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Imprinted Polymeric Materials. Insight into the Nature of Prepolymerization Complexes of Quercetin Imprinted Polymers

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Molecular imprinting techniques have proved to be a highly accessible method for producing molecule-specific recognition materials for a variety of applications, ranging from sensing to catalysis and separations. In noncovalent imprinting, it is anticipated that polymerizable complexes are created in the prepolymerization solution via self-assembly of functional monomers and template molecules resulting from inherent chemical complementarity, which will ideally form binding sites within the cross-linked matrix after polymerization. On the basis of ^{1}H NMR data and X-ray crystallographic evidence, we now infer a more important role for template self-association for the recognition properties of quercetin-imprinted polymers. While directly applicable to fundamental understanding of the molecular imprinting mechanism of this polyphenol, on a more generic scale, this work also demonstrates the utility of this strategy toward analyzing complex noncovalent interaction mechanisms between small molecules. These interactions are of particular interest for quercetin and other members of the flavone/flavonoid class of compounds, which are radical-scavenging polyphenols of substantial interest to biomedicine.

The technique of noncovalent imprinting has proven to be a prolific field of research in recent years.[1] It has previously been suggested that the typical representation of the molecular-level mechanisms governing noncovalently imprinted systems (as shown in Figure 1) is deceptively simplified and does not adequately describe how the properties of the prepolymerization system are related to the recognition properties of the finally synthesized templated materials. In recent years, evidence has emerged which indicates that processes such as template nucleation within molecularly imprinted polymers (MIPs) and template self-association play significant roles in dictating how recognition is achieved. These aspects of molecular imprinting were most notably reported by Katz and Davis[2] in their studies with the L-phenylalanine anilide imprint, an imprint system that has been investigated in detail by several groups.[3,4] A combination of X-ray crystallographic data, ^{1}H NMR data, FT-IR data, and observations of phase separation in the polymer mixture led to a proposed recognition mechanism based upon analyte interaction with template building blocks, which remain bound in the polymer matrix. Further evidence in support of this more complicated view of the imprint and recognition mechanism has emerged from the work of the groups of Baggiani[5] and Nicholls.[6] Baggiani demonstrated that the inclusion of a polymerizable derivative of the template molecule in the polymerization mixture may increase MIP affinity for the template molecule itself, which is indicative of the formation of template clusters within the binding sites as rebinding takes place. Studies by Nicholls point toward the formation of template−template complexes in the prepolymerization mixture at the typically applied concentration levels and stoichiometries of the involved polymer components. The observed cooperative effect of the functional monomer on the formation of template−template complexes facilitated the explanation of anomalous chromatographic behavior in the nicotine MIP system analyzed. Collectively, these studies are aimed at advancing our current understanding on the possible processes contributing to how a successful imprint may be achieved, as demonstrated for the quercetin system.

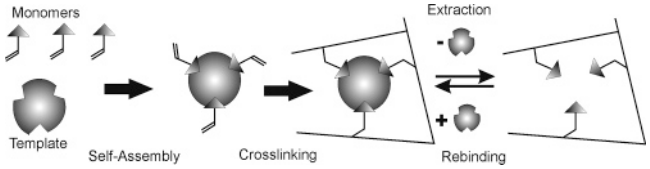

Figure 1. Schematic representation of the steps involved in the synthesis of a typical noncovalent imprint.

In the present work, we have applied a combination of ^{1}H NMR spectroscopy and X-ray crystallography to determine with the

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Figure 2. Cocrystals of quercetin and pyridine grown from a 1:8 mixture in acetone. The image on the right displays the platelike structure of the crystals at a microscopic level.

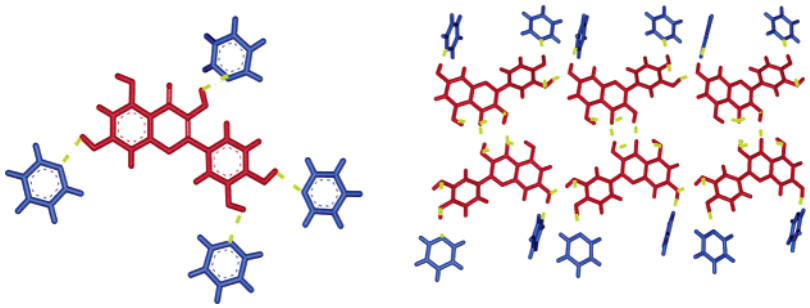

Figure 3. Anticipated quercetin−pyridine complex (left) and sheetlike structure revealed by X-ray crystallography (right).

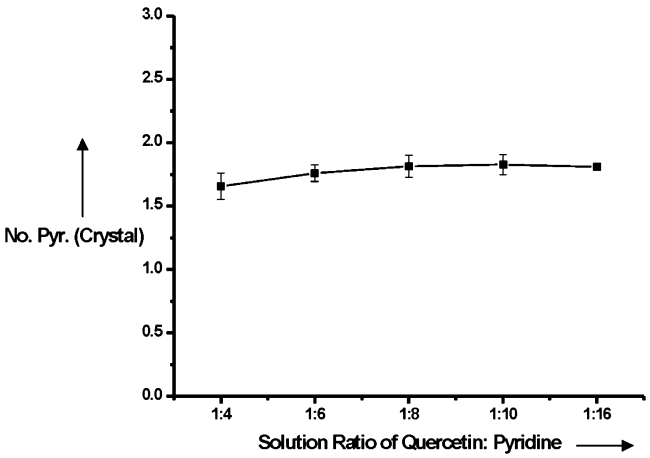

Figure 4. Average (relative) pyridine content of the quercetin−pyridine cocrystals determined after redissolving in acetone-d_6 with ^{1}H NMR spectroscopy ($n = 3$) vs the solution ratio used to grow the crystals.

greatest possible accuracy the nature and structure of the complexes that exist in the prepolymerization solution prior to the actual initiation of the polymerization reaction. It has been suggested in the past that the formation of these complexes has important ramifications for the recognition properties of the subsequently formed MIPs.[6] The system examined in this study is the quercetin (template)−4-vinylpyridine (4-VP; functional monomer) system, which has yielded MIPs of remarkably high specificity.[7] This high performance has prompted investigations into the origins of the achieved specificity; prior work[8] has demonstrated that a macroscopic phase separation—as first observed by Katz and Davis—is indeed evident in this system, with visible clusters forming as the polymerization progressed.

EXPERIMENTAL SECTION

All crystals were grown from acetone solution (10 mL) containing 1 mmol of quercetin and varying quantities (4−16 mmol) of pyridine. For ^{1}H NMR analysis of the crystals, they were redissolved in 0.75 mL of acetone-d_6. As 4-VP is subject to self-polymerization under ambient conditions, pyridine was used as a substitute. For the polymer solution from which the aggregates were recovered, a MIP composition of 15 mL of acetone, 40 mol of ethylene glycol dimethacrylate (EGDMA, cross-linker), 8 mmol of 4-VP (functional monomer), 1 mmol of quercetin dihydrate (template), and 50 mg of azobisisobutyronitrile (initiator) were used following ref 8.

^{1}H NMR analysis of clusters separated from the polymerization mixture (300 MHz, acetone-d_6, 25 °C, solvent peak at 2.05 ppm) was as follows: comparison of 4-VP peak $\delta = 8.5$ (d, 4H, *H* closest to aromatic N—corresponds to 2 protons) with quercetin $\delta = 6.27$ ppm (s, 1H, *H* at position 6 (between the hydroxyl groups on the main flavone ring)—corresponds to 1 proton). MS (clusters in methanol) (ESI-MS): calcd for 1:1 quercetin-4-VP [$C_{15}H_{10}O_7$ − C_7H_7N − 1] [M^+ − H] 406; found 406.

RESULTS AND DISCUSSION

Substituting 4-VP with pyridine—due to the tendency of 4-VP to self-polymerize—a cocrystal of quercetin−pyridine was grown from a solution (Figure 2) containing an 8-fold excess of the

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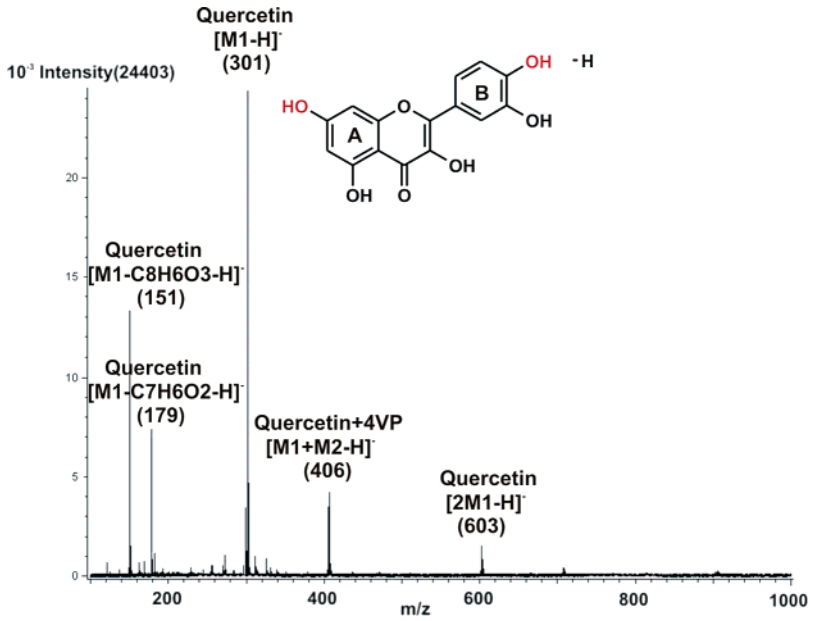

Figure 5. Electrospray ionization mass spectrum of quercetin−4-vinylpyridine complex in MeOH. The inset shows the possible fragmentation of $[M - H]^-$ for quercetin. The red-labeled OH groups indicate the moieties forming hydrogen bonds.

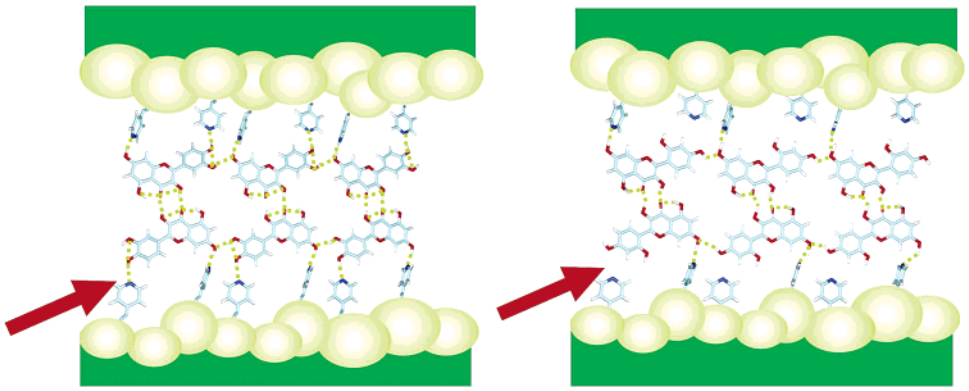

Figure 6. Model for the binding of quercetin (left) and morin (right) to the quercetin MIP assuming polymerization of the sheetlike structure shown in Figure 1 (on the right). The arrows indicate the presence of the second bond to the polymer, or (on the right) the absence of the bond.

monomer analogue (see Supporting Information for details), which corresponds to the relative quantities used during the actual MIP synthesis.

It was hypothesized that four monomer units should form H-bonds with the quercetin template, as the hydroxyl proton at the 5-position of quercetin is intramolecularly bonded and unavailable for interaction,[9] as shown in Figure 3. However, the obtained crystal structure demonstrates that only two pyridine molecules intimately interact with each quercetin molecule.

From the obtained crystallographic data it appears that self-association initiates the formation of an elaborate sheetlike structure. One of the most significant features of this arrangement is that the formation of initial H-bonds to the pyridine molecules appears to foster self-association. This is consistent with the findings of Husykens,[10] who noted the tendency of phenolic compounds to dimerize in response to the formation of a H-bond with a base, as the ability of the oxygen atom in the phenolic OH groups as an electron donor is enhanced.

Furthermore, this phenomenon readily explains earlier ^{1}H NMR titration findings by the Mizaikoff group,[11] wherein titration of 4-vinylpyridine with quercetin resulted in the rapid disappearance of all OH proton signals with the exception of the intramolecularly bonded position 5 substituent. Similarly, no $\pi-\pi$ stacking was observed via ^{1}H NMR in the initial study. The crystallographic data indicate that the sheets of quercetin−pyridine assemblies are arranged in the crystal structure at a slight offset to each other, which inhibits stacking of the aromatic moieties of quercetin and pyridine in the solid phase. The crystallographic data obtained correspond closely to what was observed in liquid-phase analysis via proton NMR, indicating that these forces of self-association, dominant in the crystal structure, may indeed be also dominant in the solution-phase structure of the monomer−template complexes.

Another significant finding in this study is the preference for quercetin−pyridine systems to adopt this arrangement; analyzing the relative content of each component in the crystals via ^{1}H NMR (Figure 4) and by X-ray crystallography for solution ratios of

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quercetin/pyridine ranging from 1:4 up to 1:16, the content of the complexed phase was consistently ~1:2 quercetin/pyridine. Furthermore, crystals that developed in a solution of 1:8 quercetin/pyridine, containing 40 mmol of EGDMA cross-linker—as applied in the actual imprinted polymer—were determined to result in the same 1:2 quercetin/pyridine ratio of the sheetlike structure, with no evidence of cross-linker molecule inclusion. These findings provide strong evidence that the cross-linker does not have a significant effect on the prepolymerization complex formation in this MIP system, as pyridine and quercetin preferentially interact even in the presence of a large excess of EGDMA.

The crystal structures obtained in the course of these studies yielded a wealth of information on the preferred interactions between pyridine and quercetin. However, to confirm that the properties observed in the solid phase are analogous the properties of the system as it polymerizes, ^{1}H NMR analysis of the clusters (as observed by O'Mahony et al.[8]) was performed. After 30 min of radical polymerization initiated in a water bath at 60 °C, the clusters that formed were isolated from the polymerization mixture. ^{1}H NMR data obtained from these clusters clearly demonstrate).

Perhaps the most important ramification of the obtained data is the consequence for interpretation of binding events when the processed MIP is used in separation applications serving as selective stationary-phase material in high-performance liquid chromatography or solid-phase extraction. Previous studies have shown that the quercetin MIP is exceptionally specific, even separating a mixture of quercetin and morin, although morin differs structurally by only one hydroxyl group.[7,8] Figure 6 shows how this difference by one OH group could affect the rebinding processes, possibly reducing the interaction to only one-point binding with the polymer itself, although extensive self-association is still possible. If this is indeed the case, morin should have a slightly smaller affinity for the quercetin MIP, which is precisely what was observed during comparative studies of these molecules.[8]

CONCLUSIONS

In summary, we conclude that X-ray crystallographic data, along with supporting ^{1}H NMR analysis, can provide insight on the molecular-level processes, which may directly and significantly affect a noncovalent molecular imprinting procedure, although it is noted that not all systems can be crystallized in this manner. In this particular system, the template molecule quercetin shows a confirmed tendency to interact with two monomer analogue subunits, which is most probably the source of the observed specificity in this system. These interactions support the observed extensive self-association in this system, indicating that the formation of discrete binding sites via formation of prepolymerization complexes between individual template molecules and multiple monomer units is unlikely. Furthermore, the presence of a cross-linking agent, which is necessary to forming a polymeric recognition material of sufficient rigidity, apparently does not affect the formation of the prepolymerization complexes. This interesting finding suggests that the cross-linker does indeed merely act as a support matrix for the binding sites created by the monomer−template interactions and does not—positively or negatively—interfere with the binding site formation. Future work will focus on analyzing other imprinted systems via similar methods to examine other possible origins of imprinting effects. The obtained data represent an excellent basis for more accurate molecular modeling of molecular imprinting processes,[12] taking phase separation and self-association effects into account for more realistic models, and thus eventually leading to modeling-assisted optimization of molecular imprinting procedures.

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SUPPORTING INFORMATION AVAILABLE

Crystallographic data of quercetin−pyridine cocrystal; NMR data; structural file for quercetin−pyridine cocrystal (CIF). This information is available free of charge via the Internet at http://pubs.acs.org.

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