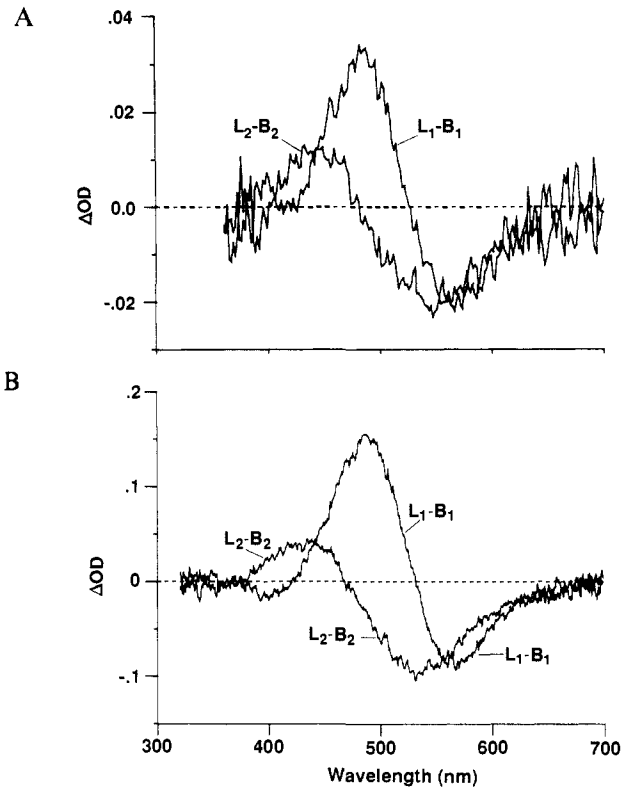


Figure 3. Difference spectra calculated for the fast and slow BATHO decays. The top figure shows the difference spectra for decay of the fast (L_2–B_2) and slow (L_1–B_1) processes which result from ISO photolysis. These were calculated from the data in Figure 1 by using the lifetimes obtained from the data in Figure 2. For comparison, the lower figure shows the comparable results obtained from RHO photolysis.[13] Although the apparent noise is greater in the upper figure, this is principally due to the reduced signal size observed for ISO photolysis. Reduced signal size occurs because of reduced absorbance of ISO at the laser wavelength compared to RHO and also because of the smaller quantum yield for ISO photolysis compared to RHO.

$LUMI_1$ spectra, since the $BATHO_2$ component is almost completely absent at 170 and 300 ns. The difference between the 30- and the 60-ns curves represents a mixture of spectra from the fast and slow decay components. Knowing the lifetimes of the two BATHO products makes it possible to subtract the $BATHO_1$–$LUMI_1$ difference spectrum to yield the $BATHO_2$–$LUMI_2$ difference spectrum. The spectra in Figure 3, scaled to the BATHO amplitudes immediately after photolysis, are identical within the noise to the corresponding spectra obtained from photolysis of RHO. The lifetimes of 195 ± 20 and 25 ± 10 ns compare well with lifetimes of 176 ± 20 ns and 36 ± 15 ns measured previously for the BATHO components of RHO. We conclude that photolysis of both RHO and ISO yield intermediates with the same spectral and kinetic properties within experimental error. The possibility that this results from a photostationary state was eliminated by experiments where ISO was excited with 477-nm light. These produced essentially identical spectra to those presented in Figure 3.

In an earlier paper, we reported similar spectral, kinetic, and photochemical properties of BATHO from RHO and ISO.[11] Spalink et al.[9] concluded from picosecond studies, however, that different bathochromic products are formed from RHO and ISO. Although our total actinic energy was in the range used by Spalink et al., the photon fluence per unit time was about 100 times higher during their 25-ps pulse. We have observed that the ratio of $BATHO_1$ to $BATHO_2$ formation is dependent on laser power.[13] Small differences in previously reported BATHO spectra could be explained by two BATHO's whose presence is affected by varying power. In fact, this model is consistent with the results of the picosecond study where spectral changes were studied as a function of excitation energy.[9] It should also be pointed out that our results are in agreement with those of low-temperature photolysis experiments on ISO.[15] There, two BATHO products with similar spectra to those seen here were observed to be produced from ISO by using nonlaser sources.

A structural explanation for two different BATHO intermediates is not available at this point. It is conceivable that the two forms comprise strained all-trans structures with slightly different arrangements within their protein pockets. Differences arising from clockwise or counter clockwise isomerization have been suggested,[15] though the fact that photolysis of the 11-cis pigment and the 9-cis pigment yields the same two BATHO products makes this explanation unlikely if, as has been suggested,[16,17] isomerization involves highly localized motions of the chromophore in the region of the isomerized bond. Proposals of different amino acid side chain conformations could also explain the existence of two batho products.[18] Alternatively, we could be seeing the effects of the formation of a di-cis product or an unstable mono-cis secondary photoproduct (such as a 13-cis photoproduct of $BATHO_2$), which decays into a different intermediate. We plan further experiments with excitation at different wavelengths and at lower laser powers as well as polarized photolysis studies to investigate these questions. It is clear that the decay of RHO and ISO is more complicated than previously assumed. However, the observation that intermediates with the same spectral and kinetic properties are observed upon photolysis of RHO and ISO support the theory that both pigments share common BATHO intermediates.

Acknowledgment. We thank the National Institutes of Health for Grant EY00983 which provided support for this work.

(15) Sasaki, N.; Tokunaga, F.; Yoshizawa, T. *Photochem. Photobiol.* **1980**, *32*, 433–441.
(16) Liu, R. S. H.; Asato, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 259–263.
(17) Warshel, A.; Barboy, N. *J. Am. Chem. Soc.* **1982**, *104*, 1469–1476.
(18) Birge, R. R.; Einterz, C. M.; Knapp, H. M.; Murray, L. P. *Biophys. J.*, in press.

Enzymatic Synthesis of Macrocyclic Lactones

Guo Zhi-Wei and Charles J. Sih*

School of Pharmacy, University of Wisconsin
Madison, Wisconsin 53706

Received October 1, 1987

It is now well established that enzymes which hydrolyze esters in aqueous media can also catalyze esterification or transesterification reactions in monophasic organic solvents or in water–organic solvent biphasic media.[1] The ester-forming ability of lipases (triacylglycerol hydrolases EC 3.1.1.3) has been known since the beginning of this century.[2] Since then, microbial lipases have been used for the regiospecific interesterification of triglycerides[3] and the preparative resolution of chiral acids and alcohols via enantiospecific esterifications.[4]

In 1984, Gatfield[5] first noted that when certain hydroxy acids were exposed to the lipase of *Mucor miehei*, lactones were formed. The macrocyclic pentadecanolide was synthesized from 15-hydroxypentadecanoic acid and γ-butyrolactone from 4-

(1) (a) Semenov, A. N.; Khmelnitski, Y. L.; Berezin, I. V.; Martinek, K. *Biocatalysis* **1987**, *1*, 3. (b) Halling, P. *J. Enzyme Microb. Technol.* **1984**, *6*, 513. (c) Kasche, V. *Enzyme Microb. Technol.* **1986**, *8*, 4 and references cited therein.
(2) Kastle, J. H.; Loevenhart, A. S. *J. Am. Chem. Soc.* **1900**, *24*, 491.
(3) Yokozeki, K.; Yamanaka, S.; Takinami, K.; Hirose, Y.; Tanaka, A.; Sonomoto, K.; Fukui, S. *Eur. J. Appl. Microbiol. Biotechnol.* **1982**, *14*, 1.
(4) (a) Langrand, G.; Secci, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Tetrahedron Lett.* **1985**, *2*, 47. (b) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072. (c) Chen, C. S.; Wu, S. H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812 and references cited therein.
(5) Gatfield, I. L. *Ann. N.Y. Acad. Sci.* **1984**, *434*, 569.

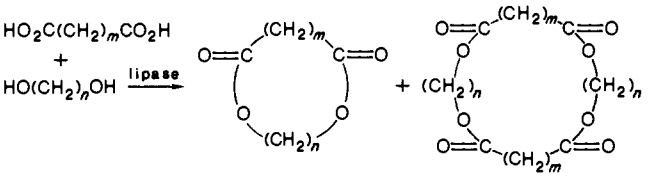
0002-7863/88/1510-1999$01.50/0

Table I. Effect of Temperature and Organic Solvents on Biocatalytic Lactonization

$$HO_2C(CH_2)_{12}CO_2H + HO(CH_2)_{10}OH \xrightarrow{K\text{-}10} [O_2C(CH_2)_{12}CO_2(CH_2)_{10}]$$

solvent	temp (°C)	monolactone (%)[13]
isooctane	33	1
isooctane	45	4
isooctane	55	23
isooctane	65	38
isooctane	75	24
isooctane	85	3
isooctane + 1% H_2O (v/v)	65	31
isooctane + 2% H_2O (v/v)	65	22
cyclohexane	65	23
carbon tetrachloride	65	21
toluene	65	6
hexane	45	8
tetrahydrofuran	65	0
1,2-dichloroethane	65	0
ethanol	65	0

hydroxybutyric acid. This observation was subequently confirmed by Yamada,[6] who reported the biocatalytic lactonization of ω-hydroxy acid methyl esters in highly diluted solutions. Very recently, Gutman et al.[7] found that porcine pancreatic lipase (PPL) in anhydrous organic solvents catalyzed the lactonization of a number of esters of γ-hydroxy acids with high degrees of enantiomeric specificity. Herein, we disclose a novel method for the construction of macrocyclic lactones via direct condensation of diacids with diols, catalyzed by lipases in nonaqueous media. In



all the cases examined, the major products of this reaction were mono and dilactones accompanied by linear oligomeric esters and a small amount of trilactones in some systems (~4%).[8] The yield of the lactonic products varied not only with different substrates but also with reaction conditions. In general, nonpolar organic solvents such as anhydrous isooctane, hexane, cyclohexane, and carbon tetrachloride were the most suitable solvents[9] for preparative syntheses (Table I). While biocatalyzed intermolecular esterification proceeded efficiently in monophasic organic solvents[4a,b] and in water–organic solvent biphasic systems,[4c] biocatalytic lactonization was less efficient in biphasic milieu. When 2% exogenous water was added to the reaction medium, the yield of the monolactone (C_{24}) decreased from 38% to approximately 22%, and the mixture of acyclic oligomeric esters increased (Table I). Similarly, at lower temperatures (<45 °C), oligomeric esters were the dominant products. Good yields of lactonic products were obtained only when the temperature was raised to 55–75 °C, which indicated that a higher energy of activation was required for lactonization; at 85 °C the lipase underwent inactivation (Table I). Although many lipases mediated this lactonization reaction depending on the particular substrates used, the most suitable lipases[10] were derived from *Candida cylindracea* (OF-360), *Pseudomonas sp.* (AK and K-10), and porcine pancreatic lipase (PPL).

Table II. Biocatalytic Condensation of Diacids with Diols

$$RO_2C(CH_2)_mCO_2R + HO(CH_2)_nOH \rightarrow \text{lactones}$$

m	n	lipase	reaction time (h)	lactone (% isolated yield) mono	di
			R = H		
12	5	K-10	48	18	17
12	6	K-10	48	24	13
12	8	K-10	48	36	12
12	10	K-10	48	40	12
12	12	K-10	48	33	6
12	16	K-10	48	30	7
10	16	K-10	48	56	15
8	16	OF-360	24	53	17
6	16	OF-360	24	52	19
4	16	OF-360	24	48	13
2	16	OF-360	48	39	9
6	6	PPL	168	6	5
			R = CH_3		
12	10	K-10	48	33	9
8	16	OF-360	24	34	9

A simple representative experimental protocol for this biocatalytic lactonization reaction follows: To a suspension of 2 mmol each of 1,10-decanedicarboxylic acid (461 mg) and 1,16-hexadecanediol (517 mg) in 200 mL of anhydrous isooctane was added 4 g of crude lipase (K-10) powder. The reaction mixture was incubated in an incubator rotary shaker (200 rpm, 2″-stroke) at 65 °C for 48 h. After filtration, the organic solvent was dried over sodium sulfate and evaporated to dryness under reduced pressure. The crude residue (0.9 g) was chromatographed over a silica gel (MN Kieselgel 60, 70–270 mesh) column (2.2 × 40 cm). Elution of the column with hexane–ethyl acetate (30:1 to 20:1) afforded 504 mg (56%) of the monolactaone, mp 64–65 °C: NMR ($CDCl_3$) δ 2.3 (*t*, *J* = 7 Hz, 4 H, CH_2COO-), 4.1 δ ppm (t, *J* = 6 Hz, 4 H, -CO_2CH_2-); molecular ion at 453 (M + 1), 435, and 213 and 132 mg (15%) of the dilactone, mp 92–93 °C; molecular ion at 906 (M + 1), 888, 453, 435, and 213.

By using the above experimental procedure, we have found that lipases can accommodate acyclic diacids (*m* = 2–12) and diols (*n* = 5–16) of various sizes (Table II). However, the optimum isolated yield of lactones was achieved when the size of the ring was in the range of 24–28. Diesters (methyl) may be used as substrates by these lipases, but the yield of lactones was lower. Moreover, cyclic diacids and diols also served as lactonization substrates. For example, the lipase of *Candida Cylindracea* catalyzed the condensation of *trans*-cyclohexane-1,4-dicarboxylic acid and 1,16-hexadecanediol (isooctane, 65 °C, 48 h) to yield a mixture of mono- (42%) and dilactones (8%). Conversely, the lipase of *Pseudomonas sp.* (K-10) catalyzed the reaction of *cis*-cyclohexane-1,2-dimethanol with 1,12-dodecanedicarboxylic acid under similar conditions to give a mixture of the corresponding monolactone (41%) and dilactone (25%) after 7 days.

It is noteworthy that several recent publications[11] reported the formation of linear ester oligomers after exposure of diacids or diesters and diols to lipases. At lower temperatures (≤45 °C) of incubation, these investigators did not detect the presence of cyclic structures of moderate ring size, an observation that is in

(6) Makita, A.; Nihira, T.; Yamada, Y. *Tetrahedron Lett.* **1987**, *28*, 805.
(7) Gutman, A. L.; Zuobi, K.; Boltansky, A. *Tetrahedron Lett.* **1987**, *28*, 3861.
(8) The PMR spectra of all the monolactones and dilactones exhibited a triplet centered at δ 4.1 ppm (*J* = 5.5–6.5 Hz) characteristic of -COO-CH_2- and a triplet at δ 2.3 ppm (*J* = 6–7 Hz, -CH_2COO- (except when *m* = 2; singlet at δ 2.6 ppm). The mono- and dilactones all gave peaks at M + 1, [(M + 1) − 18], or M − 18 and 14m + 73 (O_2C-$(CH_2)_m$-C^+O-H) [see: Biemann, K. *Mass Spectrometry-Organic Chemical Applications*; McGraw-Hill: New York, 1962; pp 55–56]. Infrared spectra showed the absence of OH absorption.
(9) See (Laane et al. Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, *30*, 81) for rules of optimization.
(10) The following lipases were products of Amano: AK and K-10 (*Pseudomonas sp.*); AP and APF-12 (*Aspergillus niger*); FAP (*Rhizopus oryzae*); MAP (*Mucor miehei*); and R-10 (*Humicola lanuginosa*). Dr. Tominaga of Amano kindly informed us that K-10 is a crude form of lipoprotein lipase 80 (*Pseudomonas sp.*) and is not derived from *Aspergillus niger* as listed in the catalog. *Candida cylindracea* (OF-360) and PPL (porcine pancreatic lipase) are products of Meito Sangyo and Sigma, respectively. All these lipases catalyzed lactone formation when *m* = 12 and *n* = 10.
(11) (a) Okumura, S.; Iwai, M.; Tominaga, Y. *Agric. Biol. Chem.* **1984**, *48*, 2805. (b) Ajima, A.; Yoshimoto, T.; Takahashi, K.; Tamaura, Y.; Saito, Y.; Inada, Y. *Biotechnol. Lett.* **1985**, *7*, 303. (c) Margolin, A. L.; Crenne, J. Y.; Klibanov, A. M. *Tetrahedron Lett.* **1987**, *28*, 1607.

basic agreement with our findings. Currently, we are examining quantitatively the sequential kinetics[12] for the resolution of enantiomeric diols to enable us to define more precisely the stereospecificity of this unique biocatalytic lactonization process.

Acknowledgment. This investigation was supported in part by Grant GM33149 from the National Institutes of Health.

(12) Wu, S. H.; Zhang, L. Q.; Chen, C. S.; Girdaukas, G.; Sih, C. J. *Tetrahedron Lett.* **1985**, *26*, 4323.

(13) The reaction mixture contained the following: 0.05 mmol each of 1,12-dodecanedicarboxylic acid and 1,10-decanediol in 5 mL of isooctane and 100 mg of lipase K-10. After incubation for 24 h at the indicated temperature, the monolactone (C_{24}) was quantitatively determined by GLC analysis on a Varian Aerograph Model 2400 instrument by using a 3-ft, OV-1 column at 245 °C.

Laser Induced Triplet Excitons in the Columnar Phases of an Octasubstituted Metal Free Phthalocyanine

Dimitra Markovitsi,* Thu-Hoa Tran-Thi, and Valérie Briois

CEA, CEN/Saclay
IRDI/DESICP/DPC/SCM UA CNRS 331
91191 Gif sur Yvette, Cedex, France

Jacques Simon and Kazuchita Ohta

ESPCI, CNRS UA 429, 10, rue Vauquelin
75231 Paris, Cedex 05, France

Received November 5, 1987

Columnar mesophases are interesting systems for the study of one-dimensional energy migration. In these phases, chromophores which constitute the central rigid part of the mesogenic molecules are stacked in columns surrounded with flexible hydrocarbon chains; the intercolumnar distance is much larger than the intermolecular distance within the columns.[1-3] Energy migration has been recently reported for the columnar phases of the 2,3,6,7,10,11-hexa-*n*-hexyloxytriphenylene; singlet excitons are generated by fusion of triplet excitons, and delayed fluorescence is observed.[4] Moreover the singlet exciton diffusion length has been determined in columnar phases formed with solid solutions of metal free and copper(II) octasubstituted phthalocyanines.[5]

The present communication describes the properties of laser induced triplet excitons observed in both the crystalline and the liquid crystalline phases of the octakis(octadecyloxymethyl)metal free phthalocyanine, **1** (Figure 1), determined by nanosecond absorption spectroscopy.[7]

Laser excitation of **1** (Q band) in homogeneous benzene solutions or in pure columnar mesophases gives transient differential absorption spectra typical of a phthalocyanine triplet–triplet absorption[8,9] (Figure 2). The triplet lifetime in benzene solutions (10^{-6} M) is 120 ± 10 μs. This value is in agreement with the ones previously reported for the nonsubstituted metal free phthalocyanine.[8,10] When the pure compound thin films are excited with low energy laser pulses a monoexponential decay of the transient absorption is observed, yielding a triplet lifetime of 7.5 ± 0.5 μs at 25 °C. This lifetime continuously decreases down to 4.2 ± 0.3 μs when the temperature increases up to 84 °C.

For high-energy pulses, the transient decay obeys second-order kinetics. The differential spectrum obtained under these conditions is the same as the one obtained with low-energy excitation. Triplet–triplet annihilation therefore occurs: $T + T \rightarrow 2S_o$. The

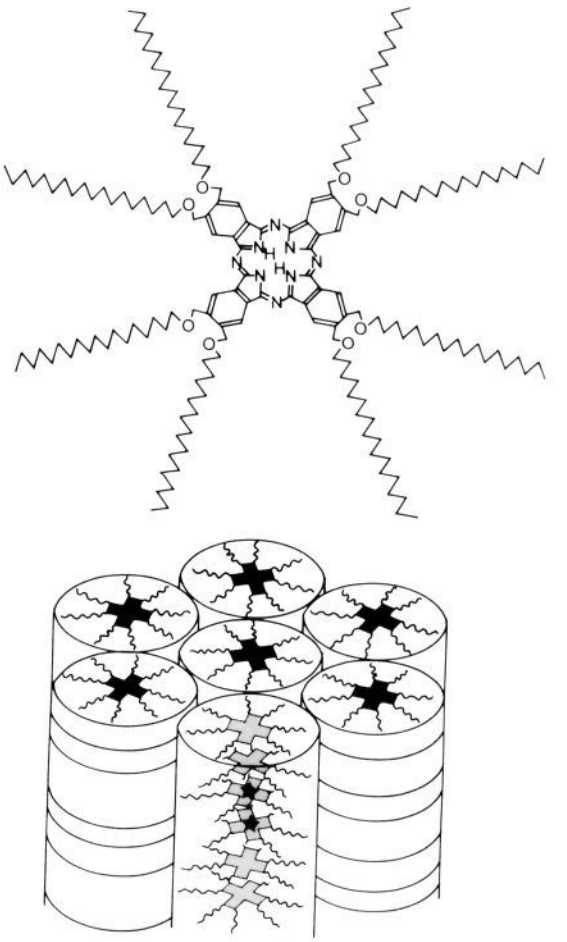


Figure 1. Schematic representation of the octakis(octadecyloxymethyl)metal free phthalocyanine used, $(C_{18}OCH_2)_8PcH_2$ (**1**).

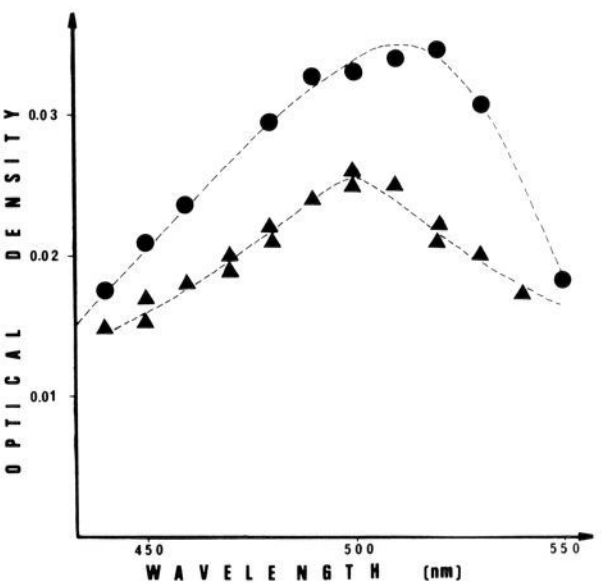


Figure 2. Transient differential absorption spectra at $t = 0.2$ μs of pure $(C_{18}OCH_2)_8PcH_2$ excited at 532 nm: (●) 25 °C, (△) 77 °C. Phase transitions: crystal $\xrightarrow{62\ °C}$ mesophase (D_{hd}) $\xrightarrow{193\ °C}$ isotropic liquid.

(1) Chandrasekhar, S.; Shadashiva, B. K.; Suresh, K. A. *Pramana* **1977**, *9*, 471.
(2) Destrade, C.; Foucher, P.; Nguyen Huu, T.; Levelut, A. M.; Malthête, J. *Mol. Cryst. Liq. Cryst.* **1984**, *106*, 121.
(3) Piechocki, C.; Simon, J.; Skoulios, A.; Guillon, D.; Weber, P. *J. Am. Chem. Soc.* **1982**, *104*, 5245.
(4) Markovitsi, D.; Rigaut, F.; Mouallem, M.; Malthête, J. *Chem. Phys. Lett.* **1987**, *135*, 236.
(5) Blanzat, B.; Barthou, C.; Tercier, N.; Andrē, J. J.; Simon J. *J. Am. Chem. Soc.* **1987**, *109*, 6193.
(6) Nonoriented samples.
(7) Tran-Thi, T. H.; Markovitsi, D.; Even, R.; Simon, J. *Chem. Phys. Lett.* **1987**, *139*, 207.
(8) McVie, J.; Sinclair, R. S.; Truscott, T. G. *J. Chem. Soc., Faraday Trans. 2* **1978**, 1870.
(9) Pyatosin, V. E.; Tsvirko, M. P. *Zh. Prikladnoi Spektroskopii* **1980**, *33*, 320.
(10) Jacques, P.; Braun, A. M. *Helv. Chim. Acta* **1981**, *64* 1800.

0002-7863/88/1510-2001$01.50/0