JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Investigation of the Absorption Mechanism of Solubilized Curcumin Using Caco-2 Cell Monolayers

Hailong Yu and Qingrong Huang*

Department of Food Science, Rutgers, the State University of New Jersey, 65 Dudley Road, New Brunswick, New Jersey 08901, United States

ABSTRACT: Curcumin is a bioactive compound with poor oral bioavailability. Low water solubility and rapid metabolism are two known limiting factors, but the absorption mechanism of solubilized curcumin remains unclear. This study investigated the permeation mechanism of solubilized curcumin using an in vitro Caco-2 cell monolayer model. It was shown that curcumin permeated across the monolayers fairly rapidly [P_{app}(A−B) = (7.1 ± 0.7) × 10^{-6} cm/s] and the permeation mechanism was found as passive diffusion [P_{app}(B−A)/P_{app}(A−B) = 1.4]. Furthermore, the permeation rates of curcumin complexed with bovine serum albumin and in the bile salts−fatty acids mixed micelles were also determined as P_{app}(mixed micelle) > P_{app}(DMSO) > P_{app}(protein complex). These results suggested that solubilization agents play an important role in the permeation of solubilized curcumin, and stronger binding between the solubilization agents and curcumin may decrease the permeation rate. The results further suggest that lipid-based formulations, which solubilize curcumin in mixed micelles after lipid digestion, are promising vehicles for curcumin oral delivery.

KEYWORDS: curcumin, Caco-2 cell monolayers, permeation, passive diffusion, solubilization

INTRODUCTION

Curcumin is a polyphenolic bioactive compound found in the spice turmeric and possesses many health-promoting benefits, such as anticancer, anti-inflammatory, antioxidation, and antimicrobial activities.[1−5] These benefits of curcumin, however, are curtailed by its low oral bioavailability.[6] Therefore, improvement of curcumin's oral bioavailability should be addressed in functional food research.

Solubilization, absorption, and metabolism are three important steps that modulate oral bioavailability. It is known that low water solubility and rapid metabolism limit the bioavailability of curcumin. Curcumin is water insoluble. The water solubility is estimated as no more than 11 ng/mL.[7] Most of the orally administered curcumin was found in feces.[8,9] Meanwhile, absorbed curcumin undergoes rapid metabolism. The major metabolites from oral administration are curcumin glucuronide and sulfate.[10,11]

On the other hand, our understanding about the absorption/permeation mechanism of solubilized curcumin is still very limited. Two research groups used an everted rat intestinal sac assay to examine the absorption of curcumin.[12,13] The results on the percentage of intestinal absorption, however, were conflicting. Moreover, the authors neither examined the absorption mechanism nor provided quantitative results on the curcumin absorption/permeation rate.

Caco-2 cell monolayers, in comparison, have been widely used to determine the permeation rate and to examine the permeation mechanisms of bioactive compounds.[14] Different studies demonstrated that in vivo absorption could be well predicted from the apparent permeation rate (P_{app}) across the Caco-2 cell monolayers.[15−17] Although the P_{app} values obtained from different laboratories are different, there is a general trend that a high P_{app} implies high absorption. Generally speaking, $P_{app} > 1 \times 10^{-6}$ cm/s means high permeation, whereas $P_{app} < 1 \times 10^{-7}$ cm/s implies low permeation.[15,16] At the same time, the absorption mechanisms can also be examined. By performing two-way [apical (A) to basolateral (B) and basolateral to apical (B−A)] permeation experiments and calculating the rate ratio, the existence of potential active efflux/uptake can be identified. In general, if P_{app}(B−A)/P_{app}(A−B) is greater than 2 or less than 0.5, the active efflux or uptake mechanisms are suggested respectively. Otherwise, the absorption mechanism may simply be passive diffusion.[18]

Because curcumin is water insoluble and solubilization is the prerequisite of absorption, many formulations such as protein complexation[19−22] and various lipid-based formulations[23−27] have been developed to increase the solubilization of curcumin. Proteins have hydrophobic cores and, thus, are able to solubilize curcumin. The binding between proteins and curcumin is strong. For example, the equilibrium constants of curcumin and different proteins are between 10^4 and 10^6 M^{-1}.[19,21,22] In comparison, lipid-based formulations do not solubilize curcumin directly in aqueous solution. Instead, after lipid digestion, triglycerides in the formulation are hydrolyzed into fatty acids, which contribute to the endogenous bile−phospholipids mixed micelles. Consequently, curcumin that is originally dissolved in the lipid-based formulation is now solublized in the micelle aqueous solution.[28] The binding between curcumin and small molecular weight micelles is much weaker than the protein complexation, with the binding constant in the magnitude of only 10^2−10^3 M^{-1}.[29,30]

In this study, the permeation rate measurements using Caco-2 cell monolayers have been carried out to investigate the absorption of solubilized curcumin. The absorption mechanism was

Received: April 12, 2011
Revised: June 27, 2011
Accepted: August 1, 2011
Published: August 01, 2011

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examined by comparing the permeation rates of curcumin in two opposite directions (A−B versus B−A) across the Caco-2 monolayers. Meanwhile, the effect of different solubilization agents, such as dimethyl sulfoxide (DMSO), bile−fatty acid mixed micelles, and bovine serum albumin (BSA) on the permeation rates of curcumin was also examined. This research may illustrate the limiting factors as well as the effect of different formulations on curcumin absorption.

MATERIALS AND METHODS

Materials. Curcumin, which contains about 85% curcumin, 11% demethoxycurcumin, and 4% bisdemethoxycurcumin,[26] was obtained from Sabinsa Corp., USA. Lucifer yellow and DMSO were purchased from Sigma-Aldrich. Glacial acetic acid, HPLC-grade water, and acetonitrile were from J. T. Baker.

Caco-2 cell line was generously provided by Dr. Judith Storch, Department of Nutrition, Rutgers, the State University of New Jersey. Dulbecco's modified Eagle medium (DMEM), Hank's buffered salt solution (HBSS), fetal bovine serum (FBS), 100× nonessential amino acids, 100× penicillin and streptomycin, 0.25% trypsin with ethylenediaminetetraacetic acid (EDTA), 1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and BSA were all purchased from Fisher Scientific. Transwell permeable polycarbonate inserts (0.4 μm) and 12-well cell culture plates were obtained from Corning.

Maintenance of Caco-2 Cell Culture. Cells were maintained in DMEM with 10% FBS, 1× nonessential amino acids, and 1× penicillin and streptomycin at 37 °C with 5% CO_2. Cells of passage 35−45 were used in this study to maintain relatively constant cellular phenotypes.

Permeation Experiments across Caco-2 Monolayers. Procedures for the determination of apparent permeation rate of curcumin across Caco-2 generally followed the detailed protocol described previously.[18] To generate Caco-2 cell monolayers in the insert filters of 12-well plates, 0.5 mL of Caco-2 cells was plated onto the insert (in the apical compartment) at the density of 6×10^5 cells/mL. One and a half milliliters of culture medium was subsequently added in the lower (basolateral) compartment of each well. Medium was changed every 2 days. Permeation experiments were performed at 21−29 days after plating.

In the permeation experiments, 20 μg/mL curcumin in the donor medium was obtained by diluting curcumin DMSO solution or BSA/mixed micelle solubilized curcumin into donor media. Two donor media were used as noted: (a) HBSS + 25 mM HEPES, pH 7.4; (b) HBSS + 10 mM methanesulfonic acid, pH 6.5. HBSS + 25 mM HEPES + 4% BSA, pH 7.4, was used as receiving medium throughout the study. BSA was added to solubilize permeated curcumin, whereas the addition of BSA to the receiving media could mimic the in vivo condition.[18] In the permeation direction of apical to basolateral (A−B) compartment, 0.4 mL of donor media with 20 μg/mL curcumin was added to the apical compartment and 1.2 mL of receiving medium was added to the basolateral compartment. In the direction of B−A, 1.2 mL of donor medium with 20 μg/mL curcumin was added to the basolateral compartment and 0.4 mL receiving media was added in the apical chamber. Plates were then put in a shaker at 100 rpm and 37 °C. After 15, 30, 45, and 60 min of permeation, half volumes of the receiving media were removed and the same volumes of fresh media were replenished.

The removed receiving media were mixed with 2 volumes of acetonitrile by vortexing briefly and centrifuged at 16000*g* in a benchtop microcentrifuge for 15 min. The supernatants were filtered through a 0.45 μm filter and analyzed with HPLC for curcumin quantification.

The cumulative quantity of curcumin permeated at each time interval was calculated and plotted against time. The initial slope was then used to calculate the apparent permeation rate (P_{app}) using the equation

$$P_{app} = \left(\frac{dQ}{dt}\right)\left(\frac{1}{AC_0}\right) \quad (1)$$

where d*Q*/d*t* is the rate of curcumin permeation, *A* is the surface area of the insert (1.1 cm^2), and C_0 is the initial curcumin concentration.

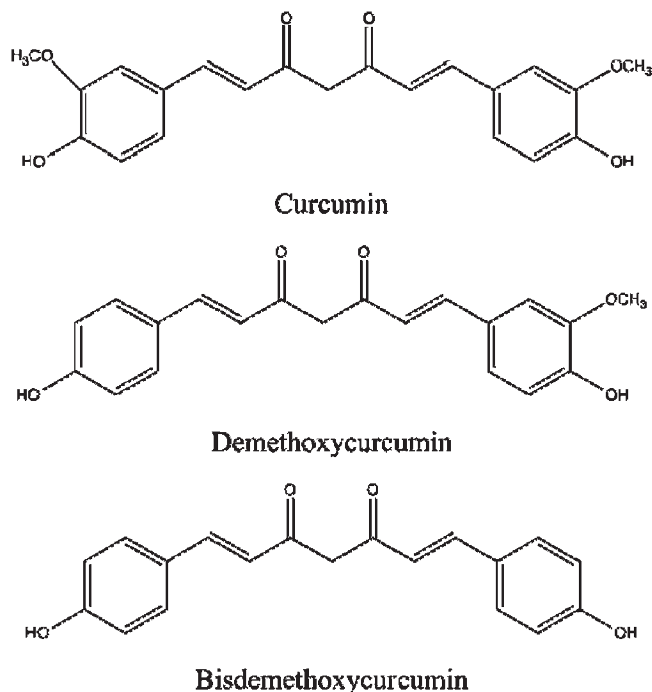

Figure 1. Chemical structures of the three major curcuminoids, curcumin, demethoxycurcumin (D-Cur), and bisdemethoxycurcumin (BD-Cur).

Quality Control of Caco-2 Cell Monolayers. To ensure the integrity of Caco-2 monolayers, the transepithelial electrical resistance (TEER) value and the apparent permeation rate of lucifer yellow were determined.

The TEER value was measured before each experiment using an Evohm2 epithelial voltmeter (World Precision Instruments) and calculated as

$$\text{TEER}\ (\Omega\cdot\text{cm}^2) = [\text{TEER}\ (\Omega) - \text{TEER}_{\text{background}}\ (\Omega)] \times \text{area}\ (\text{cm}^2) \quad (2)$$

where TEER (Ω) is the electrical resistance across Caco-2 monolayers directly read from the Evohm2 epithelial voltmeter and $\text{TEER}_{\text{background}}$ (Ω) is that across the insert only (without cells). The area (cm^2) is the area of the insert, 1.1 cm^2.

Meanwhile, the permeation rate of lucifer yellow, a paracellular permeation marker, was determined in the direction of A−B.[14] Lucifer yellow at 1 mg/mL in donor media *b* was added to the apical chamber. Permeated lucifer yellow in the receiving compartment (with no BSA added, because lucifer yellow is water-soluble) was quantified as arbitrary fluorescence emission intensity at 540 nm (with 20 nm slits) excited at 430 nm (with 10 nm slits). The P_{app} value of lucifer yellow was determined using the same equation as curcumin.

Solubilization of Curcumin in BSA and in Bile−Fatty Acid Mixed Micelles. Solubilization of curcumin in BSA (formation of curcumin−BSA complex) was achieved by pipetting curcumin DMSO solution into 4% BSA in donor solution *b*.[31]

Curcumin solubilized in bile−fatty acid mixed micelles was obtained by in vitro lipid digestion of curcumin organogel.[27] Briefly, curcumin organogel was made by dissolving curcumin in monostearin medium-chain

triglyceride organogel, which was then digested by pancreatic lipase and ultracentrifuged at 50,000 rpm (about 180,000g) to remove undigested organogel and precipitated curcumin. The resultant aqueous solution was bile−fatty acid mixed micelles with solubilized curcumin, which was further diluted to 20 μg/mL in donor solution *b* for permeation experiments.

High-Performance Liquid Chromatography (HPLC). Curcumin was quantified by HPLC, using an UltiMate 3000 HPLC system with 25D UV−vis absorption detector (Dionex). A Nova-Pak C18 3.9 × 150 mm column (Waters) was used. Mobile phase solvents were (A) 2% acetic acid in water, purged with helium, and (B) acetonitrile. Fifty microliter samples were injected into the column. Gradient elution was applied to separate the three curcuminoids: 0−2 min, 65% A and 35% B; 2−17 min, linear gradient from 35 to 55% B; 17−22 min, held at 55% B; 22−23 min, B went back to 35% linearly. The flow rate was set at 1 mL/min, and the detection wavelength was 420 nm.

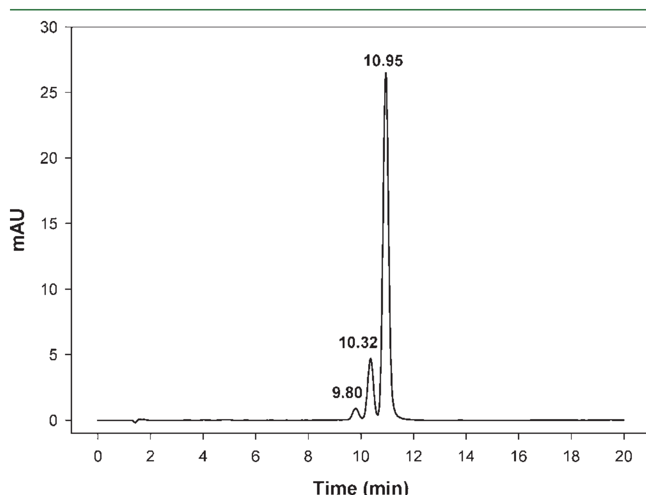

Figure 2. Typical chromatogram of three curcuminoids analyzed by HPLC. Fifty microliter injections of 0.2 μg/mL total curcuminoids are shown here.

Statistical Analysis. One-way ANOVA analysis was performed using SigmaStat with SigmaStat integration. All of the data shown are the mean ± standard deviation, $n = 3$.

RESULTS

Development of HPLC Method To Quantify Curcumin. Curcumin, demethoxycurcumin (D-Cur), and bisdemethoxycurcumin (BD-Cur) are three major curcuminoids that coexist in most curcumin products (Figure 1). The amount of D-Cur and BD-Cur could account for about 15% or more. Therefore, a sensitive HPLC method to separate and quantify the three curcuminoids was established first before the investigation of the permeation mechanism of curcumin.

Figure 2 shows a typical HPLC chromatogram separating the three compounds in the curcumin samples used in this study. The retention times for BD-Cur, D-Cur, and curcumin were approximately 9.80, 10.32, and 10.95 min, respectively. Meanwhile, because the three compounds had similar mass absorption coefficients,[32] the mass percentages of the three compounds were calculated directly from the peak areas of the three peaks: 82.1 ± 1.0% for curcumin, 14.8 ± 0.5% for D-Cur, and 3.1 ± 0.5% for BD-Cur, which is consistent with the previous paper.[26]

Different standard concentrations (10, 20, 50, 100, 200, and 500 ng/mL; 1, 2, 5, 10, 20, 50, and 100 μg/mL) of total curcuminoids were analyzed by HPLC to generate calibration curves for the three curcuminoid compounds. Because the concentration range was broad, four calibration curves of different concentration ranges were generated for each of the three compounds and the total curcuminoids (Table 1).

Examination of the Permeation Mechanism of Curcumin. Two quality control procedures were performed to confirm that the Caco-2 cell monolayers were confluent and suitable for the permeation study: (A) Only wells with TEER values >300 $\Omega \cdot cm^2$ were used;[18] (B) The permeation rate of lucifer yellow, a paracellular transport marker, is expected to be <1 × 10^{-6} cm/s,[33]

Table 1. Summary of the Equations of the Calibration Curves for Curcumin, D-Cur, BD-Cur, and Total Curcuminoids from HPLC

	X range (concentration, μg/mL)	*Y* range (peak area, mAU)	equation	r^2
curcumin	0.0082−0.082	0.0073−0.68	$Y = 8.142X + 0.0027$	0.9975
	0.082−0.82	0.68−6.95	$Y = 8.494X - 0.0725$	0.9993
	0.82−8.2	6.95−69	$Y = 8.412X - 0.2793$	0.9997
	8.2−82	69−600	$Y = 7.143X + 16.7867$	0.9993
D-Cur	0.00148−0.0148	0.012−0.12	$Y = 8X - 0.0002$	0.9954
	0.0148−0.148	0.12−1.24	$Y = 8.441X - 0.0176$	0.9986
	0.148−1.48	1.24−12.4	$Y = 8.414X - 0.0891$	0.9996
	1.48−14.8	12.4−122	$Y = 8.22X - 0.3027$	0.9992
BD-Cur	0.00062−0.0031	0.005−0.0213	$Y = 6.594X - 0.009$	0.9999
	0.0031−0.031	0.0213−0.2417	$Y = 7.904X - 0.0069$	0.9968
	0.031−0.31	0.2417−2.6387	$Y = 8.674X - 0.0682$	0.9989
	0.31−3.1	2.6387−31.668	$Y = 10.435X - 0.5751$	0.9998
total curcuminoids	0.01−0.1	0.0847−0.8203	$Y = 8.094X - 0.0017$	0.9974
	0.1−1	0.8203−8.4343	$Y = 8.468X - 0.0971$	0.9991
	1−10	8.4343−84.158	$Y = 8.421X - 0.4366$	0.9997
	10−100	84.158−753.418	$Y = 7.404X + 15.9089$	0.9997

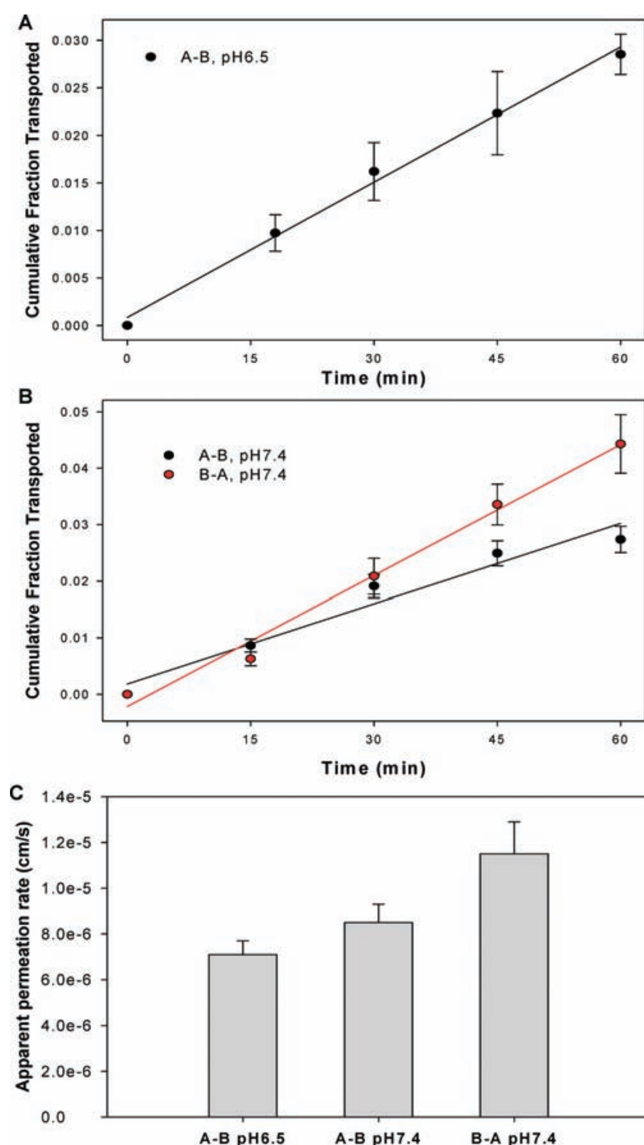

Figure 3. Transport of curcumin over time: (A) in the direction of A−B with donor compartment pH set at pH 6.5; (B) in the direction of A−B and B−A with donor compartment pH set at pH 7.4. (C) Apparent permeation rates of dimethyl sulfoxide (DMSO)-solubilized curcumin in different conditions.

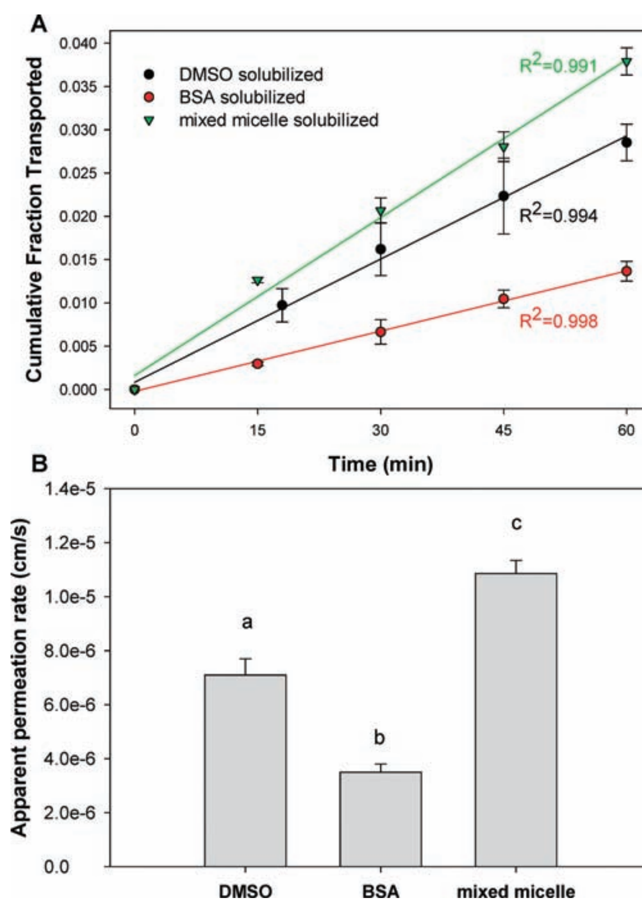

Figure 4. Effect of solubilization agents on the permeation of curcumin across Caco-2 cell monolayers: (A) transport of curcumin solubilized by dimethyl sulfoxide (DMSO), BSA, and mixed micelles; (B) comparison of the permeation rates of curcumin solublized by DMSO, BSA, and mixed micelles. Different letters (a−c) indicate significant difference.

which was determined as $(0.10 \pm 0.01) \times 10^{-6}$ cm/s in this study. Therefore, the Caco-2 cell monolayers were appropriate for use in the permeation study.

Subsequently, the permeation rate of solubilized curcumin (by DMSO dispersion) was determined in the direction of A−B, with donor media pH set at 6.5, to mimic the acidic microenvironment in the small intestine.[34] As shown in Figure 3A, >2% of curcumin was transported through the Caco-2 cell monolayers in 60 min. The corresponding P_{app}(A−B) was determined as $(7.1 \pm 0.6) \times 10^{-6}$ cm/s, which was thought to be a fast permeation rate and implied a fast absorption rate in vivo.[15,16]

The absorption mechanism of curcumin was then investigated by performing permeation experiments in both A−B and B−A directions with the pH of both donor and receiving compartments set at 7.4. As shown in Figure 3B, P_{app}(A−B) and P_{app}(B−A) were $(8.6 \pm 0.8) \times 10^{-6}$ and $(11.5 \pm 1.4) \times 10^{-6}$ cm/s, respectively and the efflux ratio $P_{app}(B-A)/P_{app}(A-B)$ was calculated as 1.4, which is <2, a common cutoff to suggest an active efflux. Therefore, the mechanism of permeation was suggested as passive diffusion with no active efflux/uptake involved.

On the basis of the investigation above, it is suggested that solubilized curcumin was able to permeate through Caco-2 cell monolayers fairly rapidly and the permeation mechanism was passive diffusion. Therefore, the permeation/absorption after solubilization was not thought to be a limiting factor for the oral bioavailability of curcumin, whereas solubilization may be the major hurdle for proper absorption. As long as curcumin is solubilized, it is expected to be able to permeate/be absorbed rapidly.

Effect of Different Solubilization Agents on the Permeation of Curcumin. Complexation with proteins and encapsulation in micelles are two direct approaches to solubilize hydrophobic compounds. Because solubilization limited the permeation of curcumin, the effect of protein complexation and micelle encapsulation on the permeation rate was examined subsequently.

As an example of protein complexation, BSA was used to form a complex with and solubilize curcumin. As shown in Figure 4, adding BSA to the donor media, while solubilizing curcumin, slowed its permeation. The permeation rate of BSA-solubilized curcumin was only $(3.5 \pm 0.3) \times 10^{-6}$ cm/s, significantly lower ($p < 0.001$) than that for DMSO-solubilized curcumin

$[(7.1 \pm 0.6) \times 10^{-6}$ cm/s]. Meanwhile, the permeation rate of micelle-solubilized curcumin was also determined. Curcumin organogel was digested by lipase in vitro, and the curcumin solubilized in the bile−fatty acid mixed micelles was used in the permeation assay. The rate for this mixed micelle-encapsulated curcumin was $(10.9 \pm 0.5) \times 10^{-6}$ cm/s, which was significantly higher ($p < 0.001$) than that for BSA-solubilized and DMSO-solubilized curcumin. The observation that different solubilization agents affected the permeation rate may be due to the different binding constants between curcumin with the solubilization agents. It was found in the literature that the equilibrium constant of curcumin binding with serum albumin was in the magnitude of 10^5 M^{-1},[19,31] whereas the constant of curcumin binding with micelles was reported to be only in the magnitude of 10^2-10^3 M^{-1}.[29,30] Therefore, it was suggested that solubilization agents may affect the permeation rate of curcumin and that strong binding between the solubilization agents with curcumin may decrease the permeation rate.

■ DISCUSSION

In the present study, the permeation mechanism of curcumin was investigated using Caco-2 cell monolayers. First, a HPLC method to quantify curcumin separately from other curcuminoids was developed. Subsequently, the mechanism of passive diffusion for the rapid permeation of solubilized curcumin was demonstrated. Finally, the effects of different solubilization agents on the permeation rates of curcumin were investigated.

In the literature, two groups used everted rat intestine model to examine the absorption of curcumin and showed conflicting results.[12,13] In Ravindranath and Chandrasekhara's work, only about 2.5% curcumin was found in the intestinal tissues after 3 h of incubation, whereas Suresh and Srinivasan found that about 40−80% curcumin was absorbed and argued that the differing observation from the previous research may be due to the improved sensitivity of the analytical methods, from thin layer chromatography to HPLC. In our study, a rapid permeation rate of curcumin across Caco-2 monolayers was observed, which is consistent with the latter work and suggests that absorption of solubilized curcumin may not be the limiting factor for curcumin's oral bioavailability.

Recently, Wahlang et al. also used Caco-2 cell monolayers to investigate the permeation of curcumin.[35] In their research, the permeation mechanism was found as passive diffusion, which is consistent with what we found here. Different from our finding, however, P_{app}(A−B) was determined as $(2.9 \pm 0.9) \times 10^{-6}$cm/s, and thus the absorption rate was considered to be low. To compare the detailed experiment methods, we found that the presence of BSA or not in the receiving solution may represent the biggest difference between the two studies. In our research, 4% BSA was added to the receiving solution, which better mimicked the in vivo environment, where the transport of hydrophobic drug in the circulatory system after absorption is through binding with plasma proteins.[34] Meanwhile, because curcumin is water-insoluble, in the absence of BSA, the partition of curcumin from the Caco-2 cells into the receiving compartments may significantly decrease the apparent permeation rate, resulting in a higher curcumin concentration inside the Caco-2 cells, where it is much better solubilized. Actually, in the paper from Wahlang et al., it was indeed found that curcumin was accumulated inside the Caco-2 cells over time. On the basis of these analyses, it is arguable that our conclusion that curcumin permeated fairly rapidly may reflect better the in vivo situation. On the other hand, we also admit that compared with some highly permeable compounds, the permeation rate of curcumin can be regarded as only moderately rapid. However, the key issue is that we do not think the absorption is the limiting factor and more effort should be put into improving the solubilization of curcumin.

Technically, it is also noted that curcumin undergoes degradation under neutral−basic conditions, which may affect its concentration in the donor compartment over time. Nevertheless, the absorption rate was measured under pH 6.5, and thus the degradation may not affect the curcumin concentration significantly. When the absorption mechanism was investigated, pH 7.4 was used in the donor compartment and degradation was expected. However, because only the ratio of permeation rates in two opposite directions was used to draw our conclusion, the impact of degradation should be minimal. On the other hand, the plots of accumulated curcumin transport over time in Figure 3 revealed a good linearity and seemed not affected by the concentration change of curcumin.

The metabolism of curcumin may occur during the absorption in the small intestine or across the Caco-2 monolayers. In our present studies, unfortunately, we were unable to identify the possible metabolites of curcumin, limited by the lack of proper instrumentation. If the biotransformation is substantial, the actual permeation rate should be even higher than the apparent rate reported here, which may further support our conclusion that solubilized curcumin is able to be absorbed rapidly.

The last part of this research investigated the effect of solubilization agents on the permeation of curcumin. The results obtained were P_{app}(mixed micelle) > P_{app} (DMSO) > P_{app}(BSA complex). Because only free solubilized curcumin is expected to be able to permeate across Caco-2 cell monolayers,[28] the partition of curcumin from within the solubilization agents to freely soluble in the donor solution may affect the apparent permeation rate. Therefore, mixed micelle solublized curcumin permeated more rapidly than solubilized BSA, as the binding between micelle and curcumin is much weaker than that with BSA. On the other hand, although DMSO, as a versatile solubilization agent, is widely used in Caco-2 monolayer experiments, after dispersion of curcumin DMSO solution into the donor medium, the curcumin aqueous solution was in supersaturated state and some curcumin may crystallize in the media. Therefore, the concentration of the actually solubilized curcumin may be <20 μg/mL and thus showed a lower permeation rate than micelle-solubilized curcumin. On the basis of these analyses, a diagram in Figure 5 summarizes our understanding of the solubilization and permeation of curcumin. The apparent permeation rate is directly determined by the free solubilized curcumin in the media, whereas solubilization agents, such as mixed micelles, protein complexes, and DMSO dispersion all provide the free solubilized curcumin. After dispersion of curcumin DMSO solution into aqueous solution, part of the supersaturated curcumin crystallizes, whereas some remains solubilized. In the case of protein complexation and mixed micelle encapsulation, free solubilized curcumin is obtained by partition. Subsequently, free solubilized curcumin permeates across Caco-2 cell monolayers (or small intestine epithelium in vivo) by passive diffusion into the basolateral compartment (or into the circulatory system in vivo).

It is possible that the excipients used to solubilize curcumin, such as DMSO, BSA protein, and bile salt−fatty acid mixed micelles, might affect the integrity of Caco-2 cell monolayers. To

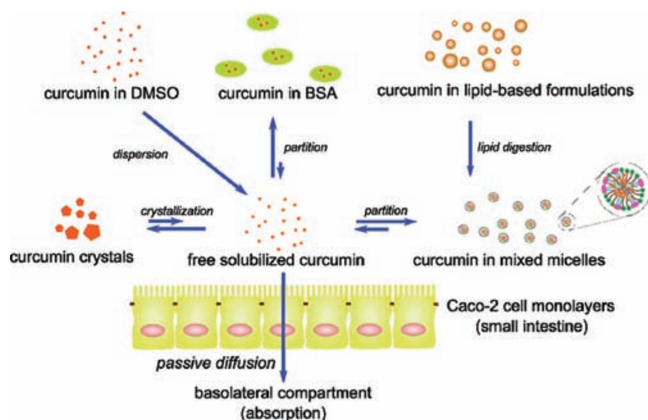

Figure 5. Diagram summarizing the solubilization and permeation of curcumin. Solubilization of curcumin is achieved by dimethyl sulfoxide (DMSO) dispersion, BSA complexation, and mixed micelle encapsulation (generated by lipid digestion of lipid-based formulations). After dispersion of curcumin DMSO solution into aqueous solution, part of the supersaturated curcumin crystallizes, whereas some remains solubilized. In the case of protein complexation and mixed micelle encapsulation, free solubilized curcumin is obtained by partition. Subsequently, free solubilized curcumin permeates across Caco-2 cell monolayers (or small intestine epithelium in vivo) by passive diffusion into the basolateral compartment (or into the circulatory system in vivo).

monitor the confluence of the monolayers, TEER values before and after the permeation experiments were monitored, and no significant decrease was observed for all three excipients, which was consistent with the literature.[34] Moreover, because curcumin was water-insoluble, it was thus thought to permeate transcellularly, but not paracellularly. Therefore, even changes in the TEER values may not affect the permeation of curcumin.

In the aspect of formulation design, on the basis of the results of this work, we suggest that solubilization should be the primary target for the formulation design and that micelle encapsulation, with weaker binding with curcumin, may be preferred. Furthermore, it is also implied that lipid-based formulations are promising in improving the solubilization of curcumin. In the lipid-based formulations, curcumin is initially solubilized in lipid phase, and after oral consumption, lipid is digested and turned into mixed micelles, which solubilize curcumin in aqueous solutions and improve the absorption and oral bioavailability of curcumin. For instance, in our previous study, it was shown that curcumin nanoemulsion is able to improve the in vivo anti-inflammatory activity of curcumin.[26]

In summary, a HPLC quantification method was developed to quantify curcumin separately from other coexisting curcuminoids. Using a Caco-2 cell monolayer model, it was revealed that solubilized curcumin permeated rapidly via the mechanism of passive diffusion, suggesting solubilization is the main limiting factor for curcumin absorption. Moreover, it was also suggested that different solubilization agents may affect the permeation rate: protein complexation slowed the permeation, whereas micelle encapsulation accelerated the permeation. This study further implied that lipid-based formulations are promising in improving the oral bioavailability of curcumin, as they are able to solubilize curcumin after digestion and also increase the permeation of solubilized curcumin.

AUTHOR INFORMATION

Corresponding Author

*Phone: (732) 932-7193. Fax: (732) 932-6776. E-mail: qhuang@aesop.rutgers.edu.

Funding Sources

This work was supported by Department of Agriculture National Research Initiative (2009-35603-05071) and in part by Advanced Orthomolecular Research, Inc. (AOR).

REFERENCES

(1) Aggarwal, B. B.; Kumar, A.; Bharti, A. C. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res.* **2003**, *23* (1A), 363–398.

(2) Joe, B.; Vijaykumar, M.; Lokesh, B. R. Biological properties of curcumin — cellular and molecular mechanisms of action. *Crit. Rev. Food Sci. Nutr.* **2004**, *44* (2), 97–111.

(3) Duvoix, A.; Blasius, R.; Delhalle, S.; Schnekenburger, M.; Morceau, F.; Henry, E.; Dicato, M.; Diederich, M. Chemopreventive and therapeutic effects of curcumin. *Cancer Lett.* **2005**, *223* (2), 181–190.

(4) Aggarwal, B. B.; Sundaram, C.; Malani, N.; Ichikawa, H. Curcumin: the Indian solid gold. In *The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease*; Spring: Berlin, Germany, 2007; p 175

(5) Sharma, R. A.; Gescher, A. J.; Steward, W. P. Curcumin: the story so far. *Eur. J. Cancer* **2005**, *41* (13), 1955–1968.

(6) Anand, P.; Kunnumakkara, A. B.; Newman, R. A.; Aggarwal, B. B. Bioavailability of curcumin: problems and promises. *Mol. Pharmaceutics* **2007**, *4* (6), 807–818.

(7) Kaminaga, Y.; Nagatsu, A.; Akiyama, T.; Sugimoto, N.; Yamazaki, T.; Maitani, T.; Mizukami, H. Production of unnatural glucosides of curcumin with drastically enhanced water solubility by cell suspension cultures of *Catharanthus roseus*. *FEBS Lett.* **2003**, *555* (2), 311–316.

(8) Sharma, R. A.; Euden, S. A.; Platton, S. L.; Cooke, D. N.; Shafayat, A.; Hewitt, H. R.; Marczylo, T. H.; Morgan, B.; Hemingway, D.; Plummer, S. M.; Pirmohamed, M.; Gescher, A. J.; Steward, W. P. Phase I clinical trial of oral curcumin. *Clin. Cancer Res.* **2004**, *10* (20), 6847–6854.

(9) Sharma, R. A.; McLelland, H. R.; Hill, K. A.; Ireson, C. R.; Euden, S. A.; Manson, M. M.; Pirmohamed, M.; Marnett, L. J.; Gescher, A. J.; Steward, W. P. Pharmacodynamic and pharmacokinetic study of oral curcuma extract in patients with colorectal cancer. *Clin. Cancer Res.* **2001**, *7* (7), 1894–1900.

(10) Ireson, C. R.; Jones, D. J.; Orr, S.; Coughtrie, M. W.; Boocock, D. J.; Williams, M. L.; Farmer, P. B.; Steward, W. P.; Gescher, A. J. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11* (1), 105–111.

(11) Ireson, C.; Orr, S.; Jones, D. J.; Verschoyle, R.; Lim, C. K.; Luo, J. L.; Howells, L.; Plummer, S.; Jukes, R.; Williams, M.; Steward, W. P.; Gescher, A. Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res.* **2001**, *61* (3), 1058–1064.

(12) Ravindranath, V.; Chandrasekhara, N. In vitro studies on the intestinal absorption of curcumin in rats. *Toxicology* **1981**, *20* (2—3), 251–257.

(13) Suresh, D.; Srinivasan, K. Studies on the in vitro absorption of spice principles — curcumin, capsaicin and piperine in rat intestines. *Food Chem. Toxicol.* **2007**, *45* (8), 1437–1442.

(14) Hidalgo, I. J.; Raub, T. J.; Borchardt, R. T. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **1989**, *96* (3), 736–749.

(15) Artursson, P.; Palm, K.; Luthman, K. Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv. Drug Delivery Rev.* **2001**, *46* (1—3), 27–43.

(16) Artursson, P.; Karlsson, J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* **1991**, *175* (3), 880–885.

(17) Stenberg, P.; Norinder, U.; Luthman, K.; Artursson, P. Experimental and computational screening models for the prediction of intestinal drug absorption. *J. Med. Chem.* **2001**, *44* (12), 1927–1937.

(18) Hubatsch, I.; Ragnarsson, E. G. E.; Artursson, P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nat. Protocols* **2007**, *2* (9), 2111–2119.

(19) Reddy, A. C. P.; Sudharshan, E.; Rao, A. G. A.; Lokesh, B. R. Interaction of curcumin with human serum albumin — a spectroscopic study. *Lipids* **1999**, *34* (10), 1025–1029.

(20) Barik, A.; Mishra, B.; Kunwar, A.; Priyadarsini, K. I. Interaction of curcumin with human serum albumin: thermodynamic properties, fluorescence energy transfer and denaturation effects. *Chem. Phys. Lett.* **2007**, *436* (1−3), 239–243.

(21) Leung, M. H. M.; Kee, T. W. Effective stabilization of curcumin by association to plasma proteins: human serum albumin and fibrinogen. *Langmuir* **2009**, *25* (10), 5773–5777.

(22) Sneharani, A. H.; Singh, S. A.; Appu Rao, A. G. Interaction of α_{S1}-casein with curcumin and its biological implications. *J. Agric. Food Chem.* **2009**, *57* (21), 10386–10391.

(23) Cui, J.; Yu, B.; Zhao, Y.; Zhu, W. W.; Li, H. L.; Lou, H. X.; Zhai, G. X. Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. *Int. J. Pharm.* **2009**, *371* (1−2), 148–155.

(24) Lin, C. C.; Lin, H. Y.; Chen, H. C.; Yu, M. W.; Lee, M. H. Stability and characterisation of phospholipid-based curcumin-encapsulated microemulsions. *Food Chem.* **2009**, *116* (4), 923–928.

(25) Sou, K.; Inenaga, S.; Takeoka, S.; Tsuchida, E. Loading of curcumin into macrophages using lipid-based nanoparticles. *Int. J. Pharm.* **2008**, *352* (1−2), 287–293.

(26) Wang, X. Y.; Jiang, Y.; Wang, Y. W.; Huang, M. T.; Ho, C. T.; Huang, Q. R. Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. *Food Chem.* **2008**, *108* (2), 419–424.

(27) Yu, H.; Shi, K.; Liu, D.; Huang, Q. Development of curcumin organogel with high in vitro bioaccessibility and loading of curcumin. *Food Chemistry* **2011**, in press (DOI: 10.1016/j.foodchem.2011.08.027).

(28) Porter, C. J. H.; Trevaskis, N. L.; Charman, W. N. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov.* **2007**, *6* (3), 231–248.

(29) Tønnesen, H. H. Solubility, chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids, XXVIII. *Pharmazie* **2002**, *57* (12), 820–824.

(30) Iwunze, M. O. Binding and distribution characteristics of curcumin solubilized in CTAB micelle. *J. Mol. Liq.* **2004**, *111* (1−3), 161–165.

(31) Kunwar, A.; Barik, A.; Pandey, R.; Priyadarsini, K. I. Transport of liposomal and albumin loaded curcumin to living cells: an absorption and fluorescence spectroscopic study. *Biochim. Biophys. Acta—Gen. Subj.* **2006**, *1760* (10), 1513–1520.

(32) Peret-Almeida, L.; Cherubino, A. P. F.; Alves, R. J.; Dufoss, L.; Gloria, M. B. A. Separation and determination of the physico-chemical characteristics of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Res Int.* **2005**, *38* (8−9), 1039–1044.

(33) Chan, E. C. Y.; Tan, W. L.; Ho, P. C.; Fang, L. J. Modeling Caco-2 permeability of drugs using immobilized artificial membrane chromatography and physicochemical descriptors. *J. Chromatogr., A* **2005**, *1072* (2), 159–168.

(34) Yamashita, S.; Furubayashi, T.; Kataoka, M.; Sakane, T.; Sezaki, H.; Tokuda, H. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. *Eur. J. Pharm. Sci.* **2000**, *10* (3), 195–204.

(35) Wahlang, B.; Pawar, Y. B.; Bansal, A. K. Identification of permeability-related hurdles in oral delivery of curcumin using the Caco-2 cell model. *Eur. J. Pharm. Biopharm.* **2011**, *77* (2), 275–282.