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Expanding the Scope of Biocatalysis: Oxidative Biotransformations on Solid-Supported Substrates

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Abstract: Oxidative biocatalytic reactions were performed on solid-supported substrates, thus expanding the repertoire of biotransformations that can be carried out on the solid phase. Various phenylacetic and benzoic acid analogues were attached to controlled pore glass beads *via* an enzyme-cleavable linker. Reactions catalyzed by peroxidases (soybean and chloro), tyrosinase, and alcohol oxidase/dehydrogenase gave a range of products, including oligophenols, halogenated aromatics, catechols, and aryl aldehydes. The resulting products were recovered following cleavage from the beads using α-chymotrypsin to selectively hydrolyze a chemically non-labile amide linkage. Controlled pore glass (CPG) modified with a polyethylene glycol (PEG) linker afforded substantially higher product yields than non-PEGylated CPG or non-swellable polymeric resins. This work represents the first attempt to combine solid-phase oxidative biotransformations with subsequent protease-catalyzed cleavage, and serves to further expand the use of biocatalysis in synthetic and medicinal chemistry.

Keywords: biotransformations; enzyme catalysis; halogenation; oxidation; solid-phase synthesis

Introduction

Advances in combinatorial chemistry have resulted largely from solid-phase chemistries with high reactivity and broad specificity.[1] Various solid supports are now commonly used and generally consist of cross-linked polymers, which provide a fluid-like environment that aids solution-phase chemistries on the solid phase. These materials have facilitated a broad array of reactions, including Ugi condensation,[2] Pummerer cyclizations[3] [2+3]cyclocondensations,[4] Beckmann rearrangements,[5] cyclization of β-diketones,[6] and Michael additions,[7] among others. As a result, combinatorial and iterative reactions are possible, including multiple reactions in sequence under different reaction conditions without the consequences of reagent holdover. Product isolation is facilitated by use of linkages that provide a labile functionality.[8] Unfortunately, many of the same attributes that make solid-phase chemistry popular cannot be readily translated to the synthesis or modification of complex lead molecules, including natural products and synthetic compounds that contain multiple functional groups. Complexities arise due to the need for selective attachment and cleavage sites and chemistries, as well as the need to perform reactions under mild conditions. For this reason, enzymatic catalysis on solid-supported substrates has been considered.

Enzymes have a long history of acting on solid substrates. For example, cellulose degradation[9] (in aqueous media) and sugar acylation[10] (in organic solvents) involve substrates with limited or no solubility, yet biocatalytic reactions are feasible. Biocatalytic transformations on solid-supported substrates have been performed since the mid-1990s. Such reactions include oligosaccharide and oligophenol synthesis *via* glycosyltransferase and peroxidase catalysis, respectively,[11,12] peptide synthesis[13] and enantioselective ester hydrolysis using proteases[14] and esterases/lipases,[15] respectively, and oligonucleotide synthesis *via*

RNA ligase.[16] These reactions are either degradative (e.g., hydrolytic) or are limited to specific oligomer formation.

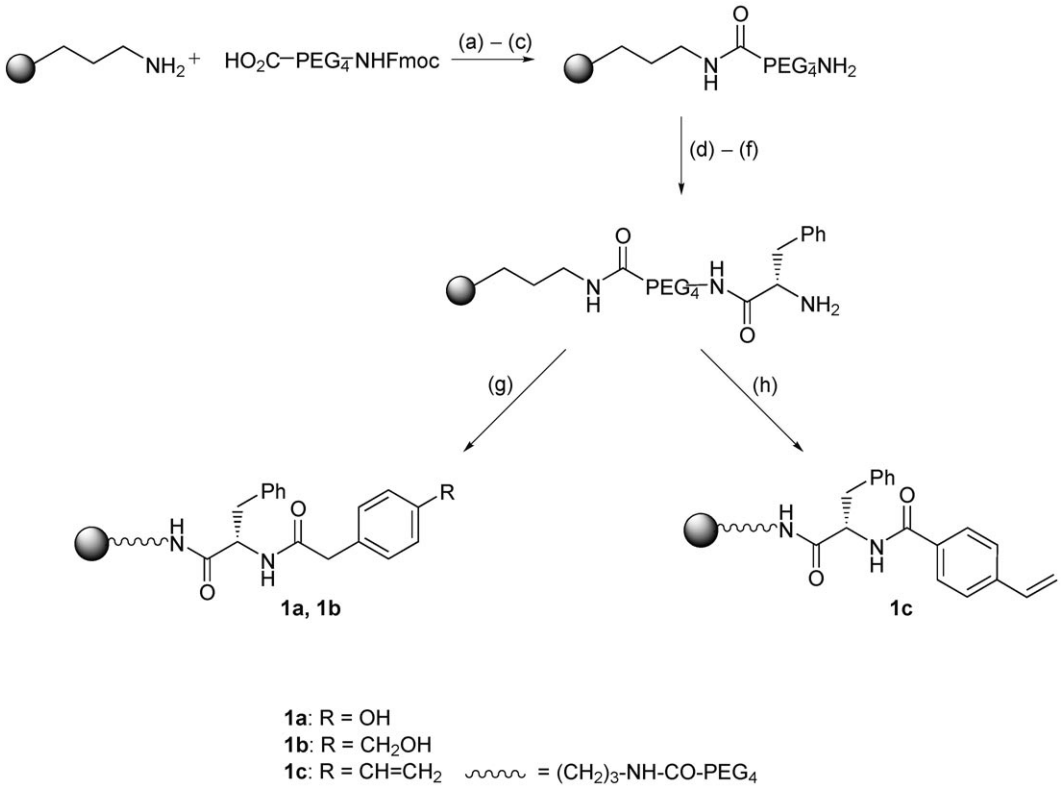
A gap in technology exists in the use of solid-phase biocatalysis for general synthetic transformations, and in particular oxidative transformations. Such reactions include phenolic coupling, as well as hydroxylation, epoxidation, halogenation, and alcohol oxidation, all of which represent examples of diverse chemistries that are difficult to achieve selectively using chemical catalysis. The current work is focused on expanding the range of solid-phase biocatalysis through the use of peroxidase, tyrosinase, and alcohol oxidase/dehydrogenase, all on solid-supported substrates. As a result, a new repertoire of solid-phase chemistries can be performed under conditions ultimately amenable to the synthesis of new chemical entities and the optimization of complex pharmaceutical lead compounds.

Results and Discussion

Our synthetic strategy involved the attachment of phenylacetic or benzoic acid derivatives, each with a different functional group at the *para* position, which served as substrates for specific oxidative enzymes. A variety of amine-functionalized supports were studied, including amino-functionalized controlled pore glass (CPG) and the PEG-based resin TentaGel® (cross-linked polystyrene containing grafted PEG). The substrates were attached *via* a linker containing L-phenylalanine (L-Phe) that enabled hydrolytic release of the reaction products from the solid support by α-chymotrypsin (CT) under ambient conditions. CT-catalyzed cleavage allows mild hydrolysis of typically recalcitrant amide-based linkers.[11,17] Such a strategy was desirable to enable structural preservation of compounds that might be unstable during acid or base cleavage.[8a]

Attachment and Cleavage of Linkers

In initial studies with CPG, an Fmoc-L-Phe linker was first attached to the surface of the support. Following Fmoc removal, the phenylacetic/benzoic acid derivatives *p*-hydroxyphenylacetic acid (HyPAA), *p*-hydroxymethyl-phenylacetic acid (HMPAA), and *p*-vinylbenzoic acid (VBA) were coupled to the L-Phe moiety (Scheme 1). In the case of PEG-functionalized



Scheme 1. Attachment of solid-supported aromatic acids to CPG and TentaGel® resins. *Reagents and conditions:* (a) *N*-Fmoc amido dPEG$_4$ (3 equiv.), DIC (4 equiv.), HOBt (6 equiv.), DMF, 60 °C; (b) 3% pyridine in AC_2O, r.t.; (c) piperidine (20%, v/v) in DMF, r.t.; (d) Fmoc-L-phenylalanine (3 equiv.), DIC (4 equiv.), HOBt (6 equiv.), DMF, 60 °C; (e) 3% pyridine in AC_2O, r.t.; (f) piperidine (20%, v/v) in DMF, r.t.; (g) 4-hydroxyphenylacetic acid or 4-(hydroxymethyl) phenylacetic acid (3 equiv.), DIC (4 equiv.), HOBt (6 equiv.), DMF, 60 °C; (h) 4-vinylbenzoic acid (3 equiv.), DIC (4 equiv.), HOBt (6 equiv.), DMF, 60 °C. Steps (a)–(c) were not carried out on TentaGel®, the L-Phe-Fmoc was attached directly to the resin.

Table 1. Enzymatic cleavage of solid-supported substrates from various supports.

Resin or Glass Type	Linker	Attachment[a] [μmol/g]	% Attachment[b]	Substrate Cleavage [%][c]		
				L-Phe-VBA	L-Phe-HyPAA	L-Phe-HMPAA
CPG	-	117.0 ± 10.0	80 ± 6.8	0.40 ± 0.03	3.0 ± 0.4	5.0 ± 0.2
CPG	PEG_4	64.0 ± 3.3	44 ± 2.2	17.0 ± 2.2	44.0 ± 1.4	66 ± 5.4
TentaGel®	-	250.0 ± 13.4	100 ± 5.5	3.0 ± 0.8	1.0 ± 0.3	-

[a] The amount of substrate attached was determined as described in the Results and Discussion.
[b] Calculated as a percentage of the measured loading of amines on the resin/beads. All resin/bead types had different amine loadings: CPG: 146 μmol/g, TentaGel: 250 μmol/g.
[c] Cleavage expressed as a percentage of attached substrate recovered following cleavage with α-chymotrypsin. All values are the mean of three independent determinations.

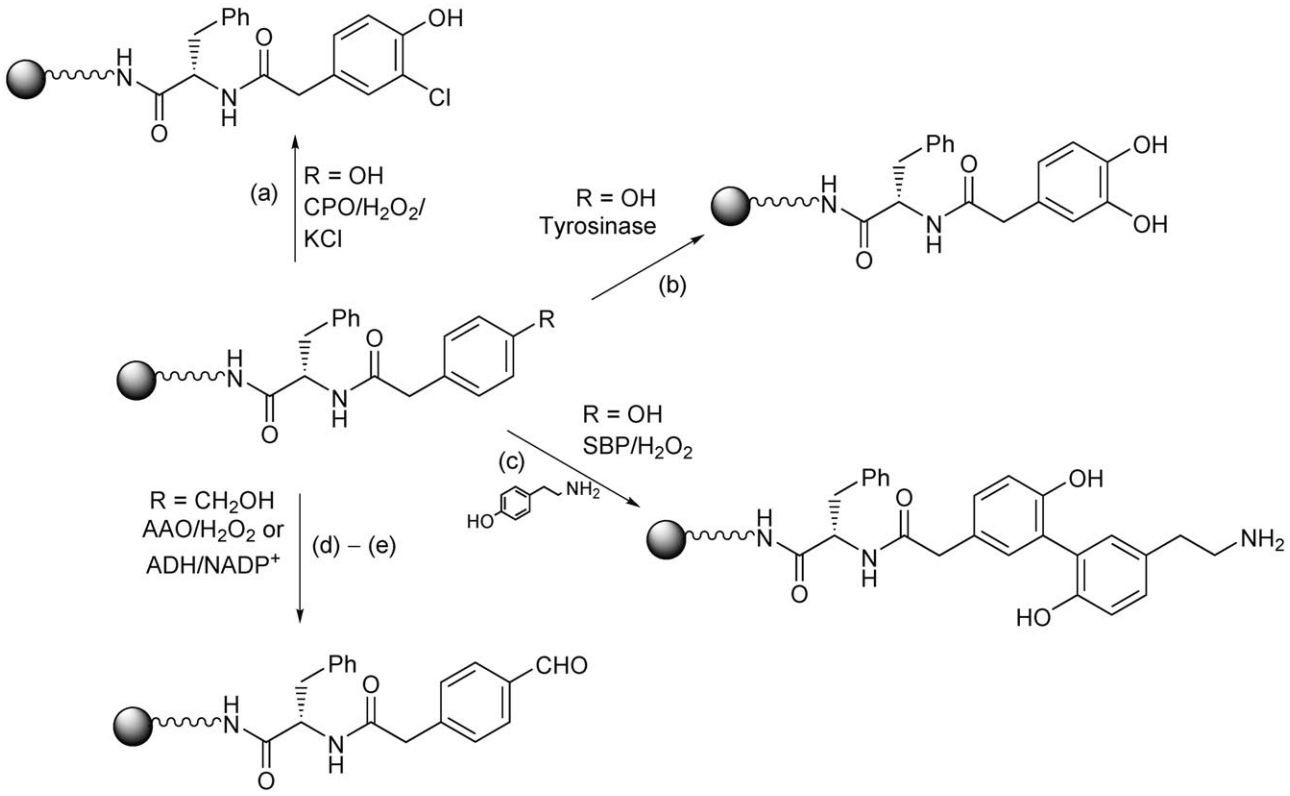
CPG, a PEG_4 amino acid (containing four ethylene glycol residues with a carboxyl group on one end and an Fmoc protected amine on the other) was first coupled to the support followed by Fmoc removal and attachment of the Fmoc-L-Phe linker. To fully characterize and quantify the degree of attachment and subsequent cleavage of the bound substrates, the Kaiser ninhydrin assay was used to measure disappearance of amine groups present on the surface and within the pores of the beads and enable the facile quantification of bound PEG, Fmoc-L-Phe, and substrate, respectively. To avoid inaccurate results due to the possibility of incomplete reactions during each attachment step, the free amines were acetylated in between each step to block the residual amino groups (Scheme 1). Attachment of the three substrates onto TentaGel® containing the Fmoc-L-Phe linker was 100% efficient (Table 1). Substrate attachment onto PEG_4-CPG was lower (~45%, Table 1). Yields of the individual attachment steps (Scheme 1) as determined by the disappearance of available amino groups were 62% for the attachment of PEG_4 to the CPG followed by Fmoc deprotection, and 70% for the subsequent attachment of the L-Phe linker to the PEG_4.

The efficiency of CT-catalyzed cleavage was determined by quantifying the cleavage products *via* LC-MS in comparison to chemically synthesized standards. In the case of TentaGel® very low cleavage was obtained (Table 1). This is consistent with previous studies by Kress et al.[14b] who evaluated enzymatic peptide cleavage from TentaGel®. Low reactivity on this support was postulated to be due to the poor accessibility of the enzyme into the pores of the supports. TentaGel® is known to poorly solvate in aqueous solution, which substantially restricts the pore size and thus provides very little accessibility of the enzyme to the bound substrate.[18,19] However, other PEG-based resins such as PEGA {poly[acryloyl-bis-(aminopropyl)polyethylene glycol]} have been widely reported to readily swell in aqueous solution. While CT-catalyzed reactions have been successfully demonstrated on PEGA-supported peptide substrates,[20,21] the resin does not allow enzymes larger than 35 kD to access the supported substrate. Thus, PEGA was not suitable for this study, which employed enzymes with molecular weights in the range of 37–118 kD. We therefore employed CPG with a pore size of 500 Å, which has been reported to be accessible to higher MW enzymes .[20]

Low cleavage was obtained with CPG (< 5% of the attached substrate in the case of HMPAA) (Table 1). While CPG has large pore sizes (500 Å), which are certainly sufficient for the 25 kD CT molecule, the limited distance of the substrates (bound to only an L-Phe linker) from the support surface may limit accessibility of the substrate into the active site of CT. The added length of the PEG_4 moiety (~20 Å including the L-Phe linker), however, improved CT-catalyzed substrate cleavage from the CPG for all three substrates, with the highest yield obtained for HMPAA (Table 1). In addition, the PEG moiety may enhance the effective solubility of the bound substrate in the aqueous phase within the pores of CPG, thereby resulting in greater substrate accessibility to the CT.[22] Finally, PEG may act as an anti-protein binding surface,[23,24] thus preventing CT or other enzymes used in this work from binding to the pore surface and blocking access of additional enzyme molecules to the bound substrate. PEG_4-CPG, therefore, was chosen as the support for subsequent oxidative biotransformation studies.

Oxidative Biocatalysis on Solid-Supported Substrates

Oxidation reactions of solid-supported substrates were catalyzed by a diverse set of enzymes (Scheme 2, Table 2), which included two peroxidases (soybean peroxidase for phenolic coupling and chloroperoxidase for halogenation, epoxidation and halohydration), tyrosinase for hydroxylation, and alcohol oxidase and dehydrogenase for benzylic alcohol oxidation. To facilitate direct comparison of reactivity among all enzymes and reactions, in all cases we quantified the bound substrate conversion. To that end, substrate conversion was determined by first



Scheme 2. Enzymatic transformation of solid-supported substrates. *Reagents and conditions:* All enzymatic reactions were performed with 50 mg of the appropriate functionalized resin. (a) Citrate buffer (50 mM, pH 5.0), CPO (10 μL, 10,000 U/mL), 30 mM H_2O_2 (final concentration, infused at a rate of 0.1 mL/h for 10 h from a 90 mM stock), KCl (20 mM), r.t.; (b) phosphate buffer (50 mM, pH 6.5), tyrosinase (0.004 mg/mL, 5,300 U/mg), ascorbic acid (5 equiv.), r.t.; (c) phosphate buffer (100 mM, pH 8.0), SBP (0.2 mg/mL, 100 U/mg), tyramine (50 mM), 4 mM H_2O_2 (final concentration, infused at a rate of 0.1 mL/h for 10 h from a 20 mM stock), r.t.; (d) MOPS-Tris buffer (100 mM, pH 7.5), AAO (2 mg/mL, 0.1 U/mg), $CuSO_4$ (0.4 mM), 30 °C; (e) Tricine-KOH buffer (100 mM, pH 9.0), ADH, (0.4 mg/mL, 0.5 U/mg), $NADP^+$ (2 mg/mL), 30 °C.

Table 2. Solid-supported oxidative biotransformations.

Substrate[a]	Enzyme	Cosubstrate/Cofactor	Reaction	% Conversion[b]
1b R = CH_2OH	Alcohol dehydrogenase (ADH)	$NADP^+$	Oxidation	12 ± 2.0
1b R = CH_2OH	Aryl alcohol oxidase (AAO)	H_2O_2	Oxidation	23 ± 2.2
1a R = OH	Tyrosinase	O_2	Oxidation	50 ± 1.5
1a R = OH	Soybean peroxidase (SBP)	H_2O_2	Phenol coupling	85 ± 3.1
1a R = OH	Chloroperoxidase (CPO)	H_2O_2 + KCl	Halogenation	100 ± 0
1c R = CH=CH_2	Chloroperoxidase (CPO)	H_2O_2 + KBr	Halohydration	100 ± 0
1c R = CH=CH_2	Chloroperoxidase (CPO)	H_2O_2	Epoxidation	30 ± 6.0

[a] Compound nomenclature relates to Scheme 1.
[b] Substrate conversion based on the amount of unreacted substrate (containing an L-Phe moiety). All values are the mean of three independent reactions.

cleaving the L-Phe linker with CT followed by quantifying the amount of unreacted substrate (containing an L-Phe moiety) using HPLC. In the absence of CT, no L-Phe-linker cleavage was observed. In the presence of CT alone, only the unreacted substrate (attached to the L-Phe moiety) was observed. This approach provides an effective conversion based on the comparison of substrate cleaved following combined oxidative and CT catalysis versus CT catalysis alone. To validate the effectiveness of this approach, we first evaluated the reactivity of aryl alcohol oxidase (AAO, 80 kD) and alcohol dehydrogenase (ADH, 100 kD) catalysis on HMPAA (Scheme 2, a), which provides an opportunity to measure independently the formation of co-products (NADPH for ADH and H_2O_2 for AAO). Substrate conversions (after 16 h in-

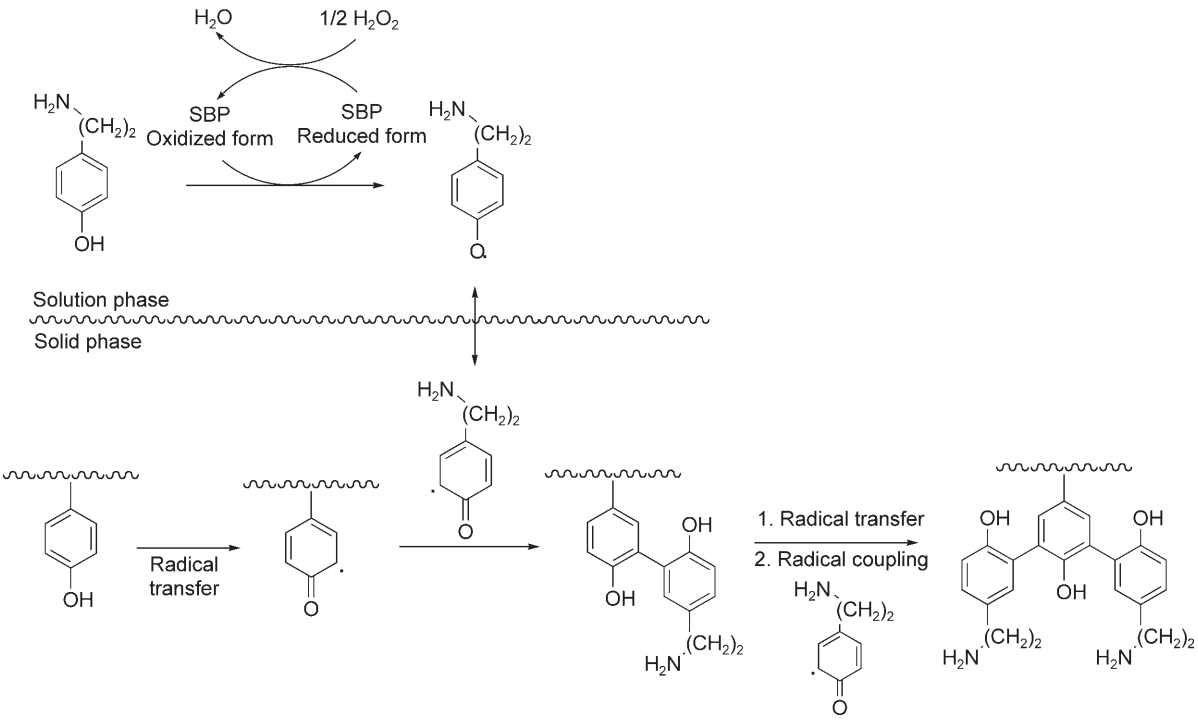


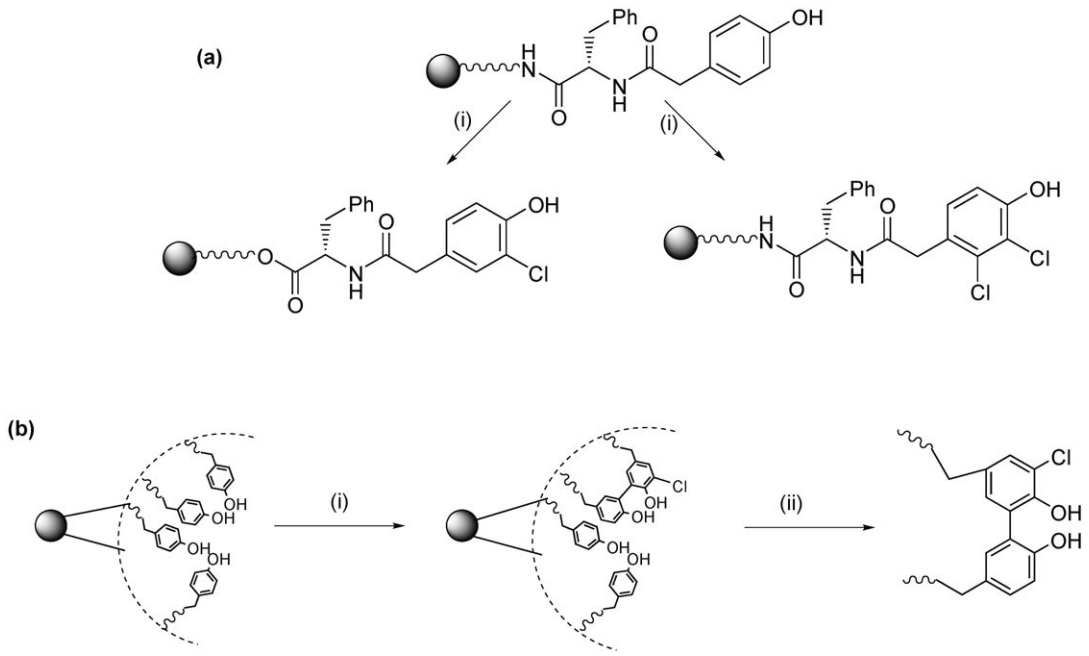
Figure 1. Soybean peroxidase-catalyzed radical coupling of tyramine to solid-supported HyPAA.

cubations) to the aldehydic product were 23 and 12% for AAO and ADH, respectively, based on the amount of HMPAA bound to CPG (Table 2). Importantly, the AAO-catalyzed formation of H_2O_2 and the ADH-catalyzed formation of NADPH as measured after 16 h matched the HMPAA conversion as determined *via* the HPLC technique. Thus, the HPLC approach and the direct product formation (in the case of ADH and AAO catalysis) were consistent.

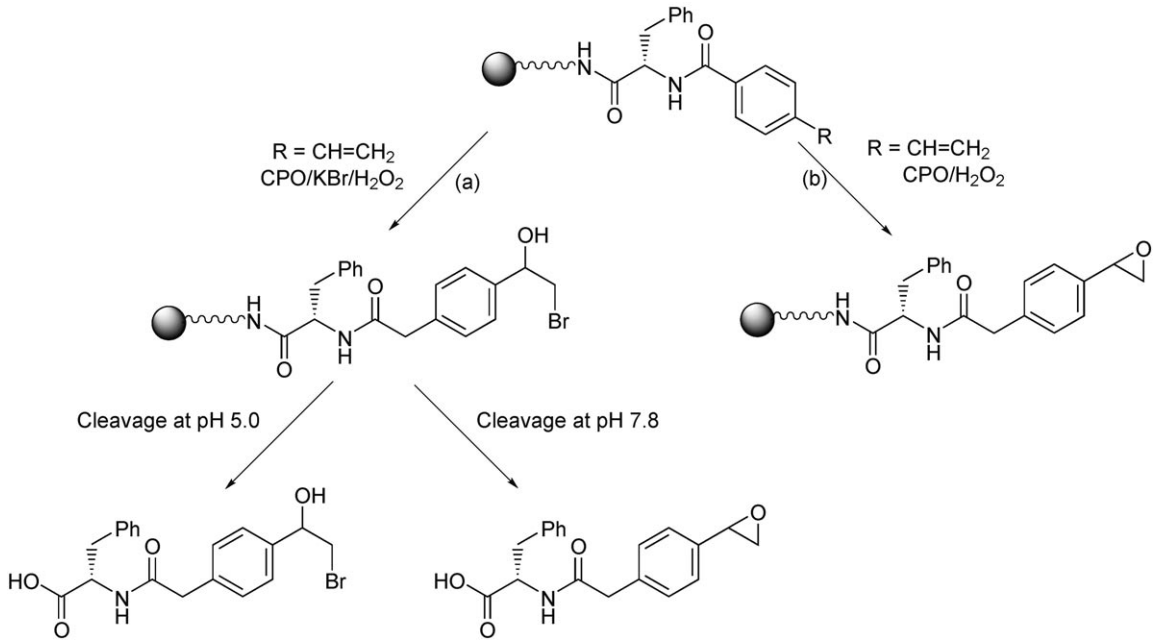
Tyrosinase (118 kD) catalysis on bound HyPAA resulted in conversion of *ca.* 50% of the bound substrate (Table 2). In the presence of ascorbic acid to reduce the quinone products from the tyrosinase reaction, cleavage resulted in identification of the catechol products. All three enzymes require direct contact with the bound substrate to catalyze the oxidation reaction. Therefore, the pores may restrict access or hinder mobility of these enzymes to only the most accessible PEG_4-CPG bound substrate molecules.

Unlike the aforementioned enzymes, peroxidases do not require direct contact of the enzyme with the bound substrate (Figure 1). As a result, biotransformations involving phenolic coupling [catalyzed by soybean peroxidase (SBP), 37 kD] and ring halogenations [catalyzed by chloroperoxidase (CPO), 42 kD] resulted in high conversions (Scheme 2, Table 2) Specifically, SBP-catalyzed phenolic coupling of tyramine in the presence of H_2O_2 resulted in the formation of coupled tyramine residues bound to HyPAA. Cleavage with CT provided *ca.* 85% conversion (Table 2). Similarly, CPO-catalyzed chlorination of HyPAA with KCl and H_2O_2 as co-substrates gave complete conversion to three distinct ring chlorination products, including mono- and dichlorinated HyPAA and a monochlorinated dimer (Scheme 3). This dimeric product was intriguing, as its formation could only come about through intermolecular ring coupling through two separate linkages within a single bead. The presence of the PEG_4 linker likely aided efficient contact of adjacent phenolic substrates within the pore to enable phenolic coupling by CPO. The high yields of SBP and CPO catalysis were most likely facilitated by the formation of diffusible products that are able to access bound HyPAA: phenoxy radicals for SBP[12] and HOCl for CPO.[25] These products are able to effectively diffuse from the enzymes within the CPG to the bound HyPAA (Figure 1 for SBP).

CPO is also capable of catalyzing non-diffusible radical-dependent reactions. Therefore, to assess the ability of CPO to catalyze reactions that require *direct* contact with the substrate, epoxidation reactions were performed using VBA in the presence of H_2O_2 (Table 2, Scheme 4,). As shown in Table 2, the conversion to the epoxide product was 30%. Conversely, in the presence of KBr, which is converted into diffusible HOBr, complete conversion of the VBA to the halohydrin product was obtained (Table 2, Scheme 4). This result confirmed that a reaction mechanism consisting of a diffusible product can be more efficient on solid phase substrates than a mechanism that requires direct contact of the substrate with the enzyme.



Scheme 3. CPO-catalyzed halogenation of bound HyPAA [putative structures based on LC-MS analysis]. **(a)** Mono- and di-chlorination of solid-supported HyPAA. **(b)** Schematic representing the formation of dimeric chlorinated products within the pore of a single bead followed by cleavage. The dashed hemispherical line indicates a pore of CPG containing bound HyPAA groups. *Reagents and conditions:* All enzymatic reactions were performed with 50 mg of the appropriate functionalized resin. (i) Citrate buffer (50 mM, pH 5.0), CPO (10 μL, 10,000 U/mL), 30 mM H_2O_2 (final concentration, infused at a rate of 0.1 mL/h for 10 h from a 90 mM stock), KCl (20 mM), r.t.; (ii) ammonium bicarbonate buffer (100 mM, pH 7.8), α-chymotrypsin (5 mg/mL, 34 U/mg), 30 °C.



Scheme 4. Chloroperoxidase-mediated transformation of solid-supported 4-vinylbenzoic acid. *Reagents and conditions:* All enzymatic reactions were performed with 50 mg of the appropriate functionalized resin. (a) Citrate buffer (50 mM, pH 5.0), CPO (10 μL, 10,000 U/mL), 30 mM H_2O_2 (final concentration, infused at a rate of 0.1 mL/h for 10 h from a 90 mM stock), KBr (20 mM), r.t.; (b) citrate buffer (50 mM, pH 5.0), CPO (10 μL, 10,000 U/mL), 30 mM H_2O_2 (final concentration, infused at a rate of 0.1 mL/h for 10 h from a 90 mM stock), r.t..

Interestingly, the halohydrin product expected from CPO catalysis in the presence of KBr and H_2O_2 was not initially observed; rather, the corresponding epoxide was generated (Scheme 4). Such epoxide formation may have been due to the CT-catalyzed cleavage conditions at pH 7.8, which promote the well-known nucleophilic dehalogenation of the halohydrin to form the epoxide.[26] To eliminate this non-enzymatic transformation, the CT-catalyzed cleavage reaction was performed at pH 5 (CT has low but measurable activity at this pH), which resulted in the formation of the expected halohydrin as the sole product (Scheme 4). Hence, in the presence of KBr, CPO catalyzed the expected formation of the HOBr product, which diffused to the vinyl moiety and, depending on the cleavage conditions, remained as the halohydrin or is dehalogenated to the epoxide (Scheme 4). This synthetic scheme, which is facilitated by retention of the halohydrin product on the solid phase, did *not* lead to the undesired aldehyde product. Hence, such a chemo-enzymatic strategy may be synthetically useful in the preparation of complex natural product epoxides.

Conclusions

In conclusion, we have demonstrated that a range of enzymatic oxidation reactions can be performed on solid-supported substrates. In the process, we have established that a combination of CPG and a PEG linker yields relatively high biocatalytic conversions on the solid phase relative to non-PEGylated CPG or non-swellable polymeric resins. The PEG_4-CPG facilitates the use of a diverse set of enzymes and serves to further expand the feasibility of solid-phase biocatalysis as a synthetic strategy. Finally, we have shown that the enzymatic cleavage conditions may alter the outcome of a given reaction, in this case a CPO-catalyzed halohydration reaction, when compared to a corresponding reaction in solution phase.

Experimental Section

Amino-functionalized controlled pore glass (CPG, 146 μmol NH_2/g) was purchased from Biosearch Technologies (Novato, CA). TentaGel® was acquired from Rapp-Polymere (Tübingen, Germany). N-Fmoc amido $dPEG_4$ acid was purchased from Quanta Biodesign (Powell, OH). SBP, CPO, tyrosinase, CT, *p*-(hydroxymethyl)phenylacetic acid, *p*-hydroxyphenyl acetic acid, and *p*-vinylbenzoic acid were obtained from Sigma–Aldrich (St. Louis, MO). Fmoc-Phe-OH was obtained from Fluka (Buchs, Switzerland). Alcohol dehydrogenase 102 and aryl alcohol oxidase 102 were obtained from Codexis (Pasadena, CA). Chemical and solvents were of the highest grade commercially available and were purchased from Sigma–Aldrich and Acros (Geel, Belgium).

General Procedure for the Preparation of CPG-Bound-PEG_4

All reagent equivalents are stated with respect to the stated loading of amine groups on the beads. 1-Hydroxybenzotriazole (HOBt, 6 equiv.), *N,N′*-diisopropylcarbodiimide (DIC, 4 equiv.), and *N*-Fmoc amido $dPEG_4$ (3 equiv.) were added to a suspension of aminopropyl-CPG resin (1 g, 0.146 mmol/g) in DMF (10 mL). The mixture was shaken at 200 rpm for 16 h at 60 °C and then filtered. The resin was washed with DMF (3×) and the procedure was repeated. After the second cycle, the resin was filtered and then washed with DMF, MeOH, and acetone (3× each). The unreacted amino groups of the resin were capped with a solution of 3% (v/v) pyridine in acetic anhydride (10 mL) at room temperature overnight. After filtration, the resin was washed with MeOH and acetone (3× each). Deprotection of the Fmoc group was carried out in piperidine [10 mL, 20% (v/v) in DMF] at room temperature for 18 h and filtered. The resin was washed with DMF, MeOH, and acetone (3× each).

General Procedure for the Preparation of Polymer-Bound L-Phenylalanine

All reagent equivalents are stated with respect to the stated loading of amine groups on the beads. HOBt (6 equiv.), DIC (4 equiv.), and L-Fmoc-Phe (3 equiv.) were added to a suspension of the appropriate amino-functionalized resin (0.146–0.25 mmol/g) in DMF (10 mL). The mixture was shaken at 200 rpm for 16 h at 60 °C. The resin was washed with DMF (3×) and the procedure was repeated. After the second cycle, the resin was filtered and then washed with DMF, MeOH, and acetone (3× each). As described above, the unreacted amino groups of the resin were capped with a solution of 3% (v/v) pyridine in acetic anhydride (10 mL) at room temperature overnight. After filtration and washing, Fmoc deprotection was achieved as described above.

General Procedure for the Attachment of Substrates to Resins

All reagent equivalents are stated with respect to the stated loading of amine groups on the beads. After attachment of L-Phe, the substrate (HyPAA, HMPAA, or VBA, 3 equiv.) was coupled to the resin in the presence of DIC (4 equiv.) and HOBt (6 equiv.) in DMF (10 mL). The mixture was shaken at 200 rpm for 16 h at 60 °C, filtered, and washed with DMF (3×), and the procedure was repeated. After filtration, the resin was washed with DMF, MeOH and acetone (3× each).

Cleavage of Bound Substrates

Enzymatic hydrolysis of bound substrates from the resins was performed with CT catalysis. The resin (50 mg) was suspended in ammonium bicarbonate buffer (100 mM, pH 7.8) in the presence of lyophilized CT (5 mg/mL, 34 U/mg). The reaction mixture was shaken at 200 rpm for 24 h at 30 °C. At the end of the reaction, the mixture was filtered off the beads by passing the liquid through a membrane filter (0.2 μm). The cleaved product was analyzed using LC-MS (Shimadzu LC-MS 2010 AD equipped with a SPD 20AV detector and LC-MS 2010 A mass spectrometer). An Alltech

(Deerfield, Il, USA) ODS C18 column was used with a gradient elution profile consisting of 0.2% formic acid:CH_3CN 95:5 to 10:90 (30 min) then back to 95:5 (10 min) and finally maintaining for 5 min at a flow rate of 0.2 mL/min. Product amounts were calculated by using calibration curves of synthesized standard products where available.

Enzymatic Reactions on Solid-Supported Substrates

Enzymatic reactions were performed with 50 mg of the appropriate functionalized resin in 20-mL vials. For ADH-catalyzed reactions, the resin-bound HMPAA (50 mg) was suspended in 5 mL Tricine-KOH buffer (100 mM, pH 9.0) in the presence of the enzyme (0.4 mg/mL, 0.5 U/mg) and $NADP^+$ (2 mg/mL). The mixture was shaken at 200 rpm and 30 °C for 16 h. For AAO-catalyzed reactions, the resin-bound HMPAA (50 mg) was suspended in 5 mL MOPS-Tris buffer (100 mM MOPS, 100 mM Tris titrated to a pH of 7.5) in the presence of $CuSO_4$ (0.4 mM) and AAO (2 mg/mL, 0.1 U/mg) followed by stirring at room temperature for 16 h. Tyrosinase-catalyzed reactions were performed in 5 mL sodium phosphate buffer (50 mM, pH 6.5) with resin-bound HyPAA (50 mg) in the presence of tyrosinase (0.004 mg mL^{-1}, 5,300 U/mg). In some cases, ascorbic acid (5 equiv. of substrate concentration) was added to the reaction mixture. The reactions were mixed at room temperature for 16 h. For SBP-catalyzed carbon-carbon coupling reactions, the resin-bound HyPAA (50 mg) was suspended in 5 mL sodium phosphate buffer (100 mM, pH 8.0) containing SBP (0.2 mg/mL, 100 U/mg) and tyramine (50 mM). The reaction mixture was stirred at room temperature and 1 mL of 20 mM H_2O_2 was infused at a rate of 0.1 mL/h for 10 h using a syringe pump (4 mM H_2O_2 final concentration). Finally, for CPO-catalyzed reactions, resin-bound HyPAA or VBA (50 mg) was suspended in 2 mL sodium citrate buffer (50 mM, pH 5.0) containing CPO (5 μL/mL, 10,000 U/mL) and in the presence or absence of KBr (20 mM). The reaction mixture was stirred at room temperature and 1 mL of 90 mM H_2O_2 was infused at a rate of 0.1 mL/h for 10 h using a syringe pump (30 mM H_2O_2 final concentration). At the end of each enzymatic reaction, the resins were filtered and washed with H_2O and acetone (3× each), dried, and weighed. The products were then cleaved from the resin as previously described.

Measurement of NADPH and H_2O_2 Formation for ADH and AAO Reactions

Enzymatic reactions were performed with 50 mg of the appropriate functionalized resin in 20 mL vials. For ADH-catalyzed reactions, the resin-bound HMPAA (50 mg) was suspended in 4 mL Tricine-KOH buffer (100 mM, pH 9.0) in the presence of ADH (0.4 mg/mL) and $NADP^+$ (2 mg/mL). The mixture was shaken at 200 rpm and 30 °C for 16 h. The amount of NADPH produced was calculated by removing aliquots (0.2 mL) of the reaction mixture and measuring the absorbance at 340 nm. For AAO-catalyzed reactions, the resin-bound HMPAA (50 mg) was suspended in 5 mL MOPS-Tris buffer (100 mM, pH 7.5) in the presence of $CuSO_4$ (0.4 mM) and AAO (2 mg/mL) followed by stirring at room temperature for 16 h. Aliquots (0.05 mL) of the reaction mixture were removed and the amount of H_2O_2 formed was quantified by adding 50 μL of a 2 mM solution of 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 100 μL of a 6 μg/mL of SBP in a microwell plate. In the presence of H_2O_2 produced as a result of AAO oxidation of bound HMPAA, SBP oxidizes ABTS resulting in a colored product, which is then measured at 405 nm.

Preparation of L-Phenylalanine Derivatives

L-Phenylalanine methyl ester hydrochloride (108 mg, 0.5 mmol) and the phenylacetic/benzoic acid derivative (0.5 mmol) were dissolved in 6 mL DMF:CH_2Cl_2 (1:3). The mixture was sequentially treated at 0 °C with $NaHCO_3$ (42 mg, 0.5 mmol), HOBt (75 mg, 0.55 mmol), and EDC (106 mg, 0.55 mmol). The reaction mixture was stirred at 0 °C for 2 h and allowed to warm to room temperature. Stirring was continued overnight and then the reaction was quenched with 15 mL H_2O followed by extraction of the aqueous phase with EtOAc (3×10 mL), after which the combined organic phases were washed with HCl 0.1 M (2× 10 mL), H_2O (10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by column chromatography (CH_2Cl_2:EtOAc, 6:4).

Hydrolysis of the methyl esters was performed as follows. To the purified L-phenylalanine methyl ester derivative (0.4 mmol) was added 1 mL KOH 1 M in MeOH. Following stirring overnight at room temperature, the solvent was evaporated under reduced pressure, and 10 mL water were added to the residue. The mixture was extracted with diethyl ether (2×10 mL) and the organic phase was discarded. The aqueous phase was acidified with HCl 1 M, extracted with diethyl ether (3×10 mL), dried over $MgSO_4$, and concentrated under vacuum. Please see Supporting Information for spectroscopic data.

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