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CIRCULAR DICHROISM OF BILIRUBIN-AMINE HETEROASSOCIATION COMPLEXES

DAVID A. LIGHTNER* AND JING-YI AN

Department of Chemistry, University of Nevada

Reno, Nevada 89557-0020

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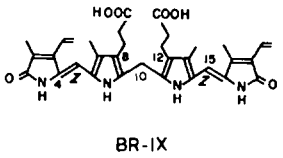
Abstract - Bichromophoric (4Z,15Z)-bilirubin-IXα, the yellow-orange cytotoxic pigment of jaundice, adopts either of two intramolecularly hydrogen-bonded enantiomeric conformations that are in dynamic equilibrium in solution. The addition of S-(+)-2-aminobutane induces the pigment solutions to exhibit intense bisignate circular dichroism [$\Delta\varepsilon^{423}_{max}$ = +19.5, $\Delta\varepsilon^{479}_{max}$ = -26.0 (benzene)] in the region of the bilirubin long wavelength UV-visible absorption band [$\Delta\varepsilon^{469}_{max}$ = 60,900 (benzene)]. As seen with serum albumin and other proteins, the optically active base acts as a chiral complexation agent to induce an asymmetric transformation of bilirubin, whose induced bisignate circular dichroism Cotton effect is characteristic of exciton splitting of the component pyrromethenone chromophores.

INTRODUCTION

(4Z,15Z)-Bilirubin-IXα (BR-IX, Fig. 1), the yellow-orange hydrophobic and cytotoxic pigment of jaundice, is a dicarboxylic acid produced abundantly in mammals by catabolism of heme.[1] The pigment forms a tightly-bound association complex with serum albumin,[2-4] which transports it to the liver for glucuronidation and excretion,[5] Although the constitutional structure of BR-IX was established long ago,[6] its conformational structure has been revealed only recently through X-ray crystallographic[7] and NMR[8] techniques. Perhaps the most important aspect of the 3-dimensional structure of BR-IX is its ability and marked tendency to form extensive intramolecular hydrogen bonds, which link the polar carboxylic acid and lactam functionalities. This governs the shape of the molecule by folding it into either of two enantiomeric conformations (A and B, Fig. 1) that interconvert by breaking and remaking all 6 H-bonds with an activation barrier of 17-18 kcal/mole.[8b,9] It also governs the polarity of the molecule and thus has very important implications for biological function.[5] As a consequence of intramolecular H-bonding, BR-IX is considerably less polar and more hydrophobic than other bile pigments which do not have propionic acid groups emanating from carbons 8 and 12,[1,5] e.g. the β, γ and δ isomers of BR-IX.[10]



BR-IX

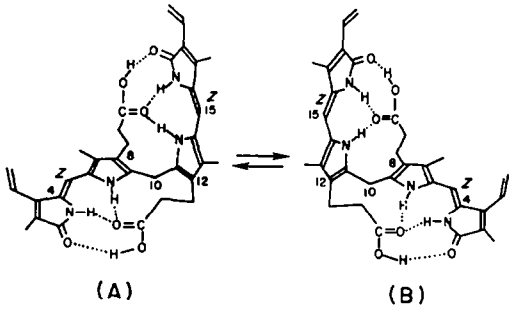


FIGURE 1. (Above) Linear representation of (4Z,15Z)-bilirubin-IXα (BR-IX). (Right) Interconverting enantiomeric, intramolecularly hydrogen-bonded conformers of BR-IX.

Bilirubin owes its well-defined chiral tertiary structure (Fig. 1) in the crystal and non-polar solvents to (i) syn-periplanar conformations of the two pyrromethenone chromophores, each possessing Z-configuration carbon-carbon double bonds at C_4 and C_{15}, (ii) two propionic acid groups at C_8 and C_{12} each capable of forming intramolecular hydrogen bonds with the opposing pyrromethenone lactam C-O/NH and pyrrole NH groups, and (iii) an sp^3 carbon at C_{10} which keeps the two pyrromethenones 98-104° apart.[1,11] Not surprisingly, rubins which differ only in their lactam ring substituents, e.g. mesobilirubin-XIIIα (MBR-XIII, Fig. 2) express the same conformational tendencies. Even when BR-IX is deprotonated at the propionic acid groups, as in salts with amines[12] or tetra-alkylammonium hydroxides,[13] the pigment retains a marked preference for the folded intramolecularly H-bonded structures of the acid form. And in solvents like dimethylsulfoxide, the pigment still retains the folded structure with the propionic acid CO_2H residues tied to their nearest pyrrole NH and lactam groups via bound solvent molecules.[8c] Its dimethyl ester (BR-IX DME, Fig. 2) also exhibits a preference for the folded conformation,[11] albeit without the benefit of intramolecular H-bonding stabilization.[11] It tends instead to hydrogen bond intermolecularly in non-polar solvents via a pyrromethenone-to-pyrromethenone dimeric arrangement akin to that observed with monochromophore pigments such as xanthobilirubic acid (XBR, Fig. 2).[14,15] In keeping with these observations, when the propionic acid groups are relocated away from C-8 and C-12, e.g. in mesobilirubin-IVα (MBR-IV, Fig. 2), the pigment is incapable of expressing intramolecular hydrogen bonding of the type shown in Fig. 1. MBR-IV is therefore considerably more polar than BR-IX and exhibits markedly different conformational,[16] solubility[17] and excretability properties.

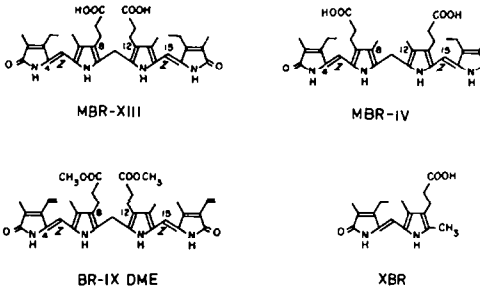


FIGURE 2. Linear representations of mesobilirubin-XIIIα (MBR-XIII) mesobilirubin-IVα (MBR-IV), bilirubin-IXα dimethylester (BR-IX DME) and xanthobilirubic acid (XBR).

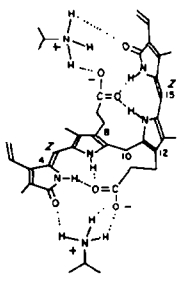


FIGURE 3. Conformational structure of the bis-isopropylammonium salt of BR-IX, as determined by X-ray.[12]

The tendency of BR-IX and MBR-XIII to adopt the folded, intramolecularly H-bonded conformation appears to persist, surprisingly perhaps, even when the carboxyl groups are deprotonated,[13] or the carboxyl hydrogens become coordinated in intermolecular H-bonding, e.g. the bis-isopropylammonium salt of BR-IX. In the latter structure, determined by X-ray crystallography,[12] the amine nitrogen participates in H-bonding to the carboxyl hydrogen, and an amine hydrogen H-bonds to the lactam oxygen of the pigment (Fig. 3) to form a stable salt linkage. This seminal observation, coupled with the fact that the salt is chiral--with two conformational enantiomers, suggests how BR-IX might bind to proteins and enzymes. It also suggests an experiment in which replacing the isopropylamine unit in the enantiomeric salt complex by an optically active amine would create diastereomeric salt complexes, with the potential for conventional separation or spectroscopic investigation by chir-optical methods. In the present work, we show that BR-IX and MBR-XIII exhibit intense optical activity in the presence of S-(+)-2-aminobutane and other optically active amines; whereas, MBR-IV, BR-IX DME and XBR exhibit weak or no optical activity. These studies provide insight into possible mechanisms for the origin of the intense optical activity of BR-IX bound non-covalently to human serum albumin (HSA)[18,19] and other proteins.[19,20]

EXPERIMENTAL

Bilirubin-IXα (BR-IX) contained less than 5% of the IIIα and XIIIα isomers, as obtained from Sigma and determined by high performance liquid chromatography.[21] Bilirubin-IXα dimethyl ester (BR-IX DME) was isolated following reaction of BR-IX with diazomethane, as described previously.[14] Mesobilirubins IVα and XIIIα (MBR-IV and MBR-XIII) and xanthobilirubic acid (XBR) were prepared by total synthesis.[15,17] The optically active amines were obtained from Norse Laboratories: S-(+)-2-aminobutane $[\alpha]_D$ +7.4° (neat), S-(-)-α-phenethylamine $[\alpha]_D$ -79° (neat); Fluka: S-(+)-(1-cyclohexyl)-ethylamine $[\alpha]_D$ +3.8° (neat); and Aldrich: S-(+)-β-phenylisopropylamine $[\alpha]_D$ +36° (neat), S-(-)-1-(1-naphthyl)ethylamine $[\alpha]_D$ -61° (c 9.5, MeOH). The organic solvents used were spectral grade. Solutions were prepared by dissolving the pigment in a freshly prepared amine solution, and the spectral data were accumulated within 30 minutes and after 24 hours of preparation. Some of the pigments are sufficiently insoluble in the solvents such that the presence of amine is required to prepare the 4.5×10^{-5} M solutions used in this study: benzene, chloroform (MBR-IV and XBR), acetone, acetonitrile and methanol. All circular dichroism spectra were recorded on a JASCO J-40 spectropolarimeter equipped with a photoelastic modulator, and all UV-visible spectra were run on a Cary 219 spectrophotometer.

RESULTS AND DISCUSSION

Complexation Equilibria, Bilirubin Conformation and Induced Optical Activity. In chloroform solution, BR-IX exhibits no optical activity as detected by circular dichroism (CD) spectroscopy. Upon the addition of S-(+)-2-aminobutane, the pigment shows CD Cotton effects (CEs) in the vicinity of the long wavelength UV-visible absorption near 460 nm (Fig 4). The CE magnitudes increase with increasing amine:pigment ratios, leveling off near 15,000:1 (Table 1). The origin of the optical activity may be understood as coming from non-equimolar concentrations of the diastereomeric salts

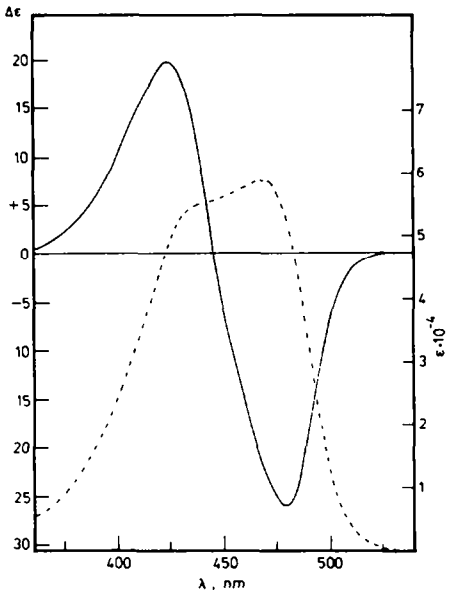


FIGURE 4. Induced circular dichroism (ICD) (——) and UV-visible (- - - -) spectra of 4.6×10^{-5} M BR-IX in benzene solution containing 0.69 M S-(+)-2-aminobutane. Data were recorded at 20°C on a JASCO J40 spectropolarimeter and a Cary 219 spectrophotometer.

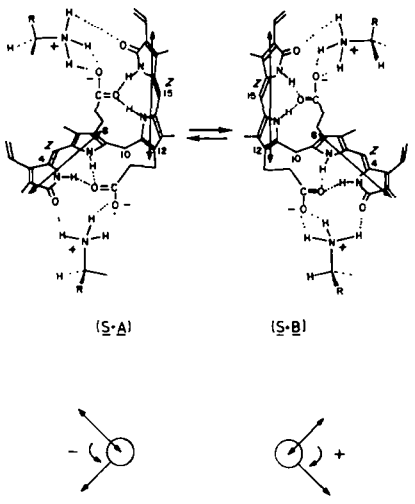


FIGURE 5. (Upper) Interconverting diastereomeric heteroassociation complexes of BR-IX with optically active α-substituted ethylamines, e.g. S-(+)-2-aminobutane. (Lower) The relative orientations of the electric dipole transition moments (viewed at C-10) of the pyrromethenone chromophores. The S·A diastereomeric complex has left-handed (-)chirality of the dipoles, the S·B complex has right-handed (+) chirality.

TABLE 1. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 4.6×10^{-5} M Bilirubin-IXα with Varying Concentrations of S-(+)-2-Aminobutane in $CHCl_3$[a] at 20°C.

Amine:Pigment Molar Ratio	Time (h)[b]	CD			UV	
		$\Delta\varepsilon_{max}(\lambda_1)$	λ_2 at $\Delta\varepsilon=0$	$\Delta\varepsilon_{max}(\lambda_3)$	ε_{max}	λ(nm)
1:1	0.5	+1.1 (420)		<<0.1	54,600	453
	24	+1.1 (420)		<<0.1		
10:1	0.5	+2.0 (415)	440	-0.6 (470)	52,400	453
	24	+2.0 (415)	440	-0.6 (470)		
100:1	0.5	+3.2 (416)	440	-2.0 (471)	53,100	455
	24	+3.2 (416)	440	-2.0 (471)		
1,000:1	0.5	+6.5 (420)	440	-7.8 (474)	57,600	455
	24	+6.1 (420)	440	-18.0 (472)		
5,000:1	0.5	+11.2 (420)	440	-14.0 (474)	61,500	458
	24	+9.8 (420)	439	-14.0 (472)		
15,000:1	0.5	+14.6 (42)	440	-19.4 (474)	62,400	459
	24	+13.8 (419)	440	-19.4 (472)		

[a] Ethanol-free, stabilized with 1% n-hexane. [b] Spectra run 0.5 hours and 24 hours after preparing solutions.

(S·A and S·B) formed in the acid-base complexation equilibria, eqs. (1) and (2), between the optically active amine (S*), e.g. (+)-2-aminobutane, and the BR-IX conformational enantiomers A and B of Fig. 1.

$$S^* + A \underset{}{\overset{K^1_{eq}}{\rightleftharpoons}} S{\cdot}A \qquad (1)$$

$$S^* + B \underset{}{\overset{K^2_{eq}}{\rightleftharpoons}} S{\cdot}B \qquad (2)$$

The values of K^1_{eq} and k^2_{eq} will be governed by the proclivity of S* to form the salt complex and its selectivity for either A or B. Although the concentrations of enantiomers A and B are expected to be equal (same ΔG°_f), barring anisotropic solvation effects, the concentrations of diastereomers S·A and S·B will not be equal (different ΔG°_f). Therefore solutions containing the diastereomeric salts are expected to show a net optical activity of the pigment, and as the equilibria of eqs (1) and (2) are driven toward salt (complex) formation by a large excess of amine, the magnitude of the ICD can be expected to increase. This is reflected in the data of Table 1. In sum, the reaction of d,l-bilirubin (A and B, Fig. 1) with a chiral amine can be viewed as a prime example of a first-order asymmetric transformation[23] where one diastereomeric complex is greatly favored over the other in an equilibrium (Fig. 5) such as:

$$\underline{d}\text{-BR-IX}\cdot\underline{S}\text{-amine} \rightleftharpoons \underline{l}\text{-Br-IX}\cdot\underline{S}\text{-amine} \qquad (3)$$

but where the BR-IX component of the complex is labile and mutarotates. (Attempts to liberate optically active BR-IX from its salt have so far proved unsuccessful, however.)

Optically active amines that bind tightly to and are highly enantio-selective in forming the salt complexes are expected to generate the largest CD CEs. This is true for any chiral complexing agent and has been noted for tightly-bound (association constant of 10^7-10^8)[2,3] BR-IX heteroassociation complexes with serum albumins, e.g. with HSA $\Delta\varepsilon^{407}_{max}$ -26, $\Delta\varepsilon^{460}_{max}$ +49.[18] With other albumins, $\Delta\varepsilon$ magnitudes as high as 250 $l\cdot mole^{-1}\cdot cm^{-1}$ have been recorded.[22] On the other hand, chiral complexing agents that either do not have a high affinity for BR-IX or exhibit little enantioselectivity for A or B can be expected to induce only weak optical activity, e.g. $\Delta\varepsilon^{408}_{max}$ +2.1, $\Delta\varepsilon^{459}_{max}$ -3.0 for BR-IX with α-cyclodextrin, a torus-shaped cycloamylose. The equilibria depicted by equations (1) - (3) can also be influenced by the choice of solvent. Non-polar solvents, e.g. benzene, should favor the

tightly bound ion-pair salt structures and drive the equilibria of equations (1) and (2) to the right. Polar, hydrogen-bonding solvents should interfere with H-bonding and facilitate dissociation. These predictions are borne out by the data of Table 2, where the $\Delta\varepsilon$ values are largest in solvents like benzene and chloroform, and smallest in dimethylsulfoxide. In the latter, the vanishingly small $\Delta\varepsilon$ values can be attributed to significantly reduced concentrations of the diastereomeric salt, either S·A or S·B.

TABLE 2. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 4.6×10^{-5} M Solutions of Bilirubin-IXα and 0.65 M S-(+)-2-aminobutane[a] at 20°C.

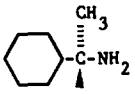
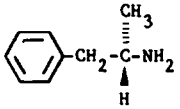
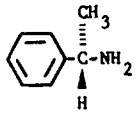
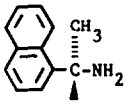
Amine	Solvent[b]	Time (h)[c]	CD $\Delta\varepsilon(\lambda_1)$	CD λ_2 at $\Delta\varepsilon=0$	CD $\Delta\varepsilon(\lambda_2)$	UV ε_{max}	UV λ(nm)
S-(+)-2-Aminobutane	C_6H_6	0.5	+19.5 (423)	445	-26.0 (479)	60,000	469
		24	+19.5 (423)	446	-26.2 (479)	60,700	469
$CH_3CH_2-\overset{CH_3}{\overset{\vert}{C}}-NH_2$ (H)	$CHCl_3$	0.5	+14.6 (420)	440	-19.4 (474)	62,400	459
		24	+13.8 (419)	439	-19.4 (472)	61,700	459
	ME_2CO	0.5	+15.1 (415)	438	-18.4 (468)	68,400	457
		24	+9.26 (415)	438	-11.1 (470)	65,400	457
	MeCN	0.5	+7.08 (416)	439	-8.17 (466)	68,100	449
		24	+7.30 (414)	439	-8.06 (471)	67,600	449
	MeOH	0.5	+1.1 (420)		<<0.1	57,800	451
		24	+1.5 (420)		<<0.1	48,200	452
	Me_2SO	0.5	<<0.1		<<0.1	70,800	460
		24	<<0.1		<<0.1	70,800	460

[a] 1:15,000 pigment:amine concentration ratio [b] C_6H_6: benzene, $CHCl_3$: chloroform, Me_2CO: acetone, MeCN: acetonitrile, MeOH: methanol, Me_2SO: dimethylsulfoxide [c] Spectra run 0.5 hours and 24 hours after preparing solutions

Molecular Structure and Induced Circular Dichroism. Previous workers have also noted that BR-IX will exhibit CD in the presence of optically active amines, but the data were not interpreted in terms of pigment molecular structure.[24] The only published induced CD (ICD) spectra of BR-IX with chiral amines show $\Delta\varepsilon^{407}_{max}$ +6.0, $\Delta\varepsilon^{462}_{max}$ -7.8 for a 100:1 molar ratio of acetyl-L-lysine-N'-methylamide: BR-IX in dichloromethane solvent and $\Delta\varepsilon^{420}_{max}$ +5.8, $\Delta\varepsilon^{462}_{max}$ -9.9, $\Delta\varepsilon^{502}_{max}$ +7.1 for a 1:1 molar ratio of poly-L-lysine: BR-IX in water at pH 11.4.[25] The phenomenon of ICD with optically active amines is a general one, as is illustrated in Table 3 for substituted ethyl amines of the S-configuration. The ICDs are all bisignate with a long wavelength (-) CE followed by a short wavelength (+) CE, except for the naphthyl amine in benzene solvent. The origin of the sign reversal here is unclear; a change in solvent for this amine appears to reverse the order of stability of S·A and S·B. Except with the naphthyl amine, the aralkyl amines generally give larger $\Delta\varepsilon$ values than the alkyl amines, with the largest ICD coming from the β-phenylisopropylamine. In fact, its $\Delta\varepsilon$ values are remarkably high and exceed those recorded[18] for BR-IX HSA solutions near physiologic pH (see above). They suggest a high degree of enantio-selectivity of the amine for one of the BR-IX conformational enantiomers and strong coordination to it--the same characteristics normally accorded to protein and enzyme binding. The strong preferential binding exhibited by the phenyl amines probably involves "multiple point" binding[26,27] from van der Waals attractions between the amine's π-electron-rich aromatic ring and the pigment's pyrromethenone moieties in addition to H-bonding in the salt binding site (Fig. 3). This sort of pi-system stacking has been shown to occur with purines and pyrimidines[28] and was shown to be important in complexes of β-arylethylamines with acridines.[27]

The heteroassociation complexation of eqs. (1) and (2) apparently requires acid functional groups in the pigment and is thus expected to apply to MBR-IV and XBR, as well as to BR-IX, all of which contain propionic acid groups and should therefore be capable of making diastereomeric salt complexes. With XBR, which has only one pyrromethenone chromophore, only a weak monosignate ICD CE can be detected (Table 4). It is clear, however, that some sort of complexation interaction must

TABLE 3. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 4.6_a - 4.9×10^{-5} M Solutions of Bilirubin-IXα and 0.6 - 0.7 M Optically Active Primary Amines at 25°C.

Amine	Solvent	Time (h)[b]	CD $\Delta\varepsilon(\lambda_1)$	λ_2 at $\Delta\varepsilon=0$	$\Delta\varepsilon(\lambda_2)$	UV ε_{max}	λ(nm)
S-(+)-α-Cyclohexyl ethylamine	C_6H_6	0.5	+15.0 (427)	446	-21.6 (480)	57,600	470
		24	+14.1 (427)	445	-21.9 (480)	54,400	470
	MeCN	0.5	+14.5 (413)	435	-20.5 (467)	58,000	453
		24	+14.3 (413)	435	-20.5 (467)	58,000	453
	Me_2SO	0.5	<<0.1		<<0.1	70,100	460
		24	<<0.1		<<0.1	70,100	460
S-(+)-β-Phenyl-Isopropylamine	C_6H_6	0.5	+49.8 (424)	445	-69.4 (480)	61,200	466
		24	+42.9 (424)	445	-60.6 (479)	57,100	466
	MeCN	0.5	+24.6 (413)	435	-35.1 (468)	66,400	452
		24	+25.7 (414)	436	-36.3 (468)	66,400	452
	Me_2SO	0.5	+1.37 (413)	435	-3.08 (470)	70,800	460
		24	+0.91 (413)	435	-3.42 (467)	69,600	460
S-(-)-α-Phenethyl-amine	C_6H_6	0.5	+28.6 (420)	441	-40.6 (476)	54,200	470
		24	+24.5 (422)	443	-37.0 (476)	53,300	468
	MeCN	0.5	+38.1 (415)	436	-43.3 (468)	67,100	451
		24	+30.2 (414)	436	-40.8 (469)	66,100	451
	Me_2SO	0.5	<<0.1		<<0.1	70,200	460
		24	<<0.1		<<0.1	70,100	460
S-(-)-α-(1-naphthyl)-ethylamine	C_6H_6	0.5	-7.1 (435)	470	+12.9 (490)	48,600	465
		24	-5.6 (424)	478	+7.8 (490)	47,700	461
	MeCN	0.5	+4.0 (413)	433	-6.7 (465)	50,000	449
		24	+3.9 (413)	433	-6.6 (465)	50,100	449
	Me_2SO	0.5	<<0.1		<<0.1	69,100	458
		24	<<0.1		<<0.1	69,000	458

[a] 1:15,000 pigment:amine concentration ratio [b] Spectra run 0.5 hours and 24 hours after preparing solutions

TABLE 4. Circular Dichroism and Ultraviolet-Visible Spectral Data for 4.6×10^{-5} M Rubinoid Pigments in the Presence of S-(+)-2-Aminobutane in Chloroform[a] at 20°C. (Pigment:Amine Ratio is 1:15,000.)

Pigment	Time (h)[b]	CD $\Delta\varepsilon_{max}(\lambda_1)$	λ_2 at $\Delta\varepsilon=0$	$\Delta\varepsilon_{max}(\lambda_3)$	UV ε_{max}	λ(nm)
BR-IX	0.5	+14.6 (420)	440	-19.4 (474)	62,400	459
	24	+13.8 (419)	439	-19.4 (472)	61,700	459
MBR-XIII	0.5	+15.1 (396)	414	-21.2 (445)	56.600	434
	24	+15.3 (396)	415	-20.3 (444)	55,100	434
MBR-IV	0.5	+1.64 (383)	407	-0.82 (450)	48,800	386
	24	+1.84 (383)	407	-0.41 (445)	46,500	387
BR-IX DME	0.5	<<0.1	-	<<0.1	59,300	410
	24	<<0.1	-	<<0.1	57,100	410
XBR	0.5	+1.09 (400)	423		21,000	410
	24	<<0.1 (400)	-		17,300	410

[a] Ethanol-free, stabilized with 1% n-hexane. [b] Spectra run 0.5 hours and 24 hours after preparing solutions.

take place between the amine and XBR because XBR is extremely insoluble and will not dissolve in chloroform solvent at the indicated concentration without the amine being present. Similarly MBR-IV, which like XBR is insoluble in chloroform and like BR-IX is a bichromophoric molecule, cannot adopt the H-bonded chiral conformations of Fig. 1 and therefore gives only weak ICD CEs. BR-IX DME, which is not known to form amine salt complexes or adopt the H-bonded chiral conformations of Fig. 1,[11] shows no ICD. However, in marked contrast, strong well-defined bisignate CEs are seen only for BR-IX and MBR-XIII, both of which are capable of adopting and prefer to assume the enantiomeric conformations of Fig. 1. The evidence therefore supports the importance of such conformations in the diastereomeric salt complexes, e.g. Fig. 5, formed by equilibria expressed in equations (1) and (2).

In summary, the heteroassociation complexation (eqs. 1 and 2) apparently requires acid functional groups and is not due uniquely to other forces, e.g. micellar, electrostatic or pi-interactions, because the dimethyl ester of BR-IX gives only vanishingly small CD CEs. It also requires pigment that can adopt enantiomeric conformations (A and B) because MBR-IV, which is a diacid incapable of adopting the intramolecularly H-bonded conformations of Fig. 1, shows only weak CD CEs. Although MBR-IV is capable of forming amine salts, such diastereomeric complexes are free to assume other conformations, with no apparent strong preference for either of the unique, chiral conformations expressed by BR-IX and MBR-XIII. The ICD of MBR-IV may have its origin in general asymmetric solvation effects involving the amine, possibly as a chiral template, possibly of a nature similar to that which produces bisignate CD CEs for BR-IX DME in ethyl (S)-(-)-lactate ($\Delta\varepsilon^{410}_{max}$ -3, $\Delta\varepsilon^{430}_{max}$ +2.5), and in (R,R)-(-)-2,3-butanediol ($\Delta\varepsilon^{405}_{max}$ -0.4, $\Delta\varepsilon^{430}_{max}$ +0.8).[29]

Bisignate Cotton Effects and Exciton Coupling. Bisignate CEs are characteristic of the ICD spectra of the various bichromophoric pigments (BR-IX, MBR-XIII and MBR-IV) studied here. The bisignate nature of the induced CD, with two oppositely-signed CEs straddling the UV-vis transition(s), is typical of excited state interaction in weakly coupled electronic systems (molecular exciton[30]).[31] Here, two pyrromethenone chromophores with strongly allowed long wavelength electronic transitions (ε_{max} -30,000), have only a small interchromophoric electron overlap but interact through their (locally) excited states by resonance splitting (electrostatic interaction of the local transition moment dipoles). The exciton splitting gives rise to the long wavelength UV-vis transitions, one higher in energy and one lower in energy, with the separation dependent on the strength and relative orientation of the pyrromethenone electric dipole transition moments (Fig. 5).[31] As seen in their UV-vis spectra, the two electronic transitions overlap to give the characteristically broadened absorption bands of bilirubinoids.[32] As seen in their CD spectra, however, the two exciton transitions are always oppositely signed, as predicted by theory,[31] and thus give rise to bisignate CEs. In contrast to UV-vis absorption bands, which may show only slight broadening when the exciton splitting energy is small, when two oppositely-signed curves overlap in the CD, there is considerable cancellation in the region between the band centers with the net result that the observed bisignate CE maxima are displaced from the actual locations of the (uncombined) CD transitions[33] and typically are seen to flank the corresponding UV-vis band(s). This is amply illustrated with ICD and UV-vis spectra of BR-IX in the presence of S-(+)-2-aminobutane (Fig. 4).

Bisignate CDs might also arise in bichromophoric bilirubins if each pyrromethenone acted independently to produce CEs of opposite signs. The optical activity might arise from asymmetric perturbation or induced dissymmetry of the chromophore--through the action of chiral solvation ligating agents. However, we tend to believe that this mechanism is unimportant for explaining the large ICD CEs of BR-IX and MBR-XIII for the following reasons. (1) The monochromophore molecular analog, XBR, shows no ICD with added amine. (2) Bichromophoric MBR-IV and BR-IX DME give either a very weak or no ICD, although they can adopt the chiral conformations of Fig. 1--albeit without the benefit of intramolecular H-bonds to hold the pyrromethenone moieties in place. (3) The CD couplets for the bichromophoric molecules are always of opposite sign, as required by the exciton model. If the chromophores were acting independently, one should expect to find both monosignate and bisignate ICDs for, e.g. BR-IX. But this has not been observed.

Absolute Configuration. Exciton coupling theory provides a way to assign the absolute configuration, either S•A or S•B (Fig. 5) of the predominant salt complex. The handedness or screw sense that the electronic transition moments of the coupled pyrromethenone chromophores make with each other (Fig. 5) correlates with signed order of the bisignate CD CEs.[31] A right-handed screw sense (positive chirality) of the transition moment leads to a (+) longer wavelength CE followed by a (-) shorter wavelength CE. For a left-handed screw sense (negative chirality), the CE signs are inverted: (-) at the longer wavelength and (+) at the shorter wavelength component of the bisignate CE. Since the direction of the electric dipole transition moment in the pyrromethenone chromophore has been calculated in theoretical studies[34] to lie along the longitudinal axis of the planar conjugated π-system, the exciton model can predict the CE signs of the structurally well-defined diastereomers, S•A and S•B, and the enantiomers A and B. In these intramolecularly hydrogen bonded conformations, the relative orientations of the two pyrromethenone electric dipole moments constitute a left-handed chirality for S•A and A and a right-handed chirality for S•B and B. Thus, theory predicts a predominance of the left-handed diastereomeric complex S•A for solutions of BR-IX in the presence of the (S)-configuration amines of Tables 2 and 3 (except the naphthylamine in benzene) since the induced bisignate to CDs show a (-) CE near 480 nm followed by a (+) CE near 420 nm. By analogy, formation of the right-handed diastereomeric complex S•B is favored for BR-IX in the presence of (S)-(-)-α-(1-naphthyl)ethylamine in benzene or human serum albumin at pH 7.3.[18]

Just as the absolute configuration can be assigned by exciton coupling theory, so can it be used to calculate the magnitudes of the CEs for enantiomers A and B and, by rough analogy, the CEs of the diastereomeric complexes S•A and S•B. Following the example of Harada and Nakanishi,[31] we computed the $\Delta\epsilon_{max}$ values for the two transitions of A to be -260 and +190, as observed in the calculated CD for the long and short wavelength exciton components, respectively.[35] Using these calculated values as references for 100% diastereomerically pure complexes, the maximum experimental $\Delta\epsilon$ values (-26 at 479 nm) for BR-IX + S-(+)-2-aminobutane and (-69 at 480 nm) for BR-IX + S-(+)-β-phenylisopropylamine in benzene (Tables 2 and 3) correspond, respectively, to 10% and 26%, diastereomeric excess of the complex corresponding to S•A.

CONCLUDING REMARKS

BR-IX and related rubins with propionic acid or propionate groups located at C-8 and C-12 tend to adopt either of two intramolecularly H-bonded enantiomeric conformations (Fig. 1) and as such may be viewed as racemic mixtures of interconverting mirror image structures. Chiral amines bind to produce diastereomeric complexes in which one diastereomer is favored over the other. As a consequence, BR-IX solutions become optically active and exhibit bisignate CD CEs, the intensity of which depends on the binding constant and enantioselectivity. The very large bisignate CEs previously characteristic only of albumin and other protein complexes with BR-IX can be detected for certain amines, which must also bind to BR-IX with a high degree of enantioselectivity.

Conformations such as A and B and S•A and S•B (Figs. 1 and 5) appear to be essential in understanding the origin of the intense bisignate ICD CEs. For those bichromophoric pigments (MBR-IV, BR-IX DME) that cannot or do not adopt them give only weak or vanishingly small ICD CEs in the presence of chiral amines. Consequently, although we view all the bichromophoric pigments of this study as potential molecular excitons, only BR-IX and MBR-XIII show bisignate ICD data characteristic of exciton systems wherein the two chromophores are held in the well defined geometry necessary for near optimal orientation (skew angle of ~100°) of their relevant electric dipole transition moments.

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