Considerations in the Use of an Infinite-Dose System for Studying Surfactant Effects on Diffusion in Isolated Cuticles

Moritz Knoche† and Martin J. Bukovac*

Department of Horticulture, Michigan State University, East Lansing, Michigan 48824-1325

The effect of Triton X-100 [TX-100, α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl)] at concentrations below and above the critical micelle concentration (cmc, cmc = 0.019% w/w) on diffusion of 2-(1-[^{14}C]naphthyl)acetic acid (NAA, 1 μM) through isolated tomato (*Lycopersicon esculentum* Mill. cv. Pik Red) fruit cuticular (CM) and dewaxed cuticular membranes (DCM) was studied using an infinite-dose system. TX-100 significantly increased time lags of NAA diffusion from 5 h (NAA only) and 5.8 h (0.01% w/v TX-100) to 7.4 h (0.1% TX-100) and 7.1 h (1% TX-100) when averaged over CM and DCM. Diffusion time lags in the absence and presence of TX-100 were greater for CM (6.1 h) than for DCM (5.2 h). In CM, 0.01% TX-100 increased NAA flux, while 0.1% had no effect and 1% TX-100 decreased the flux. In DCM, both 0.01 and 0.1% TX-100 enhanced the rate of NAA penetration, while flux was reduced in the presence of 1% TX-100. Two different approaches for calculating partition and diffusion coefficients are presented, and their suitability for analyzing effects of spray additives on cuticular penetration of an active ingredient is discussed using the example of TX-100 effects on NAA penetration.

INTRODUCTION

Surfactants are commonly used in formulations of agrochemicals (Holloway and Stock, 1990). They may increase (Lownds et al., 1987; Knoche and Bukovac, 1991; Stevens and Bukovac, 1987), have no effect on, or decrease foliar uptake of the active ingredient (ai) (Knoche et al., 1991). The mode of surfactant action in enhancing foliar penetration is not well understood (Holloway and Stock, 1990). Surface tension related phenomena, i.e., increase of contact area between spray droplet and absorbing surface, modification of droplet drying, etc., can only account for part of the surfactant effect on foliar uptake. In field and laboratory studies surfactants frequently increase efficacy of agrochemicals at concentrations above the critical micelle concentration (cmc) (Holloway and Stock, 1990; Holloway et al., 1992; Jansen, 1964). Since surface tension remains constant at concentrations above the cmc, this increase cannot be attributed to surface tension related properties. Instead, this observation suggests a surfactant/cuticle interaction leading to enhanced permeability and, thus, ai penetration.

Cuticular penetration is the rate-limiting step in foliar uptake (Jansen, 1964), and isolated cuticles are often used to study the interactions between ai and cuticle occurring during cuticular penetration (Bukovac and Petracek, 1993; Bukovac et al., 1990; Schönherr and Riederer, 1989). Infinite-dose systems have been used to investigate cuticular penetration of agrochemicals (Kerler et al., 1984; Schönherr and Riederer, 1989). In these systems, diffusion of an ai is followed from a donor solution across an interfacing cuticle into a receiver solution (Kerler et al., 1984). Infinite-dose systems eliminate problems associated with penetration studies involving living tissue and the drying of spray droplets and provide a high degree of control under defined conditions (Bukovac and Petracek, 1993). The steady-state conditions obtained in such systems allow for a mechanistic analysis of the penetration process. Provided that certain assumptions are fulfilled, the penetration process may be divided into various components and meaningful transport coefficients, e.g., partition, diffusion, and permeability, can be calculated (Bukovac and Petracek, 1993). These coefficients are useful in comparing and analyzing penetration characteristics of various agrochemicals and plant cuticles.

Expanding the use of infinite-dose diffusion studies to assess the effect of formulation additives on ai penetration may be equally useful. For example, relating the effect of a surfactant to different components of the penetration process (i.e., effects on sorption, diffusion, and desorption) may be an effective strategy for improving screening procedures to optimize pesticide formulations.

The purpose of our contribution was to investigate how infinite-dose studies and steady-state analysis of penetration may be applied to quantify effects of surfactants on transcuticular movement of pesticides. Data on the short-term and long-term effect of Triton X-100 (TX-100) on penetration of 2-(1-[^{14}C]naphthyl)acetic acid (NAA) through an isolated tomato fruit cuticular membrane served as a model.

MATERIALS AND METHODS

Cuticle Isolation. Cuticular membranes (CM) were enzymatically isolated from locally field-grown mature tomato (*Lycopersicon esculentum* Mill. cv. Pik Red) fruit (Orgell, 1955; Yamada et al., 1964), rinsed thoroughly in deionized water, dried, and stored at room temperature. Cuticular waxes were extracted from part of the isolated CM by batch extraction in chloroform/methanol (1:1 v/v) at 45 °C. Extraction solutions were changed 10 times over a 72-h period, and cuticles were subsequently dried. CM with wax removed will be referred to as dewaxed CM (DCM).

Chemicals. Donor solutions, simulating agricultural spray solutions, were prepared with NAA (specific activity 2.3 GBq mmol^{-1}, 98.7% radiochemical purity by TLC, Amersham Corp., Arlington Heights, IL) at a concentration of 1 μM NAA and buffered with 20 mM citric acid. The pH was adjusted to 3.2 using NaOH, which is 1 pH unit below the pK_a of NAA. Addition of 1 mM Na_3N prevented microbial growth. For surfactant treatments, a commercial preparation of TX-100 (Rohm and Haas, Philadelphia, PA; cmc = 0.019% w/w) with an average of

† Present address: DLO-Centre for Agrobiological Research (CABO-DLO), P.O. Box 14, 6700 AA Wageningen, The Netherlands.

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9.5 oxyethylene units was added at concentrations of 0.01, 0.1, and 1.0% w/v in the short-term study and 0.01% w/v in the long-term study. This surfactant was selected because (1) it is a common constituent of agricultural pesticide formulations and spray solutions and (2) this and closely related surfactants have been shown to enhance foliar uptake of agrochemicals (Lownds et al., 1987; Knoche and Bukovac, 1991; Stevens and Bukovac, 1987). Buffer solutions without NAA and surfactant were used as receiver solutions.

Experimental Procedure. The diffusion apparatus and experimental procedure have been described previously (Kerler et al., 1984). Briefly, cuticles were mounted in plexiglass holders between two glass half-cells using vacuum grease. Cuticles were prehydrated and leak tested by adding 10 and 5 mL of buffer to the donor and receiver cells, respectively. This created a slight hydrostatic pressure (water column height ca. 30 mm) on the morphological outer side of the cuticle. Diffusion cells were held in a thermostated water bath at 25 ± 1 °C. Upon initiation of the experiment, the buffer solution of the donor cell was replaced with 5 mL of the appropriate treatment solution. The morphological outer surface of the cuticle was always oriented to the donor solution. Solutions were stirred vigorously with magnetic stir bars. At selected time intervals (generally 2-h intervals between 16 and 26 h after initiation of the short-term experiment) 1-mL aliquots were removed from the receiver cell, radioassayed by liquid scintillation spectrometry (Model 1211 Rackbeta, LKB Wallac, Turku, Finland) and replaced with cold receiver solution. This sampling time period was selected since flow was expected to be at steady state within 2.7 times the diffusion time lag (Rogers, 1985) and preliminary studies have shown that the time lags for NAA diffusion were less than 6 h (Knoche and Bukovac, unpublished results). In the long-term study, receiver solutions were sampled at 2-h intervals over five time periods, namely, 16–26, 40–52, 64–70, 88–100, and 112–124 h after initiation of the experiment.

Data Analysis. After steady state was achieved (i.e., when flow rate becomes independent of time), NAA flux (J) was calculated on the basis of the exposed cuticle area (A) and the flow rate (F) was obtained from the slope of a regression line fitted to the linear portion of a plot of the amount of NAA penetrated vs time (eq 1, Figure 1). Diffusion time lag (t_e) was calculated from the x-axis intercept of the extrapolated regression line. Permeance (P) and diffusion (D) coefficients were calculated using eqs 2 and 3, respectively (Figure 1; Crank and Park, 1968). Equation 4 describes the relationship between the permeance, diffusion, and partition (K) coefficients (Figure 1). Equation 4 may also be used to calculate the steady-state partition coefficient (K_{trans}). Cuticle thickness (ΔX) was calculated from cross-sectional areas of cuticular disks, cuticle weights, and specific densities (CM, 1.21 mg cm^{-3}; DCM, 1.19 mg cm^{-3}; Petracek, 1991). Data were subjected to analysis of variance where appropriate. Unless otherwise specified, treatment means were compared by Duncan's multiple-range test (P = 0.05).

RESULTS AND DISCUSSION

Considerable variation of NAA penetration was observed across all experiments (Figures 2 and 4), although all cuticles were isolated from fruits collected from a common plot during the same growing season. Similar variability has been reported in other studies (Geyer and Schönherr, 1988; Knoche and Bukovac, 1993; Petracek, 1991). NAA diffusion in the absence and presence of TX-100 was linearly related with time in both CM and DCM between 16 and 26 h after initiation of the experiment (Figure 2). Below the cmc, at 0.01%, TX-100 increased the flux of NAA across the CM, while concentrations above the cmc had no effect on (0.1% TX-100) or decreased (1.0% TX-100) the flux (Figure 2). A similar relationship between TX-100 concentration and NAA flux was observed for DCM at 0.01 and 1.0% TX-100 (Figure 2). However, at 0.1%, TX-100 doubled the flow rate of NAA in DCM, while there was no significant effect in the CM (Figure 2).

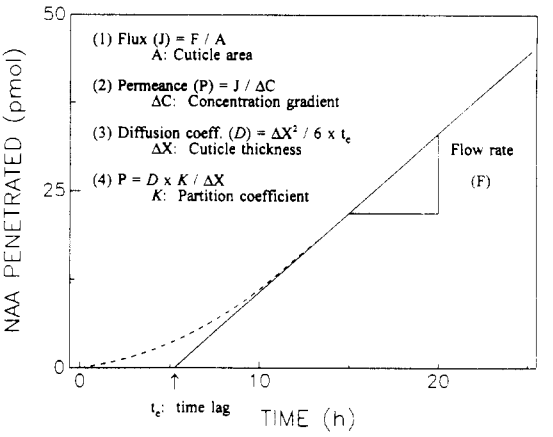

Figure 1. Example of analysis of the time course of an infinite-dose diffusion experiment (for explanation of equations see text).

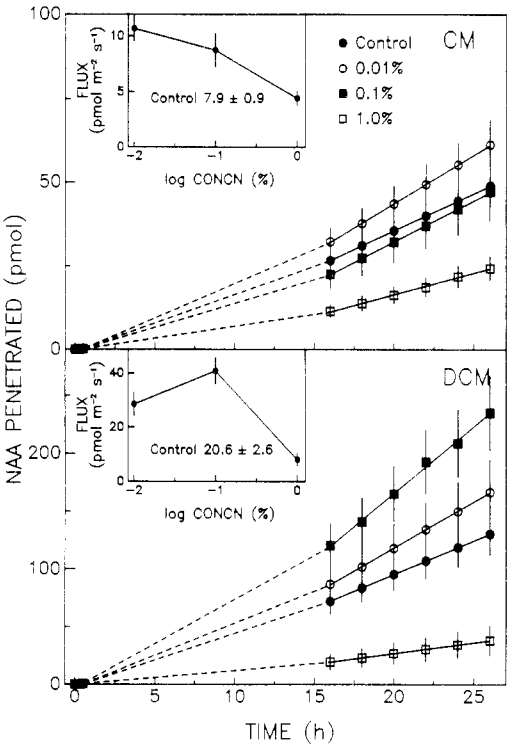

Figure 2. Effect of TX-100 on NAA diffusion across cuticular (CM) and dewaxed cuticular (DCM) membranes. (Insets) Flux of NAA across CM and DCM as a function of TX-100 concentration. Bars represent standard errors of the mean. Numbers of observations were 85, 29, 41, and 20 for CM without and with 0.01, 0.1, and 1.0% TX-100 and 22, 14, 9, and 5 for the DCM treatments, respectively.

The permeance coefficient for NAA diffusion paralleled the change in NAA flux (Figure 3B) when calculated using the total amount of NAA in the donor solution as the concentration gradient (eq 2, Figure 1). The relative surfactant effect was similar for CM and DCM at 0.01 and 1% TX-100, while 0.1% TX-100 increased NAA permeance in DCM but not in CM (Figure 3A). Generally, permeance coefficients were 2–3 times greater for DCM compared to those for CM (Figure 3B).

Mechanistically, the surfactant-induced change in the NAA permeance coefficient may be related to a change in D and/or a change in K (eq 4, Figure 1). The diffusion coefficient describes the mobility of NAA in the cuticle. The partition coefficient characterizes the affinity (solubility) of NAA for the cuticle and, thus, the steepness of the concentration gradient in the cuticle. Two approaches are generally used in calculating D and K:

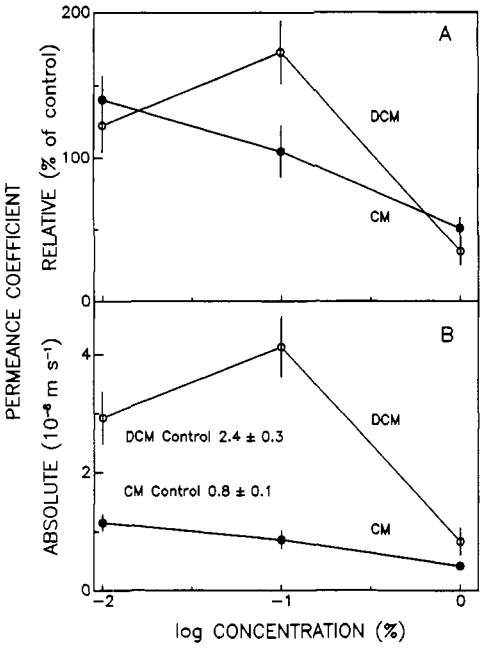

Figure 3. Effect of TX-100 concentration on relative (A) and absolute (B) permeance coefficients of NAA in cuticular and dewaxed cuticular membranes. Bars represent standard errors of the mean.

Table 1. Effect of TX-100 Concentration on Time Lag (t_e) of NAA Diffusion through Cuticular (CM) and Dewaxed Cuticular Membranes (DCM)[a]

	diffusion time lag, h		
TX-100, % (w/v)	CM	DCM	mean[b]
0	5.2	4.1	5.0b
0.01	5.7	5.9	5.8b
0.1	7.7	6.2	7.4a
1.0	7.3	6.4	7.1a
mean[b]	6.1a	5.2b	

[a] Numbers of observations were 85, 29, 41, and 20 for CM without and with 0.01, 0.1, and 1.0% TX-100 and 22, 14, 9, and 5 for the DCM treatments, respectively. [b] Means followed by the same letter are not significantly different, Duncan's multiple-range test, $P = 0.05$.

(i) Time Lag Concept. Calculate time lag (t_e), cuticle thickness (ΔX), and D (eq 3, Figure 1). The *steady-state flux partition coefficient* K_{trans} can then be calculated using eq 4 (Figure 1) after solving for K.

(ii) Direct Measurement. The *equilibrium partition coefficient* K_{sorp} is determined in a separate sorption experiment by solving eq 4 (Figure 1) for D.

Time Lag Derived D. In homogeneous ideal membranes, D is inversely related to t_e (eq 3, Figure 1). DCM can be considered ideal for many active ingredients, while CM generally are not (Schönherr and Riederer, 1989). Time lags for NAA diffusion increased significantly at 0.1 and 1.0% TX-100 and were greater for CM than for DCM (Table 1). Hence, D for DCM derived from eq 3 (Figure 1) decreased from 3.0×10^{-15} m^2 s^{-1} (NAA only) to 1.3×10^{-15} m^2 s^{-1} (NAA + 1% TX-100) upon addition of increasing concentrations of TX-100 (Table 2). The steady-state flux partition coefficient K_{trans} increased at 0.01 and 0.1% TX-100 but decreased at 1% TX-100. These calculations would suggest that the increase in permeance of DCM at 0.01 (t-test significant at $P = 0.12$) and 0.1% TX-100 (t-test significant at $P = 0.0001$) was related to increased NAA affinity (K) for the cuticle and decreased NAA mobility (D) in the cuticle. Accordingly, a decrease of K and D caused the decrease in permeance at 1% TX-100 (t-test significant at $P = 0.002$).

Table 2. Effect of TX-100 Concentration on Permeance (P), Diffusion (D), and Partition (K) Coefficients of NAA in Dewaxed Cuticular Membranes[a]

	transport coefficients				
		time lag method[b]		sorption method[c]	
TX-100 % (w/v)	P, 10^{-8} m s^{-1}	D, 10^{-15} m^2 s^{-1}	K_{trans}	D, 10^{-15} m^2 s^{-1}	K_{sorp}
0	2.4 ± 0.3	3.0 ± 0.5	125	1.7	194
0.01	2.9 ± 0.4	1.9 ± 0.3	244	2.1	201
0.1	4.1 ± 0.5	2.1 ± 0.2	321	4.4	151
1.0	0.8 ± 0.2	1.3 ± 0.1	89	4.6	22

[a] Numbers of observations were 22, 14, 9, and 5 for DCM without and with 0.01, 0.1, and 1.0% TX-100, respectively. [b] Time lag method: D was calculated from the time lag of diffusion (eq 3, Figure 1) and the steady-state K_{trans} obtained from eq 4 (Figure 1). [c] Sorption method: equilibrium K_{sorp} was obtained from a sorption experiment (Shafer and Bukovac, 1989), and D was derived using eq 4 (Figure 1).

Derivation of D Using K_{sorp}. Sorption studies on the effect of TX-100 concentration on NAA partitioning into cuticles revealed that, below the cmc, K_{sorp} was not significantly affected by surfactant. However, above the cmc, increasing TX-100 concentration decreased K_{sorp} (Shafer and Bukovac, 1989). This decrease of K_{sorp} was related to solubilization, i.e., trapping, of NAA in surfactant micelles (Heredia and Bukovac, 1990, 1992; Shafer and Bukovac, 1989). Because micelles are too large to penetrate the cuticle, solubilization of NAA decreased the effective NAA concentration in solution, thus the driving force for NAA sorption. According to eq 4 (Figure 1) a change in P for a given membrane can be caused by an altered D and/or K. On the basis of this relationship a D was calculated using K_{sorp} derived from sorption studies and using P measured in our penetration experiments. Derivation of D using K_{sorp} showed that the change in P in DCM was the net effect of an increase in D (control vs 0.01 vs 0.1% and 1% TX-100, respectively) and a marked decrease in K_{sorp} at concentrations above the cmc (control and 0.01 vs 0.1 vs 1.0% TX-100; Table 2).

The discrepancies between the values based on the two methods are striking and deserve further discussion. A comparison of steady-state K_{trans} and equilibrium K_{sorp} revealed marked differences between the two partition coefficients, which—in an ideal system—should be identical (Table 2). In our study K_{trans} was smaller than K_{sorp} in the absence of TX-100, while K_{trans} exceeded K_{sorp} by 1.2-, 2.1-, and 4.0-fold in the presence of TX-100 at 0.01, 0.1, and 1%, respectively (Table 2). Several factors need to be considered.

First, the assumption that DCM represent an ideal membrane needs to be re-evaluated. The equilibrium K_{sorp} could exceed steady-state K_{trans} if sorption "sites" were not quantitatively involved in the diffusion process, for example, due to irreversible sorption. Although covalent binding, hence irreversible association, of NAA to epoxy groups in pepper fruit cuticles has been observed, there was no indication for covalent binding of NAA in tomato fruit cuticles (Shafer and Bukovac, 1987). Alternatively, if the relative contribution of sorption sites to diffusion differed among sites (e.g., by varying binding strength), deviations between equilibrium K_{sorp} and steady-state K_{trans} would also be expected. Varying binding strength, however, is unlikely to lead to linear, constant partitioning type isotherms for the system NAA/cuticle (Bukovac et al., 1990). Therefore, it seems reasonable to assume that the tomato fruit DCM behave as ideal membranes.

Second, K_{trans} was derived indirectly from a measured P and a calculated D; thus, any errors inherent in the

Table 3. Effect of Overestimating Cuticle Thickness (ΔX) on the Diffusion Coefficient (D) and Partition Coefficient (K_{trans}) of NAA in a Dewaxed Cuticular Membrane (for Details See Text)

cuticle thickness		transport coefficient	
relative, %	absolute, μm	D, 10^{-15} m^2 s^{-1}	K_{trans}
100	13.9[a]	1.7[a]	125[a]
80	11.1	1.1	156
60	8.4	0.6	208
40	5.6	0.3	312
20	2.8	0.1	624

[a] These data are based on a specific cuticle (no. 4) because its permeability characteristics matched the average (flux, 14.3 pmol m^{-2} s^{-1}; time lag, 5.2 h; permeance coefficient, 1.5×10^{-8} m s^{-1}) and, thus, this cuticle was considered representative of the cuticle population used in the short-term penetration study.

calculation of D affect K_{trans}. The estimation of D using time lag involves the calculation of ΔX from membrane area, weight, and specific density (eq 3, Figure 1). This "gravimetric" thickness, however, may not correspond to the actual length of the diffusion path, if cuticles—as in mature tomato fruits (Bukovac et al., 1990)—encase epidermal cells. Extensive hypodermal development would increase the weight per unit area but not necessarily contribute to diffusive resistance. Also, the length of the path through the membrane may not be related to cuticle thickness. An overestimation of ΔX would lead to overestimation of D (eq 3, Figure 1) and, hence, underestimation of K_{trans} (eq 4, Figure 1). If ΔX was overestimated by about 30%, K_{trans} approximately equals K_{sorp} (Table 3), which was reported to be 188 for the system NAA/ DCM (Bukovac et al., 1990). An error of about 30% in estimation of ΔX would be in agreement with direct thickness measurements of (a different batch of) tomato fruit cuticles, revealing total averages of 10.5 μm, while thickness between epidermal cell wall and outer morphological surface averaged 7.0 μm (Petracek, 1991). The difference between K_{trans} and K_{sorp} in the absence of a surfactant therefore, at least in part, may be attributed to the gravimetric thickness estimation.

However, the above explanation does not account for the discrepancy between K_{trans} and K_{sorp} in the presence of TX-100. Since the same ΔX is involved, K_{trans} would even further increase compared to K_{sorp} (Table 2). Theoretically, K_{trans} cannot exceed K_{sorp} unless the transport mechanism changes dramatically (e.g., from diffusion to mass flow). There is no evidence that such changes occur. Alternatively, K_{trans} would be overestimated if D was underestimated (e.g., by an overestimation of time lag). In turn, t_e may be overestimated if the flux of NAA across the cuticle increases with time. For the sampling times of our short-term experiments (16–26 h), NAA flux appeared to be linear (in most cases $r^2 \geq 0.99$) and independent of time (Figure 2). However, changes of NAA flux may occur over an extended period of time (>26 h) if the surfactant alters cuticle properties. Therefore, we conducted a long-term penetration study and followed the time course of NAA penetration up to 120 h (Figure 4). The long-term study demonstrated that when 0.01% surfactant and NAA were added simultaneously to the donor cell, NAA flux through CM and DCM slowly increased with time up to 94 h (Figure 4). In contrast, in the absence of a surfactant or when cuticles were pre-equilibrated with 0.01% surfactant (for 48 h, surfactant present in donor and receiver solution), NAA penetration was linearly related with time and NAA flux was constant (Figure 4). Since cuticles were pre-equilibrated with surfactants present in both donor and receiver solutions,

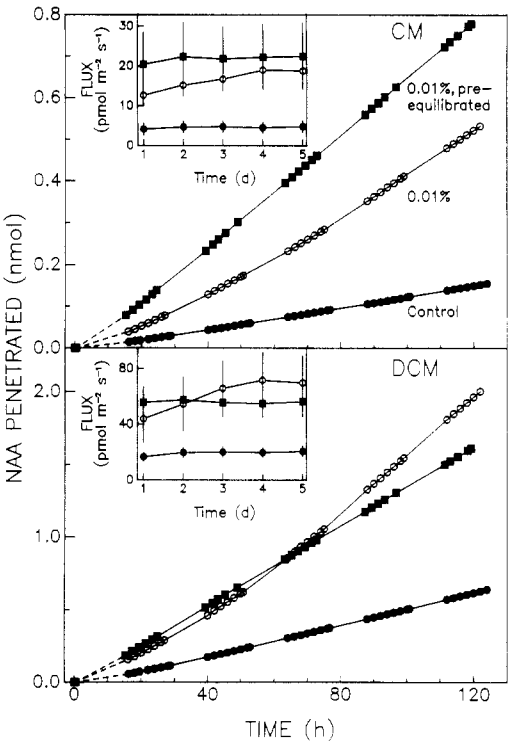

Figure 4. Effect of TX-100 (0.01%) on long-term time course of NAA diffusion across cuticular (CM) and dewaxed cuticular (DCM) membranes. Preequilibrated cuticles were equilibrated with surfactant in donor and receiver for 48 h prior to initiation of the NAA penetration study. (Insets) Flux of NAA across CM and DCM in the presence and absence of TX-100 as a function of time. Data symbols represent averages of five to six replications.

a 48-h pre-equilibration period was sufficient to ensure a constant NAA flux. Without pre-equilibration of the cuticle with surfactant, t_e changed, i.e., increased, with the time period used for the calculation; thus, under these conditions a meaningful D cannot be calculated from diffusion time lags. The nonlinear time course of NAA penetration in the presence of surfactant (without a pre-equilibration period) is most likely related to direct effects of the surfactant on the cuticle. First, sorption of surfactant monomers may cause swelling or plasticize the CM and/or DCM, which in turn would increase cuticle permeability (Knoche and Bukovac, 1993; Schönherr, 1993). Second, surfactant micelles were shown to solubilize cuticular wax and wax solubilization would increase permeability of the CM (Tamura and Bukovac, 1990). Both effects are expected to be time dependent and therefore could account for the increase of NAA flux with time. On the other hand, surfactant interaction with the ai in solution, i.e., solubilization of the ai by micelles, will occur almost instantaneously and, hence, would not account for the time dependence of NAA penetration. Although at present we do not know the molecular mechanism of the surfactant effect, direct effects of surfactant on the cuticle are the more likely explanation.

The advantage of approach ii over approach i is that the transport coefficients are not dependent on the time to establish a constant flow rate. In the hypothetical situation depicted in Figure 5, approach ii, i.e., calculation of D using K_{sorp}, will yield identical transport coefficients irrespective of pre-equilibration of the cuticle with surfactant (Figure 5, curves A–C). In contrast, approach i, i.e., time lag derived D, would only yield a meaningful D if the cuticle was pre-equilibrated with surfactant for a sufficient period of time (Figure 5, curve C). Further, for any compound that interacts directly with the cuticle and

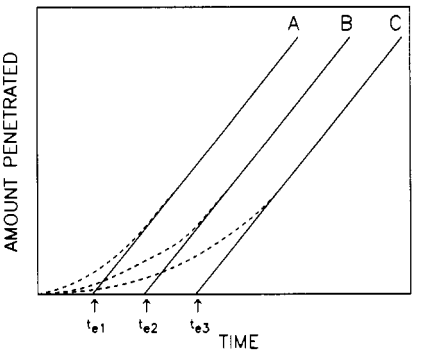

Figure 5. Hypothetical curves depicting the effect of pre-equilibration of the cuticle with surfactant on diffusion time lag. (Curve A) Cuticle pre-equilibrated; (curve B) not fully equilibrated: (curve C) cuticle not pre-equilibrated.

alters its permeability, a meaningful time lag derived D requires successful pre-equilibration of the cuticle prior to initiation of the penetration study. Clearly, achieving a constant ai flux remains an essential requirement also for approach ii, since the time course for establishing equilibrium in sorption studies may not be representative for penetration studies. In sorption studies cuticles are usually incubated in donor solutions and, thus, the ai can be sorbed simultaneously from both the morphological outer and cell wall sides of the cuticle. In penetration studies, however, ai sorption to the cuticle can only proceed unilaterally from the donor solution through the morphological outer surface of the cuticle. Hence, equilibrium is attained more rapidly in sorption than penetration studies.

Since only an apparent steady-state flux was established in our short-term studies in the presence of the surfactant, transport coefficients cannot be quantified. However, on the basis of our data and the arguments presented above, the following qualitative conclusions can be drawn. The biphasic surfactant concentration response of NAA permeance in DCM was the net effect of two opposing processes. (1) NAA mobility (D) must have increased with increasing surfactant concentration up to the cmc (control vs 0.01 vs 0.1 and 1.0% TX-100), and (2) the amount of NAA sorbed to the cuticle (K_{sorp}) decreased as the concentration of TX-100 micelles was increased at concentrations above the cmc (control and 0.01% TX-100 vs 0.1 and 1.0% TX-100; see also sorption method in Table 2). Similarly, the same qualitative explanations must hold for CM. Below the cmc, NAA mobility must have increased with increasing concentration of surfactant monomers, while above the cmc surfactant micelles decreased the effective NAA concentration. The differential response between CM (no effect on NAA penetration) and DCM (increased NAA penetration) at 0.1% TX-100 may have been related to the nonequilibrium conditions in the short-term time course study. Time lags for TX-100 diffusion through tomato fruit cuticles were longer for CM than for DCM (8.2 vs 5.1 h; Petracek, 1991) and, hence, DCM will equilibrate faster with TX-100 than CM. Therefore, the penetration-enhancing effect of surfactant monomers probably was greater, i.e., closer to steady state, in DCM than in CM, while micelles instantaneously decreased the effective driving force for NAA penetration in CM and DCM. This interpretation is supported in part by data from a long-term study of NAA penetration through CM in the presence of Triton X surfactants (Knoche and Bukovac, 1993) demonstrating that, upon surfactant addition, micelles decreased NAA flux instantaneously (immediate decrease of K_{sorp}), while the penetration-enhancing effect of surfactant monomers (effect on D) always developed with time. In fact, when TX-165 (16 EO) was added at concentrations 2.5-fold above the cmc, NAA flux only temporarily decreased but increased again and approached a new steady-state thereafter (Knoche and Bukovac, 1993). A similar response was observed with TX-100 at 0.1% (Knoche and Bukovac, unpublished results), where a temporary decrease of NAA flux was followed by an increase of NAA penetration. The NAA flow approached a new steady state at a higher rate than in the absence of the surfactant (Knoche and Bukovac, unpublished results).

Clearly, further studies are necessary before final conclusions can be drawn. The following two questions are particularly important: First, what is the effective driving force of an ai at surfactant concentrations above the cmc, where micelles solubilize the ai? Unless the distribution of the ai between micelle and the bulk (nonmicellar) solution can be quantified, only *apparent* permeance coefficients can be calculated on the basis of the total ai in the system. Second, what is the role of surfactant micelles in enhancing cuticular permeability? Our data for DCM suggested indirectly that the role of micelles was limited to solubilization of the ai, since D remained essentially constant at TX-100 concentrations above the cmc (Table 2; D derived using K_{sorp}). At present we do not know whether this observation is limited to DCM. On the basis of the concentration dependence of wax solubilization (Tamura and Bukovac, 1990), one would expect the diffusion coefficient of NAA in CM to increase when the surfactant concentration is increased above the cmc. For these reasons, further studies should focus on the ai flux in the presence of a surfactant over an extended period of time. Additionally, selection of surfactant concentrations bridging a close range around the cmc as well as concentrations well above the cmc may become a critical key to a better understanding of the role of surfactant micelles on the surfactant effect of ai penetration.

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