And they point to the ability of cyclodextrin to associate preferentially with **1A/C** or **1B/D** (Figure 1).

The conclusions reached here do not depend on the fact that bilirubin IXα has a nonsymmetric ordering of methyl and vinyl groups, for the symmetric IIIα and XIIIα isomers show a qualitatively similar behavior with cyclodextrins.[29] Rather, they depend crucially on the ability of the bilirubin to hydrogen bond intramolecularly (to give enantiomeric conformations), as is amply demonstrated by the strong bisignate ICD of symmetric mesobilirubin XIIIα (Figures 2 and 4) and the failure of both its position isomer, mesobilirubin IVα, and its pyrromethenone chromophoric unit, xanthobilirubic acid, to exhibit anything more than weak or vanishingly small monosignate ICD CEs under the experimental conditions.

The CD data, showing bisignate CEs on either side of the UV–vis λ_{max}, are characteristic of chromophore–chromophore interaction in the excited state (exciton coupling).[30] Here, the two pyrromethenone chromophores of bichromophoric bilirubin interact (couple) to give two electronic transitions, one higher in energy and one lower in energy. These two transitions are not widely separated[31] and overlap to give the somewhat broadened characteristic absorption bands of bilirubin IXα (Figure 3) and mesobilirubin XIIIα (Figure 4) seen in their UV–vis spectra. In the CD spectra, however, when two close-lying transitions have oppositely signed CEs, the CEs will overlap with considerable cancellation to produce oppositely signed, widely separated maxima flanking the overlapped electronic transitions.[32] This, in fact, is just what one sees with the characteristic bisignate ICD of bilirubins complexed with cyclodextrins (Figures 3 and 4) and proteins.[13]

Exciton coupling theory also allows for assignment of absolute configuration of the chiral bilirubins. The handedness or the skew sense of the electronic transition moments of the coupled pyrromethenone chromophores (Figure 1) correlates with the signed order of the bisignate CD CEs.[30] Thus, a left-handed screw sense (negative chirality) of the transition moments leads to a (−) longer wavelength CE followed by a (+) shorter wavelength CE, and for a right-handed screw sense (positive chirality) the CE signs are inverted in the same order.[33] The direction of the electric dipole transition moment in the pyrromethenone chromophore has been determined from theoretical studies[9] to lie along the longitudinal axis of the planar conjugated π system. Thus, in accordance with the exciton model[30] or the C_2 rule,[34] in the intramolecularly H-bonded conformations of bilirubin (Figure 1), the relative orientations of the two pyrromethenone electric dipole moments (Figure 1) consitute a left-handed chirality for **1A/C** and a right-handed chirality for **1B/D** (Figure 1). The observed bilirubin–cyclodextrin ICD data [(−) CE near 460 nm, (+) CE near 405 nm; UV λ_{max} ~436 nm, Figure 3 and Tables I–III] and mesobilirubin XIIIα–cyclodextrin ICD data [(−) CE near 435

(29) Gawroński, J. K.; Ma, J.-S.; Lightner, D. A., unpublished results.
(30) For leading references and examples, see: Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry"; University Science Books: Mill Valley, CA, 1983.
(31) The separation of the coupled electronic transitions will depend on the nature and relative orientation of the chromophores (and their electric dipole transition moments), ref 30 and 34.
(32) This phenomenon has been discussed in detail previously: Wellman, K. M.; Lauer, P. H. A.; Briggs, W. S.; Moscowitz, A.; Djerassi, C. *J. Am. Chem. Soc.* **1965**, *87*, 66–72.
(33) If the transition moments lie nearly in the same plane with the vector connecting them, as in ~0° and ~180s skew angles between the transition moments (Figure 1), then the simple form[30] of the exciton chirality rule cannot be applied with certainty, and one must determine exactly the relative orientations of the transition moments to the connecting vector. See: Hansen, Aa. E.; Bouman, T. D. *Adv. Chem. Phys.* **1980**, *44*, 545–644.
(34) Hug, W.; Wagnière, G. *Tetrahedron* **1972**, *28*, 1241–1248.

nm, (+) CE near 385 nm; UV $\lambda_{max} \sim 415$ nm] of Figure 4 and Table IV are in accord with selective complexation of cyclodextrin with the left-handed chiral conformation, viz., **1A/C** (Figure 1).

Similar conclusions favoring predominance of the left-handed chiral conformation (Figure 1) are in accord with the following: (1) the previously reported ICD bisignate CE [$\Delta\epsilon_{466} \sim -4.7$, $\Delta\epsilon_{416} \sim +3.0$] of 3.4×10^{-5} *M* bilirubin IXα in 7.24×10^{-2} M sodium deoxycholate at pH 8.0,[14] and (2) the CD data of Harmatz and Blauer[35] for selected albumin-bilirubin complexes, e.g., the powerful bisignate CE [$\Delta\epsilon_{467} \sim -80$, $\Delta\epsilon_{415} \sim +21$] for 2.5×10^{-5} M bilirubin IXα in 5.0×10^{-5} M goat albumin at pH 9.8. However, a predominance of a right-handed chiral conformation appears to be indicated [bisignate ICD CE: $\Delta\epsilon_{460} \sim +53$, $\Delta\epsilon_{410} \sim -33$] for bilirubin IX$\alpha$ (2.5×10^{-5} M) bound to human serum albumin (5.0×10^{-5} M) at pH 9.8. The order of magnitude larger ICDs associated with the protein-bound bilirubin suggest a relatively larger enantiomeric excess of pigment in the aqueous albumin solutions than in the cyclodextrin or sodium deoxycholate solutions. The stereochemical facets of the interaction of bilirubins and pyrromethenones with protein (chiral solute), sodium deoxycholate (chiral micelle), and similar systems are currently under further investigation in our laboratory.

The major, bisignate CEs observed for chiral conformations of bilirubin and discussed above are not the only CEs observed in bilirubin-cyclodextrin solutions. Other weak CEs can be seen near 520 nm for solutions of **1** with C7A and C8A, but not C6A (Tables I-III). These data suggest weak intermolecular electric dipole coupling with other bilirubin pyrromethenone chromophores—possibly the presence of dimers or higher aggregates[3,26] complexed with the larger cyclodextrins. Compared with the aqueous solution data, however, complexation of bilirubin to cyclodextrins in Me_2SO solvent gives relatively weak monosignate ICDs with CE λ_{max} nearly coincident with the UV-vis λ_{max}. It thus appears that in Me_2SO well-defined minimum-energy conformers of **1** are not available for complexation with cyclodextrin or complexation is more limited or less discerning than in aqueous buffers.[36] Curiously, when the Me_2SO solutions are slightly basified, weak ICDs that have signs opposite to those in aqueous buffers can be detected.

Conclusion

Our CD studies show that enantiomeric intramolecularly hydrogen-bonded conformations of bilirubin can be complexed selectively with cyclodextrins, with the left-handed chirality enantiomer (**1A/C**) complexed preferentially. These results find close parallels with and can explain the previously observed ICD of bilirubin in sodium deoxycholate solution.[14] Similar parallels may be drawn to the complexation of bilirubin to albumin,[13] where bisignate ICDs are also found, and where selective association of the protein with enantiomeric conformations of bilirubin is probably also important.

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Registry No. 1, 635-65-4; mesobilirubin IVα, 94732-74-8; xanthobilirubic acid, 15770-19-1; mesobilirubin XIIIα, 79719-28-1; α-cyclodextrin, 10016-20-3; β-cyclodextrin, 7585-39-9; γ-cyclodextrin, 17465-86-0.

(35) Harmatz, D.; Blauer, G. *Arch. Biochem. Biophys.* **1975**, *170*, 375-383.

(36) Me_2SO is expected to interfere with intramolecular H bonding and probably destabilizes conformations like **1A** and **1B**, see ref 5 and 6. Bilirubin is much more soluble in ME_2SO than in water.

Structure of Hexamethylene Triperoxide Diamine

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Abstract: The structure of 1,6-diaza-3,4,8,9,12,13-hexaoxabicyclo[4.4.4]tetradecane (hexamethylene triperoxide diamine or HMTD) has been determined. The compound has a most unusual, exactly planar 3-fold coordination about the two bridgehead nitrogen atoms, with N-C distances of 1.421 (8) Å. It crystallizes in the trigonal space group *R*3*m*: $a = 10.417$ (5) Å, $c = 6.975$ (3) Å, $z = 3$. The structure was refined by least squares to an *R* index of 0.034.

1,6-Diaza-3,4,8,9,12,13-hexaoxabicyclo[4.4.4]tetradecane (hexamethylene triperoxide diamine or HMTD) was first synthesized in 1885 by Legler.[1] It was soon found to be a powerful initiating explosive and as such was studied by Taylor and Rinkenbach of the Bureau of Mines.[2] Although it is inexpensive, easy to synthesize, relatively insensitive to shock (it requires a 3-cm drop of a 2-kg weight to detonate, as compared to 0.25 cm for mercury fulminate), and more powerful than most initiating explosives, HMTD slowly decomposes when stored and so is not now of commercial or military importance.

We were first attracted to HMTD by a stick model of the molecule that suggested there might be a central cavity toward which some lone pair electrons from the oxygen atoms were pointing (Figure 1). The possibility of capturing protons in the cavity, as is known for 1,6-diazabicyclo[4.4.4]tetradecane,[3] immediately came to mind. We began a crystallographic study to determine the size and configuration of the cavity; our results are reported in this paper.

Table I. Crystal Data for Hexamethylene Triperoxide Diamine

$N(CH_2O_2CH_2)_3N$ ($C_6H_{12}N_2O_6$)	
M_r 208.17	
space group *R*3*m* No. 160	
$a = 10.417$ (5) Å	rhombohedral axes
$c = 6.975$ (3) Å	$a = 6.448$ (3) Å
$V = 655.5$ (12) Å^3	$\alpha = 107.76$ (3)°
$F(000) = 330e$	$V = 218.5$ (4) Å^3
$Z = 3$	$Z = 1$
ρ(calcd) = 1.58 g cm^{-3}	ρ(obsd) = 1.57 g cm^{-3} (ref 2)
Mo Kα, 0.71073 Å	μ(Mo Kα), 1.53 cm^{-1}
$T = 21$ °C	($\mu r_{max} = 0.03$)

Expermental Section

Caution: HMTD is a powerful explosive. The synthesis of HMTD itself is quite facile.[4] Fourteen grams of hexamethylenetetramine is

(1) Legler, L. *Ber.* **1885**, *18*, 3343-3351.
(2) Taylor, C. A.; Rinkenbach, W. H. *Army Ordnance* **1924**, *5*, 463-466.
(3) Alder, R. W., Orpen, A. G.; Sessions, R. B. *J. Chem. Soc., Chem. Commun.* **1983**, 999-1000.

(4) Davis, T. L. "The Chemistry of Powders and Explosives"; Angriff Press: Hollywood, CA, 1943.

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