Self-Association of Plant Wax Components: A Thermodynamic Analysis

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Excess specific heat, \( C_p^E \), of binary mixtures of selected components of plant cuticular waxes has been determined. This thermodynamic parameter gives an explanation of the special molecular arrangement in crystalline and amorphous zones of plant waxes. \( C_p^E \) values indicate that hydrogen bonding between chains results in the formation of amorphous zones. Conclusions on the self-assembly process of plant waxes have been also made.

Introduction

The surface of the aerial parts of plants are covered by the cuticle, which serves as a barrier between the plant and its environment. The outermost part of the cuticle is mainly composed by a complex mixture of acyl lipids, commonly named cuticular waxes. Cuticular waxes play important roles in helping plants resist drought, pathogens, and insects and in protecting them from UV irradiation. They also constitute the main barrier limiting the transport across the plant—atmosphere interface.1,2

Cuticular waxes are complex mixtures of very long chain fatty acids, alcohols, aldehydes, esters, and \( n \)-alkanes.3 In some plants, other compounds such as cyclic terpenoids and phenolics can be also present. These mixtures of nonpolar compounds are deposited at continuous or very frequent intervals of time in the outer surface of leaves and fruits forming crystalline and amorphous regions and follow a mechanism that still constitutes one of the mysterious aspects of cuticular wax production. On the other hand, it has been established that epicuticular crystalline-amorphous structures most likely result from the physical-chemical properties of the wax rather than as a result of a specific transport process.4

According to Reynhardt and Riederer,5 the structure of a wax can be described in terms of crystalline and amorphous zones. The crystalline fraction of a wax5 (termed zone A) is the most important one. In this zone, the chain molecules are stacked in a regular fashion. These crystalline aggregates (assembled in an orthorhombic or hexagonal crystal lattice) form laterally extended structures such as platelets or flakes within the wax. The distribution in chain lengths results in an amorphous region B between the chain ends of adjacent layers of chains. Since the chains of a plant wax consist of a number of different compounds, the differences in the chains lead to steric hindrances and disruption of the geometric homogeneity of the structure. Wax compounds of low melting point (short-chain aliphatics) or cyclic compounds are excluded from zone A and they form the zone C, a solid rigid amorphous region.5

Information and study on the driving force of the self-assembly and arrangement of the different components of plant epicuticular waxes is important in order to understand its function as the transport barrier of the plant cuticle on a molecular level. For this reason, detailed research focused on the influence of the concentration and types of specific components in the final structure of plant waxes is necessary.

It is well established that the specific heat (or heat capacity), \( C_p \), of a molecule is the most sensitive thermodynamic indicator of structure.6 Depending on the nature of their individual components, the thermodynamic behavior of a solid—solid solution often shows pronounced nonideality owing to the formation of intermolecular interactions. The \( C_p^E \) change on mixing, i.e., the excess heat capacity, gives useful structural information on the behavior of the corresponding solute in solution.7 It might be particularly interesting for the case of solid solutions or mixtures formed by the different components of plant waxes. In this sense, the present work specifically deals with the determination of the specific heat of mixtures of plant wax components of different nature. Conclusions on the self-assembly process of plant epicuticular waxes are also made.

Experimental Section

Wax Components. All chemicals used (oleanolic acid, \( n \)-hexacosanol, \( n \)-tetracosanoic acid, tritiated and the wax ester of 42 carbon atoms, arachidyl tetracosanoate) in this work were obtained from Sigma or Aldrich Chemical Co. Chemical structures of these compounds are showed in Figure 1. Their stated purities were always better than 98% and used without further purification. Binary mixture samples were made by solving the corresponding amount of each wax component in a small volume of chloroform (Aldrich, purity > 99%). The organic solvent was evaporated at room temperature and the dry residue constituted the wax mixture that was used in the standard calorimetric assay.
Specific Heat Measurement. Calorimetric experiments were performed on a differential scanning calorimeter (DSC) from Shimadzu Corp, model DSC-50. Scan rate of 5 °C per minute was used in the temperature range 273–330 K. The quantities used of wax samples were between 3 and 10 mg. All specific heat traces are given in units of J/g K. The apparent specific heat of component 1 of the binary mixture was calculated using the equation

$$\phi_{C1} = \left[ C_p(mixture) - \chi_2 C_p^2 \right] / \chi_1 \quad (1)$$

where $\phi_{C1}$ is the apparent specific heat of component 1, $C_p(mixture)$ is the specific heat of the mixture at 298 K, $\chi_2$ is the weight fraction of the component 2, $\chi_1$ the corresponding weight fraction of component 2, and $C_p^2$ is the specific heat of component 2 at 298 K. Specific heat of individual wax compounds were measured at 298 K using aluminum oxide as reference.

Excess specific heat of each component of the corresponding mixtures, $C_p^E$, was calculated from the equation

$$C_p^E = \chi_1 (\phi_{C1} - C_p^1) \quad (2)$$

Results and Discussion

In the present work two main type of wax mixtures, in terms of specific heat determinations, were analyzed and discussed. The first one corresponded to the different mixtures of the long chain alcohol $n$-hexacosanol with two long chain aliphatic compounds frequently found in plant waxes: the paraffin wax $n$-tritiacontane (the C33 alkane) and the wax ester arachidyl tetracosanoate shown in Figure 1. The $C_p$ of these binary mixtures at 298 K is shown in Figure 2 for different weight percentages. The shape of the curves indicates that the mixtures of these wax components do not represent an ideal solution of the two wax components (solid–solid solution) since the measured heat capacity does not represent the mean average of the $C_p$ contribution of each compound. This is due to the existence of intermolecular interactions between the two components of the binary mixture. The mixtures formed between the long chain ester and the long chain alcohol show more deviation from an ideal mixture than the mixtures with the long chain alkane and with the long chain alcohol.

On the other hand, two-component mixtures of the cyclic triterpenoid oleanolic acid, present in some plant waxes, and the three wax compounds mentioned above exhibited specific heat values at 298 K shown in Figure 3. In these cases, except for one unique mixture, we can affirm that the corresponding solid–solid solutions were near the ideality, i.e., the $C_p$ values measured are, between the experimental errors, the mean average of the $C_p$ contributions of each compound.

The excess specific heat, $C_p^E$, at 298 K of the mixtures discussed above are presented in Table 1. These values were calculated from the experimental results of the apparent specific heat of each wax compound at different weight percentages (eq 1) and from eq 2. Since $C_p^E$ is a sensitive measure of the forming and breaking of structure in solid–solid solutions, it is interesting to compare the situation for the two types of mixtures aboved described. $C_p^E$ is clearly negative throughout the concentration range for the mixtures.

![Figure 1](image1.png)

**Figure 1.** Chemical structures of the plant wax compounds studied in this work.

![Figure 2](image2.png)

**Figure 2.** Specific heat, $C_p$, of the different mixtures of $n$-hexacosanol and the $n$-alkane tritiacontane (●) and the wax ester arachidyl tetracosanoate (○) at 298 K.

![Figure 3](image3.png)

**Figure 3.** Specific heat, $C_p$, of the different mixtures of the triterpenoid oleanolic acid and $n$-hexacosanol (●), the wax ester arachidyl tetracosanoate (○) and the $n$-alkane tritiacontane (▽) at 298 K.

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<tr>
<th>Table 1. Calculated Excess Specific Heat, $C_p^E$ (J/g K), for the Different Wax Mixtures at 298 K</th>
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<td>binary mixtures</td>
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* A mixtures: oleanolic Acid + $n$-hexacosanol (A1), tritiacontano (A2) and long chain wax ester (A3). B mixtures: $n$-hexacosanol + tritiacontano (B1) and long chain wax ester (B2). For more details, see text.
of aliphatic wax compounds (Figure 2 and Table 1). Negative $C_p^E$ is an indication that there is a decrease in structure of the short-range orientational order. For B mixtures this is due to the interaction of the $n$-hexacosanol with the two other aliphatic compounds.7 In this case, the molecular order is destroyed by the aliphatic compounds, and the self-association of the long chain alcohol can produce a zone type A (see Introduction) can be accompanied by the formation of a more solid amorphous zone, type B, where differences in the chains lead to steric hindrances and disruption of the geometric homogeneity of the structure. This situation is the chains lead to steric hindrances and disruption of the geometric homogeneity of the structure. This situation is more evident in the case of the $n$-hexacosanol/long chain ester mixture (B2) where $C_p^E$ becomes more negative as the content of long chain ester increases, probably due to the possible formation of intermolecular hydrogen bonds between the alcohol and the ester. As some authors have recently reported, the formation of hydrogen bonds in plant waxes should prevent phase separation of the shorter and longer chain distributions, reducing the crystallinity and increasing the melting point of the waxes.9,10 In this sense, confirmation of the coexistence of A and B zones have been confirmed in the cuticular waxes isolated and reconstituted from leaves of Hordeum vulgare (barley).9 X-ray powder diffraction of this type of waxes indicated than the presence of intermolecular hydrogen bonding in the wax influences in its structure in two main ways: first, preventing phase separation of the shorter and longer chains of the components and, second, resulting in a relatively large amorphous zone.

$C_p^E$ values for mixtures of the cyclic triterpenoid oleanolic acid with aliphatic wax compounds were slightly negatives or practically null (Table 1). Only the mixture with $n$-hexacosanol seems to induce a lowering in structure, probably due to some hydrogen bond interactions between the alcohol and the ester. As some authors have recently reported, the formation of hydrogen bonds in plant waxes should prevent phase separation of the shorter and longer chain distributions, reducing the crystallinity and increasing the melting point of the waxes.9,10

Following the structural model of plant waxes above exposed, in these cases the two mixture components follow, independently of each other, their respective molecular characteristics producing near ideal solid–solid solutions. In this case the coexistence of both zones A, high crystallinity region, and C, rigid amorphous region takes place. This observation agrees with previous data, published by our research group, on grape berry epicuticular waxes, a type of plant wax that contains high amounts of oleanolic acid.11 X-ray diffraction analysis indicated the existence of crystal-line planes, mainly consisting of the long chain alcohol $n$-hexacosanol, together a less ordered matrix of oleanolic acid dimers showing different orientations.11 X-ray diffraction patterns of the two individual compounds and the isolated berry wax practically presented the same shape and characteristics.11

Conclusion

This paper reveals that the measurement of the specific heat of distinct plant wax components and the evaluation of the excess heat specific of binary mixtures gives a thermodynamic explanation of the self-assembly or self-association process of wax formation. It has been showed that hydrogen bonding between chains or molecules with different structures and functional groups results in the formation of amorphous discrete zones. Physiological implications of the existence of these zones are important because they are accessible to water and small permeating molecules, which can easily diffuse in this molecular arrangement. Further studies on the $C_p^E$ variation with temperature will help to understand the molecular dynamics and interactions of these important lipophilic molecules that constitutes the real last barrier between the plant and the atmosphere.

References and Notes

(8) Bhattacharyya, S. N.; Patterson, D. J. Phys. Chem. 1979, 83, 2979.
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