JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10959–10963 10959
DOI:10.1021/jf902266m

Partition of Indicaxanthin in Membrane Biomimetic Systems. A Kinetic and Modeling Approach

Maria Liria Turco Liveri,[†] Luciana Sciascia,[†] Mario Allegra,[‡] Luisa Tesoriere,[‡] and Maria A. Livrea*[,‡]

[†]Dipartimento di Chimica Fisica "F. Accascina", Viale delle Scienze, Pad. 17 and [‡]Dipartimento Farmacochimico Tossicologico e Biologico, Via Cipolla 74, Università degli Studi di Palermo, 90128 Palermo, Italy

The solubilization site of indicaxanthin (Ind) in lipid bilayers was investigated by the kinetics of Ind oxidation by peroxyl radicals in water and in aqueous/L-α-dipalmitoyl-phosphatidylcholine (DPPC) vesicles, pH 7.4, and 37.0 and 48.0 °C, that is, in a gel-like and a crystal liquidlike bilayer state, respectively. The time-dependent Ind absorbance decay, matched with a successful simulation of the reaction kinetic mechanism by Gepasi software, supported a multistep pathway. Computer-assisted analysis allowed calculation of the rate constants associated with the reactions involved, the values of which decreased with increasing DPPC concentration. The binding constant calculated according to a pseudo two-phase distribution model did not vary with the physicochemical state of the vesicle, indicating location of Ind in a region whose state is not affected by temperature changes, at the interface between hydrophobic core and hydrophilic head groups. Other measurements carried out in the presence of dimyristoyl-phosphatidylcholine vesicles, indicated that the phytochemical was confined to the aqueous phase.

KEYWORDS: Betalain pigments; biomimetic membranes; DPPC; vesicles; phospholipids; Gepasi simulation.

INTRODUCTION

Betalain pigments are a quite small group of dietary phytochemicals having in common the structure of betalamic acid. This is conjugated with either amino acids or amines to form betaxanthins or with cyclo-DOPA derivatives to form betacyanins (**Figure 1**). Because of their chemistry, including charged portions and ionizable groups, as well as lipophilic moieties, these molecules may behave as amphiphilic-like compounds at physiological pH. Indeed, they have been found capable of interacting with human lipoproteins (*1*, *2*), and binding to biological membranes (*3*) and to large unilamellar soybean-phosphatidylcholine liposomes (*4*).

The yellow indicaxanthin (Ind, **Figure 1**), the major betaxanthin of the cactus pear (*Opuntia ficus indica*) fruit, is the immonium derivative of proline with betalamic acid. Its reducing cyclic amine group makes this molecule a potential natural antioxidant (*5*). Protective effects of Ind against lipid oxidation have been shown in various biological models, from liposomes (*4*) to either healthy or pathological red blood cells (*6*, *7*), as well as cell cultures (*8*). Studies on the interactions of Ind with lipid bilayers may help to rationalize its antioxidative effects. Recently the characteristic absorbance spectrum of Ind in the visible light and its variation as a function of temperature and phospholipid concentration have been exploited in our laboratory to investigate the location of the pigment in vesicles of either L-α-dipalmitoyl- phosphatidylcholine (DPPC), or L-α-dimyristoyl-phosphatidylcholine (DMPC) (*9*). While ruling out that the pigment partitioned at the hydrophobic interior, that study did not allow to unequivocally conclude on the location of Ind in these vesicles.

Reaction kinetics of molecules in aqueous solution as compared with analogous reactions in the presence of aggregated media such as lipid bilayers, can fruitfully be exploited to gain useful hints on the solubilization site of the reacting species, when monitored within a temperature range including the main transition temperature (T_m) of the bilayer. Since Ind is an effective peroxyl radical-scavenger (*4*), the kinetics of Ind oxidation by the thermogenerated peroxyl radicals from the water-soluble azo-initiator 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) have been studied in water, and in a heterogeneous aqueous/DPPC, as well as /DMPC, liposomal system. A Gepasi modeling approach (*10*) to calculate rate constants and their variation as a function of phospholipid concentration and temperature was a tool to reinvestigate the partition of indicaxanthin between water and vesicular pseudo phases. Quantitative analysis of the kinetic data led us to establish the solubilization site of the pigment.

EXPERIMENTAL PROCEDURES

Materials. Pure L-α-dimyristoyl-phosphatidylcholine (DMPC) and L-α-dipalmitoyl-phosphatidyl choline (DPPC) were from Avanti Lipids. Indicaxantin was isolated from local cactus pear fruits (*Opuntia ficus indica*), as described (*4*). As reported in previous spectrophotometric studies (*9*), Ind was lost less than 10% when exposed for 60 min at the highest temperature used in the present work (48 °C). 2,2′-Azobis(2-amidinopropane)hydrochloride

*To whom correspondence should be addressed.

Published on Web 10/27/2009

(AAPH) was obtained from Polyscience Inc. and was used without further purification. All solutions have been prepared by using deionized water (Culligan Pharma System 20, High Wycombe, U.K.).

Preparation of Vesicles. Stock aqueous vesicular dispersions were prepared by sonication of either DMPC or DPPC suspensions in 5 mM phosphate buffer saline, pH 7.4 (PBS), as previously described (*9*). Briefly, lipid suspensions in PBS were sonicated by a titanium probe connected to a 100 W Pabish High Intensity Ultrasonic Processor, using 5 s pulses of 60 W, with a 1 s interval for 30 min. Temperature was kept constant at 55 and 37 °C for DPPC and DMPC, respectively. Samples were subsequently filtrated using Millex-HV filters (PDVF) with pore diameter of 45 μm to remove eventual titanium particles. Vesicle dispersions at the required lipid concentration were obtained by diluting the stock dispersion with PBS and used immediately. By this method, small unilamellar vesicles are obtained, whose size is independent of time for at least 3 h (*11*−*13*), with aqueous dispersions maintaining a clear aspect and no turbidity from adhesion or fusion processes evident. When required, indicaxanthin was added to the vesicular preparations as a PBS solution to obtain a 2.0×10^{-6} mol dm^{-3} final concentration. Under these conditions, the addition of the pigment did not change the ionic strength of the medium nor cause osmotic effects at the vesicles so that the vesicular stability was not affected.

Kinetic Measurements. Ind at 2.0×10^{-6} mol dm^{-3} in PBS was incubated under air in the presence of 0.01 mol dm^{-3} AAPH in the absence and in the presence of variable concentrations of either DMPC or DPPC vesicular preparations. The kinetic runs were performed in 1.0 cm optical path cuvettes of a Beckman DU-640 spectrophotometer (Palo Alto, CA), equipped with thermostat Heto Therm ±0.1 °C (Thermo Karlsruhe, Germany) and appropriate magnetic stirring devices. The spectrophotometer was interfaced to a computer for both data collection and analysis. The time-course of the reaction between Ind and AAPH-generated peroxyl radicals was monitored by the disappearance of the pigment at 482 nm. Phospholipid concentration was varied in the range $0.2-1 \times 10^{-3}$ mol dm^{-3}. The assays were carried out at either 37.0 or 48.0 °C for the DPPC vesicles (T_m = 41.3 °C) and at 37.0 °C for DMPC (T_m = 24.4 °C). The rate constants were obtained by best fits of the absorbance data to suggested reaction kinetic models using the Gepasi software package (*10*). All fits (n = 5) varied within ±5%.

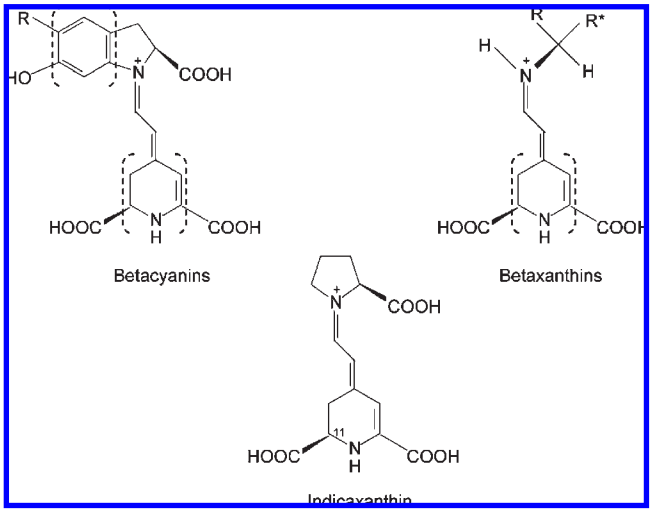


Figure 1. Basic structure of betacyanins and betaxanthins and molecular structure of indicaxanthin. In brackets the parts with lipophilic character.

RESULTS AND DISCUSSION

The reaction kinetics of Ind with thermally generated peroxyl radicals from AAPH at 37.0 °C in buffer pH 7.4 were spectrophotometrically monitored at 482 nm, and the absorbance of the pigment as a function of time is presented in **Figure 2A**. The time-course of the pigment decay did not fit first-order, nor second order rate laws, suggesting a multistep pathway, the mechanism of which was simulated by Gepasi modeling. The suggested set of reactions (**Scheme 1**) and the simultaneous solving of the relevant rate laws generated a kinetic curve fitting satisfactorily the experimental data (**Figure 2A**). In the simulated model, decomposition of AAPH (AN = NA) is followed by immediate reaction of carbon-centered radicals (A·) with oxygen to form peroxyl-radicals (AOO·), which in turn may abstract H from both Ind and a product of intramolecular Ind transformation (Ind*).

The calculated rate constants for the reactions are reported in **Table 1**. It may be worth noting that k_1 calculated for the dissociation of AAPH at 37 °C is comparable with the constant measured by other authors (*14*).

The kinetics of the reaction between Ind and peroxyl radicals from AAPH were spectrophotometrically monitored in the presence of varied DPPC vesicle concentrations,at 37.0 °C, so that

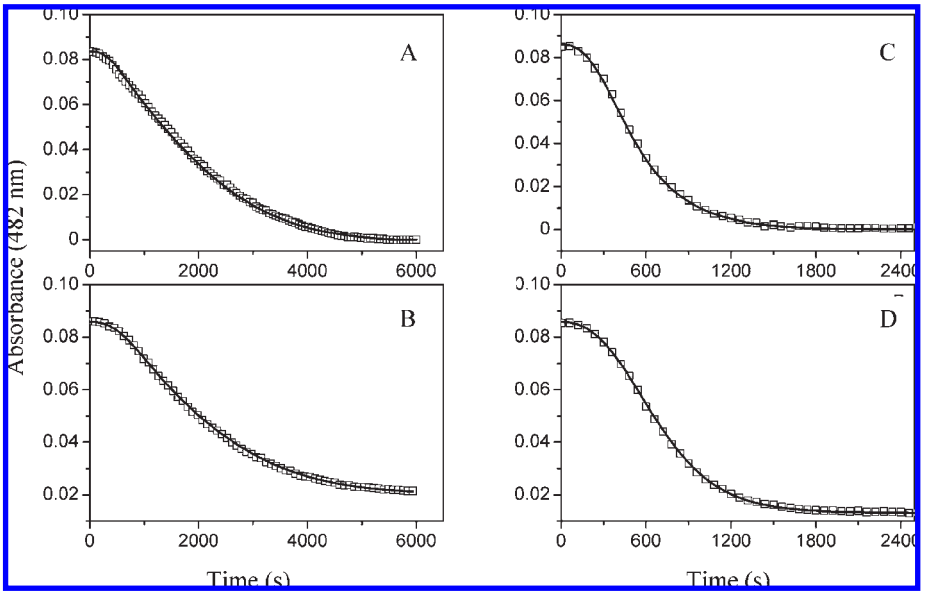


Figure 2. Time-course of absorbance decay of indicaxantin during reaction with AAPH-derived peroxyl radicals, monitored at 37 °C (**A**,**B**), or at 48 °C (**C**,**D**), either in the absence (**A**,**C**) or in the presence (**B**,**D**) of 1.0 mmol dm^{-3} DPPC vesicles; (□) experimental points, and (—) data calculated according **Scheme 1**. Incubation conditions are as reported in Experimental Procedures.

Scheme 1

$$AN=NA \xrightarrow[O_2]{k_1} AOO\cdot + AOO\cdot + N_2 \qquad v_1 = k_1[AN=NA] \qquad \text{Eq.1}$$

$$Ind + AOO\cdot \xrightarrow{k_2} P_1 + AOOH \qquad v_2 = k_2[Ind][AOO\cdot] \qquad \text{Eq.2}$$

$$Ind \xrightarrow{k_3} Ind^* \qquad v_3 = k_3[Ind] \qquad \text{Eq.3}$$

$$Ind^* + AOO\cdot \xrightarrow{k_4} P \qquad v_4 = k_4[Ind^*][AOO\cdot] \qquad \text{Eq.4}$$

Table 1. Rate Constants Associated to the Reactions in **Scheme 1** in the Absence or in the Presence of DPPC Vesicles

temp	[DPPC] mmol dm^{-3}	k_2[a] s^{-1}	k_2[b] dm^{3}mol^{-1} s^{-1}	k_4[c] s^{-1}	10^{-3} k_4[d] dm^{3}mol^{-1} s^{-1}
37 °C	0	3.50	0.301	2.501	13.16
	0.20	3.45	0.267	2.422	6.98
	0.25	3.51	0.265	2.406	6.87
	0.30	3.51	0.264	2.402	6.37
	0.40	3.50	0.265	2.392	5.98
	0.65	3.48	0.260	2.380	5.78
	0.85	3.51	0.256	2.378	5.51
	1.00	3.50	0.254	2.377	5.40
48 °C	0	5.10	1.492	15.90	7.21
	0.20	5.05	1.341	15.53	8.22
	0.30	4.90	1.294	15.40	7.24
	0.40	5.00	1.276	15.39	6.93
	0.60	5.10	1.244	15.33	6.43
	0.80	4.95	1.248	15.32	6.33
	1.00	5.00	1.233	15.32	6.14

[a] Calculated by eq 1. [b] Calculated by eq 2. [c] Calculated by eq 3. [d] Calculated by eq 4.

measurements were performed in the gel-like vesicular state (*15*). A representative Ind absorbance decay in the presence of 1.0×10^{-3} mol dm^{-3} DPPC is shown in **Figure 2B**. In analogy with the reaction in the absence of vesicles, the kinetic data were analyzed according to the multistep model in **Scheme 1**. The simultaneous solving of the rate laws generated kinetic curves fitting the experimental data for all vesicular concentrations. The calculated rate constants associated to the reaction steps are listed in **Table 1**. With the exception of the azo-initiator dissociation, an inverse relationship was observed between the rate constants and the DPPC vesicle concentration, indicating that the reactivity of Ind was affected by the vesicular environment. The kinetic data were then analyzed according to a pseudo two-phase distribution model (*16*), where the hydrophilic peroxyl radicals are confined to the aqueous pseudophase, whereas the pigment can distribute between the vesicular pseudo phase (v), and the aqueous one (w), in accordance with the reactions in **Scheme 2**.

An implication of the pseudo two-phase distribution model is that the rate constants are related to the relevant rate constants in either water (k_w), or vesicles (k_v), to the binding constants (K), and to the surfactant concentration according to eqs 5−7

$$k_2 = \frac{k_{2,w} + k'_{2,v}K_{Ind}[\text{DPPC}]}{1 + K_{Ind}[\text{DPPC}]} \qquad (5)$$

$$k_3 = \frac{k_{3,w} + k'_{3,v}K_{Ind}[\text{DPPC}]}{1 + K_{Ind}[\text{DPPC}]} \qquad (6)$$

$$k_4 = \frac{k_{4,w} + k'_{4,v}K_{Ind*}[\text{DPPC}]}{1 + K_{Ind*}[\text{DPPC}]} \qquad (7)$$

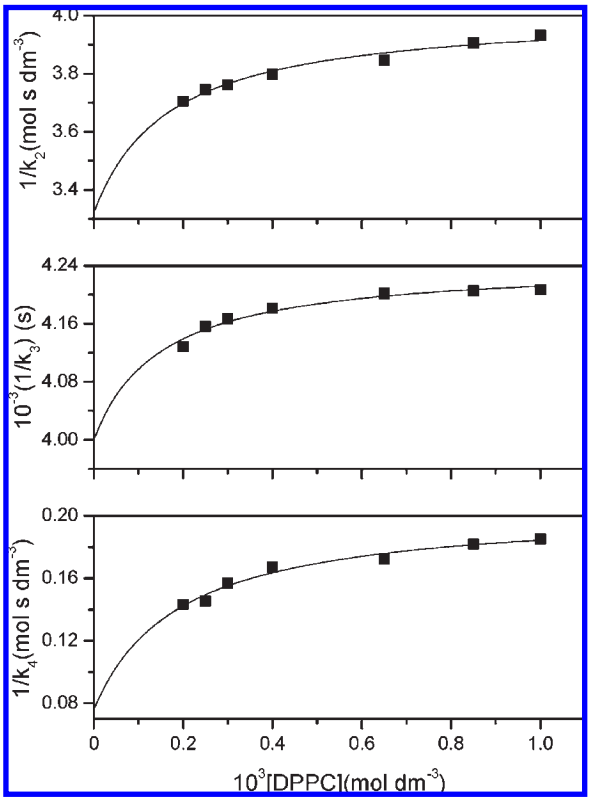
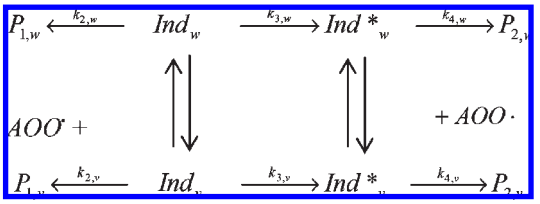


Figure 3. Relationships between reciprocal of rate constants of **Scheme 1** and DPPC vesicle concentrations at 37 °C. (■) Experimental data and (—) curve calculated by a nonlinear least-squares fit to eqs 5−7. Incubation conditions are reported in Experimental Procedures.

Scheme 2



where $k'_{(i),v} = k_{(i),v}/V_{\text{DPPC}}$, and V_{DPPC} is the molar partial volume of the DPPC vesicle. Graphical representations of the relationships between the rate constants and DPPC vesicle concentration, rearranged as $1/k$ versus [DPPC], are shown in **Figure 3**. The plots are expressed as a curve tending to a plateau, indicating that the reactivity of Ind varies in accordance with the vesicular environment, so that at high surfactant concentrations, the rate constants are comparable with the relevant $k'_{(i)v}K_{ind}$[DPPC].

The rate constants in either water phase or vesicles were calculated from nonlinear least-squares fits of the kinetic data to eqs 5−7 and are reported in **Table 2**. The values of $k_{(i),w}$ are comparable with those measured in the absence of DPPC vesicles (see **Table 1**), thus confirming the appropriateness of the kinetic analysis according to the pseudo two-phase distribution model adopted. In addition, the reactivity of Ind (and Ind*) as expressed by the values of $k'_{(i)v}$, smaller than the relevant $k_{(i)w}$, may be consistent with the difficulty of the species to react with peroxyl radicals confined in a different pseudophase.

In accordance with the pseudo two-phase distribution model, Ind is distributed between the vesicular pseudophase (Ind_v), and the aqueous one (Ind_w), with a partition coefficient $P = [\text{Ind}_v]/[\text{Ind}_w]$, and a binding constant $K_{Ind} = [\text{Ind}_v]/\{[\text{Ind}_w]\cdot[\text{DPPC}]\}$, that expresses the affinity of Ind for the vesicular

Table 2. Rate Constants in Aqueous Phase ($k_{(i)w}$) or in DPPC Vesicles ($k'_{(i)v}$), Associated to the Reactions in **Scheme 2**

	$k_{(i)w}$			$k'_{(i)v}$		
temp	k_2[a] $dm^3\ mol^{-1}\ s^{-1}$	k_3[b] $10^3\ s^{-1}$	k_4[c] $dm^3\ mol^{-1}\ s^{-1}$	k'_2[a] $mol^{-1}\ s^{-1}$	k'_3[b] $10^3\ dm^{-3}\ s^{-1}$	k'_4[c] $mol^{-1}\ s^{-1}$
37 °C	0.30 ± 0.01	0.25 ± 0.04	13.1 ± 0.50	0.25 ± 0.06	2.4 ± 0.7	5.0 ± 0.3
48 °C	1.5 ± 0.01	1.59 ± 0.02	17.2 ± 0.60	1.20 ± 0.50	1.7 ± 0.06	5.7 ± 0.2

[a] Calculated from nonlinear least squares fits of the kinetic data to eq 5. [b] Calculated from nonlinear least squares fits of the kinetic data to eq 6. [c] Calculated from nonlinear least squares fits of the kinetic data to eq 7.

pseudophase. K_{Ind} was calculated as $(7.2 \pm 0.9) \times 10^3\ dm^3\ mol^{-1}$ ($n = 5$), and $(7 \pm 1) \times 10^3\ dm^3\ mol^{-1}$ ($n = 5$), according to eqs 5 and 6, respectively. The consistency of these values further supports the validity of the suggested reactions.

Nonlinear least-squares analysis according to eq 7 allowed the calculation of $K_{Ind*} = (14 \pm 2) \times 10^3\ dm^3\ mol^{-1}$ ($n = 5$), showing that the product of the intramolecular Ind transformation binds to the DPPC bilayer with a higher affinity than Ind.

Variation of the values of the binding constants at varying of the vesicular physicochemical state may provide indication on the solubilization site of compounds in lipid bilayers. The kinetics of the reaction between Ind and AAPH-derived peroxyl radicals were monitored at 48.0 °C, a temperature at which the DPPC bilayer is in its liquid-crystal-like state. First, the absorbance decay of Ind at 482 nm was monitored in the absence of phospholipids, and the curve profile is shown in **Figure 2C**. Spectral data were analyzed by Gepasi, according to the suggested multistep simulation model (**Scheme 1**), which fitted satisfactorily the experimental data (**Figure 2C**). The calculated kinetic parameters are collected in **Table 1**. The reaction between Ind and AAPH-derived peroxyl radicals at 48.0 °C was carried out in the presence of DPPC vesicles with varied phospholipid concentrations. A representative Ind absorbance decay, monitored in the presence of 1.0×10^{-3} mol dm^{-3} DPPC, is shown in **Figure 2D**. The spectral data were analyzed by Gepasi, and the simultaneous solution of the rate laws generated kinetic curves fitting the experimental data for all DPPC vesicle concentrations. The calculated rate constants associated to the reaction steps at varying of the vesicular concentrations are reported in **Table 1**. With the exception of the dissociation constant of AAPH, the rate constants for the reaction of Ind with AAPH-derived peroxyl radicals were inversely correlated with the DPPC vesicle concentration. In accordance, the two-pseudophase partition model shown in **Scheme 2** was considered, to which eqs 5−7, relating rate constants and kinetic binding constants to the DPPC vesicle concentration, were applied. Graphical representations of the relationships between the reverse of rate constants and DPPC vesicle concentration are shown in **Figure 4**. The curved plots, showing that the reactivity of Ind at 48.0 °C is affected by the vesicular environment, were analyzed by nonlinear least-squares fits of the kinetic data to eqs 5−7. The values of the rate constants $k_{(i),w}$ and $k_{(i),v}$ are reported in **Table 2**. The calculated $k_{(i),w}$ are quite comparable with those obtained by kinetic measurements in the absence of DPPC vesicles (see **Table 1**) thus confirming that the two-pseudophase partition model was applied appropriately even at 48 °C. The binding constants to DPPC vesicles at 48.0 °C were calculated as $K_{Ind} = (6 \pm 1) \times 10^3\ dm^3\ mol^{-1}$ and $K_{Ind} = (6 \pm 1) \times 10^3\ dm^3\ mol^{-1}$, according to eqs 5 and 6, respectively, and $K_{Ind*} = (17 \pm 2) \times 10^3\ dm^3\ mol^{-1}$ according to eq 7.

Collectively, our data show that the binding constants calculated for Ind at 37.0 and 48.0 °C are quite similar, so that the compound is considered to be partitioned in a region of the DPPC bilayer, whose state is not affected by temperature changes, the so-called "palisade domain". This membrane portion, laying between the hydrophilic head-groups and the hydrophobic core, seems to be an ideal solubilization site for molecules such as Ind, sharing hydrophilic as well as lipophilic moieties.

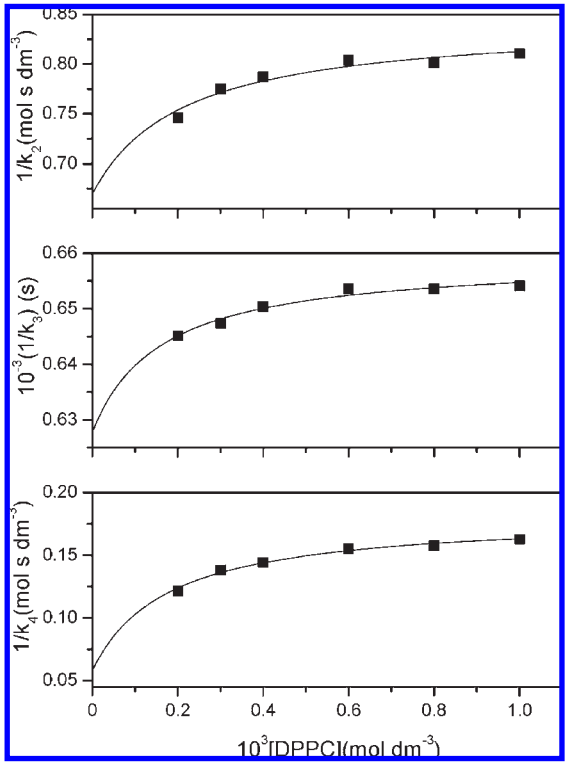


Figure 4. Relationships between reciprocal of rate constants of **Scheme 1** and DPPC vesicle concentrations at 48 °C. (■) Experimental data, and (—) curve calculated by a nonlinear least-squares fit to eqs 5−7. Incubation conditions are reported in Experimental Procedures.

The simulated reaction model matching the experimental data included an intramolecular arrangement of Ind. Betaxanthins are known to undergo modifications such as decarboxylation and isomerization (*17*), however decarboxylated products from Ind have never been isolated (*18*). The molecule instead is known to be prone to C-11 isomerization (*17*). According to our modeling approach, K_{Ind*} that appeared to be unaffected by temperature changes indicates partition of this molecular species at the palisade level as well but with a binding constant higher than Ind. In accordance, the reactivity of Ind*, as expressed by k_4, appears to be more strongly affected by [DPPC], suggesting a more hydrophobic character of such a compound. A varied stereochemistry, such as transformation of the C-11(*S*/*S*) in the C-11 (*S*/*R*) Ind epimer, could reasonably be considered to meet the modification required in the kinetic model.

The kinetics of the reaction between Ind and peroxyl radicals from AAPH were studied at 37 °C in the presence of DMPC vesicles, a saturated phospholipid shorter than DPPC by two CH_2 groups, at concentrations varied from 0.2×10^{-3} to 1.0×10^{-3} mol dm^{-3}. Gepasi analysis of the spectrophotometric data revealed that Ind decay kinetics, and rate constants for the reactions in **Scheme 1** were independent of the DMPC vesicle concentration (not shown), indicating that Ind is confined to the aqueous compartment. Kinetic measurements at a temperature

lower than DMPC T_m (T_m = 24.4 °C) were not carried out because of two main reasons, that is, at such a low temperature the vesicular dispersions are not stable for the time-interval required for the kinetic run, and the rate of the initiator decomposition is too low. In considering that Ind does not partition in a DMPC vesicle when in a crystal liquid-like phase, some interaction of the betalain with the same vesicle in a gel-like state seems less likely. Finally, in support of the present kinetic measurements, our previous spectrophotometric studies showing that the pigment spectrum and absorbance were unaffected at varying of the DMPC vesicle concentration within a temperature range between 25.0 and 48.0 °C (*9*), allow us to be confident about the conclusion that Ind does not partition in the DMPC bilayer. The length of the fatty acid chain may be a crucial factor for partitioning of Ind at least in saturated phospholipid bilayers.

Ind is a dietary highly bioavailable phytochemical (*2*) capable of binding to biological membranes (*3*), and liposomes of either saturated or unsaturated lipids (*4*). An important implication of the present study is that Ind, located in a bilayer at a level between the hydrophobic region and the polar head groups, can react with aqueous peroxyl radicals adsorbed onto the vesicular surface (*19*). This location is consistent with the antioxidant effects observed in soybean phosphatidylcholine bilayers (*4*) and suggests that protective effects of Ind may result from scavenging of radicals from the water phase as well as propagating lipoperoxyl radicals floating to the bilayer polar interface (*20*).

LITERATURE CITED

(1) Tesoriere, L.; Butera, D.; D'Arpa, D.; Di Gaudio, F.; Allegra, M.; Gentile, C.; Livrea, M. A. Increased resistance to oxidation of betalain-enriched human low density lipoproteins. *Free Radical Res*. **2003**, *37*, 689–696.

(2) Tesoriere, L.; Allegra, M.; Butera, D.; Livrea, M. A. Absorption, excretion, and distribution in low density lipoproteins of dietary antioxidant betalains. Potential health effects of betalains in humans. *Am. J. Clin. Nutr*. **2004**, *80*, 941–945.

(3) Kanner, J; Harel, S; Granit, R. Betalains-A new class of dietary cationized antioxidants. *J. Agric. Food Chem*. **2001**, *49*, 5178–5185.

(4) Tesoriere, L.; Allegra, M.; Butera, D.; Gentile, C.; Livrea, M. A. Kinetics of the lipoperoxyl radical scavenging activity of indicaxanthin in solution and in unilamellar liposomes. *Free Radical Res*. **2007**, *41*, 226–233.

(5) Butera, D.; Tesoriere, L.; Di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A. M.; Kohen, R; Livrea, M. A. Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxantin. *J. Agric. Food Chem*. **2002**, *50*, 6895–6901.

(6) Tesoriere, L.; Butera, D.; Allegra, M.; Fazzari, M.; Livrea, M. A. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo-induced oxidative hemolysis in humans. *J Agric. Food Chem* **2005**, *53*, 1266–1270.

(7) Tesoriere, L.; Allegra, M.; Butera, D.; Gentile, C.; Livrea, M. A. Cytoprotective effects of the antioxidant phytochemical indicaxanthin in beta-thalassemia red blood cells. *Free Radical Res*. **2006**, *40*, 753–761.

(8) Gentile, C.; Tesoriere, L.; Allegra, M.; Livrea, M. A.; D'Alessio, P. Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. *Ann. N.Y. Acad. Sci*. **2004**, *1028*, 481–486.

(9) Turco-Liveri, M. L.; Sciascia, L.; Lombardo, R.; Tesoriere, L.; Passante, E.; Livrea, M. A. Spectrophotometric evidence for the solubilization site of betalain pigments in membrane biomimetic systems. *J. Agric. Food Chem*. **2007**, *55*, 2836–2840.

(10) Mendes, P. Biochemistry by numbers: simulation of biochemical pathways with Gepasi3. *Comput. Appl. Biosci*. **1993**, *9*, 563–567.

(11) Cabaleiro-Lago, C.; Garcia-Rio, L.; Herves, P.; Perez-Juste, J. Effects of zwitterionic vesicles on the reactivity of benzoyl chlorids. *J. Phys. Chem. B* **2006**, *8524*, 491–8530.

(12) Andersen, M.; Hammarstrom, L.; Edwards, K. Effect of bilayer phase transitions on vesicle structure and its influence on the kinetics of viologen reduction. *J. Phys. Chem*. **1995**, *99*, 14531–14538.

(13) Walde, P.; Ichikawa, S. Enzymes inside lipid vesicles: preparation, reactivity and applications. *Biomol. Eng*. **2001**, *18*, 143–177.

(14) Wahl, R. U. R.; Zheng, L; Madison, S. A.; DePinto, R. L.; Shay, B. J. Mechanistic studies on the decomposition of water-soluble azo-radical-initiators. *J. Chem. Soc., Perkin Trans*. **1998**, *2*, 2009–2017.

(15) Koynova, R.; Caffrey, M. Phases and phase transitions of the phosphatidylcholines. *Biochim. Biophys. Acta* **1998**, *1376*, 91–145.

(16) Romsted, L. S. *Surfactants in Solution*; Plenum Press: New York, 1984.

(17) Herbach, K. M.; Stintzing, F; Carle, R. Betalain stability and degradation: structural and chromatic aspects. *J. Food Sci*. **2006**, *71*, R41–R50.

(18) Mosshammer, M. R.; Maier, C; Stintzing, F; Carle, R. Impact of thermal treatment and storage on color of yellow-orange cactus pear (Opuntia ficus indica [L.] Mill cv gialla) juice. *J. Food. Sci*. **2006**, *71*, C400–C406.

(19) Barclay, L. R. C.; Baskin, K. A.; Locke, S. J.; Schaefer, T. D. Autoxidation of model membranes. The kinetics and mechanism of autoxidation of mixed phospholipid bilayers. *Can. J. Chem*. **1987**, *65*, 2529–2540.

(20) Barclay, L. R. C. Model biomembranes: quantitative studies of peroxidation, antioxidant action, partitioning, and oxidative stress. *Can. J. Chem*. **1993**, *71*, 1–16.

Received for review July 2, 2009. Revised manuscript received October 13, 2009. Accepted October 14, 2009. This work has been carried out by a grant from Assessorato Agricoltura e Foreste Regione Sicilia