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Chapter 11
Synthesis of Vinyl Polymers via Enzymatic Oxidative Polymerisation

W. Zhang and F. Hollmann

Abstract Enzymatic methods for the polymerisation of vinyl monomers are presented and critically discussed. Vinyl monomers can be polymerised initiated by enzyme-catalysed radical formation. The most widely used initiators for this purpose are β-diketo compounds, which can be transformed into the corresponding radicals via peroxidase- or laccase-catalysed oxidation. For this, peroxidases use hydrogen peroxide as oxidant, while laccases rely on molecular oxygen. Both enzyme classes comprise specific advantages and disadvantages that are discussed in this chapter. Also, parameters to control the polymer properties are introduced and discussed.

Keywords Polymerisation of vinyl monomers · Laccase · Peroxidase · Biocatalysis

11.1 Introduction

Polymers obtained from vinyl monomers represent an important class of plastics with widespread applications. The most predominant mechanism for their synthesis relies on radical initiation followed by radical chain propagation and termination yielding the final product.

Next to the classical radical chain initiators, enzymatic radical initiation has received growing interest (mostly from the academic world) as possibly milder and more benign alternative.

In this chapter we will outline the current mechanistic understanding of the most important enzyme-initiated vinyl polymerisation reactions, present some recent application examples and discuss the advantages and drawbacks of these methods compared to the current chemical state of the art.

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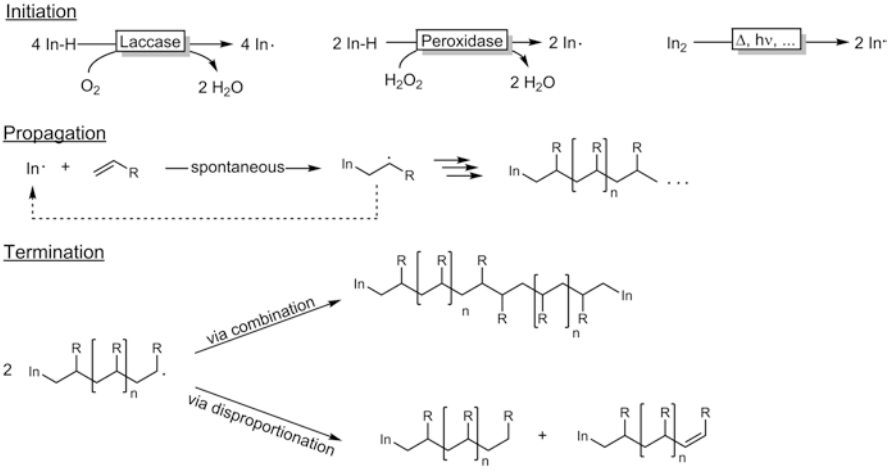
11.2 General Topics

The term 'enzymatic polymerisation of vinyl monomers', which is frequently found in the literature, is somewhat misleading as it suggests the biocatalyst being involved in the actual polymerisation reaction. In fact, the biocatalysts discussed in this chapter exclusively catalyse the first step of the polymerisation reaction (i.e. the initiation reaction), while the polymer formation occurs spontaneously. Hence, classical benefits of biocatalysis such as stereoselectivity [1] cannot be expected from this sort of polymerisation reactions. In essence, the course of an enzyme initiated polymerisation differs from a 'classical' chemical polymerisation reaction only in the initiation reaction (Scheme 11.1).

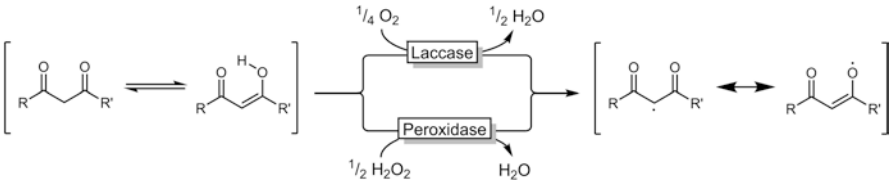
11.2.1 Mechanism of Enzyme-Initiated Polymerisations

Laccases and peroxidases are the enzymes most widely used for the enzyme-initiated polymerisation of vinyl monomers [2–4]. Their 'natural' substrates are phenolic (and related) compounds, and the enzymes catalyse a H-atom abstraction yielding reactive radical compounds. Therefore, laccases and peroxidases are also widespread used in the polymerisation of phenolics (Chaps. 9 and 10).

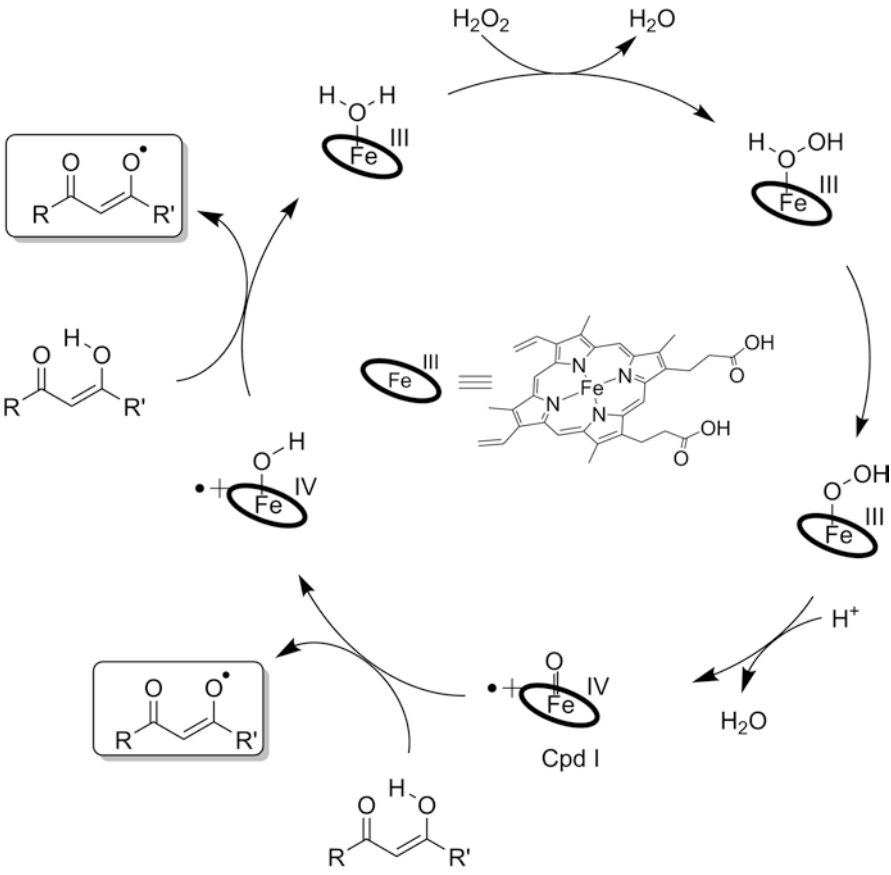
Next to phenols, laccases and peroxidases also mediate H-atom abstraction reactions from other activated starting materials, especially from β-diketo compounds (Scheme 11.2). The resulting radicals function as radical initiators (In•) for the polymerisation of vinyl monomers as discussed throughout this chapter.



Scheme 11.1 Essential steps of the radical polymerisation of vinyl compounds consisting of (1) initiation, (2) propagation and (3) termination. 'Classical chemical' and enzymatic polymerisations differ mostly in the first step (chain initiation)

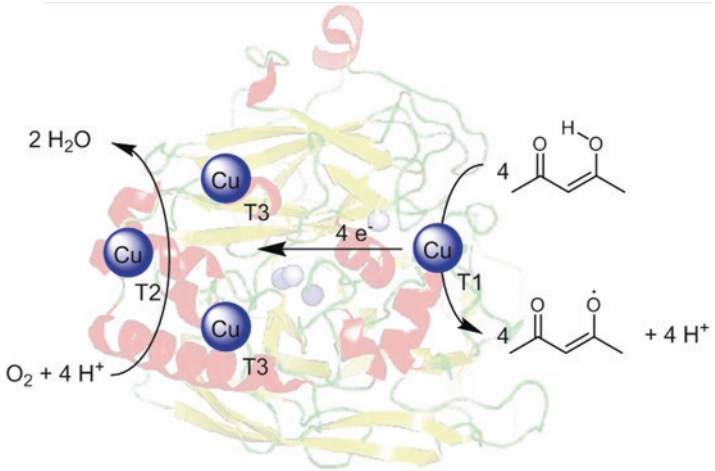


Scheme 11.2 Laccase- or peroxidase-catalysed H-atom abstraction from a β-diketone substrate

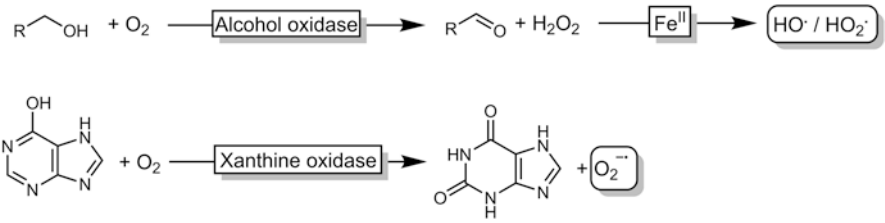


Scheme 11.3 Catalytic mechanism of the peroxidase-catalysed H-atom abstraction of β-diketones

The catalytic mechanisms of peroxidases and laccases differ considerably. *Peroxidases* are generally heme-dependent enzymes, which in the presence of hydrogen peroxide (or other organic peroxides) form a highly oxidised (formal) Fe^{V}-oxo-species (compound I). Compound I is best described as oxyferryl (Fe^{IV}) embedded in a porphyrin radical cation [5]. Compound I performs two successive H-atom abstraction reactions from activated substrates (phenols or β-diketones) forming two radical initiators (Scheme 11.3).



Scheme 11.4 Simplified reaction scheme of laccase-catalysed oxidation of β-diketones



Scheme 11.5 Miscellaneous enzymatic systems to generate polymerisation initiators

Laccases also catalyse H-atom abstraction reactions from substrates very similar to those of the aforementioned peroxidases. In contrast, however, laccases utilise molecular oxygen instead of hydrogen peroxide as oxidant for this reaction. Laccases contain four copper ions (which is why they are also called blue-copper oxidases) classified as T1, T2 and T3 [6, 7]. Generally speaking, the T1 Cu ion performs four successive single-electron oxidation steps on the starting material transferring the reducing equivalents to the T2/T3-Cu ions. O_2 reduction occurs in the T2/T3 cluster (which also very tightly binds the intermediate, partially reduced oxygen species, Scheme 11.4).

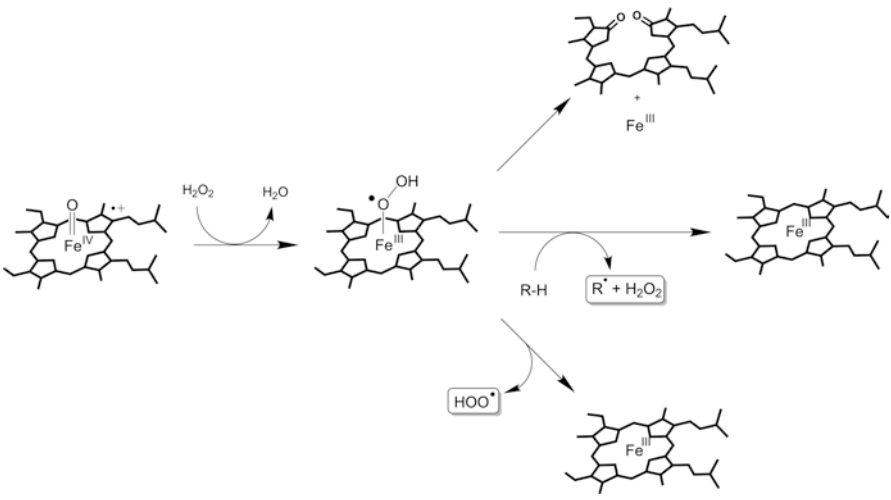
Next to the predominant peroxidases and laccases, also a few other enzymatic systems to generate polymerisation initiators are worth mentioning here. Alcohol oxidases catalyse the aerobic oxidation of alcohols to the corresponding carbonyl groups yielding hydrogen peroxide as by-product. In the presence of Fe^{II} ions, the latter can initiate Fenton-like reactions with reactive oxygen species (ROS) as initiators (Scheme 11.5) [8]. Similarly, xanthine oxidase can be used for the generation of ROS; in contrast to alcohol oxidases, this enzyme generates superoxide directly (Scheme 11.5) [8].

11.2.2 *Factors Influencing the Outcome of Enzyme-Initiated Polymerisation of Vinyl Monomers*

As for every radical polymerisation reaction, the yield and properties of the final product largely depend on the ratio of radical initiator to the monomer and the presence of possible chain growth inhibitors.

The in situ concentration of the active initiator radical can be influenced by parameters such as the enzyme concentration (its activity, respectively). Lalot and coworkers have investigated the effect of enzyme and initiator concentration on the polymer size of the HRP-initiated polymerisation of acrylamide (AAm, Scheme 11.7) [9]. These authors confirmed that a lower in situ concentration of the active initiator molecule (Acac radical) favours high molecular weights. This concentration directly correlates (increases) with the concentration of Acac and HRP. Qualitatively, the same trend was also found for the laccase-initiated polymerisation [10]. Overall, controlling the in situ concentration of the initiator radical via overall initiator concentration and/or enzyme concentration is a very good handle to control the polymer weight of the final product.

Also the oxidant concentration can play an important role in the polymerisation reaction but needs careful adjustment. In the case of peroxidases, H_2O_2 should not be applied in too high concentrations as H_2O_2 also is an efficient inactivator of the heme-enzymes [11]. The exact mechanism is not defined yet, and probably different inactivation pathways exist (Scheme 11.6), but it is clear that high in situ concentrations of H_2O_2 should be avoided.



Scheme 11.6 Different pathways of inactivation of heme enzymes by H_2O_2. Both oxidative destruction of the heme prosthetic group and formation of reactive oxygen species (highlighted) leading to enzyme inactivation are discussed

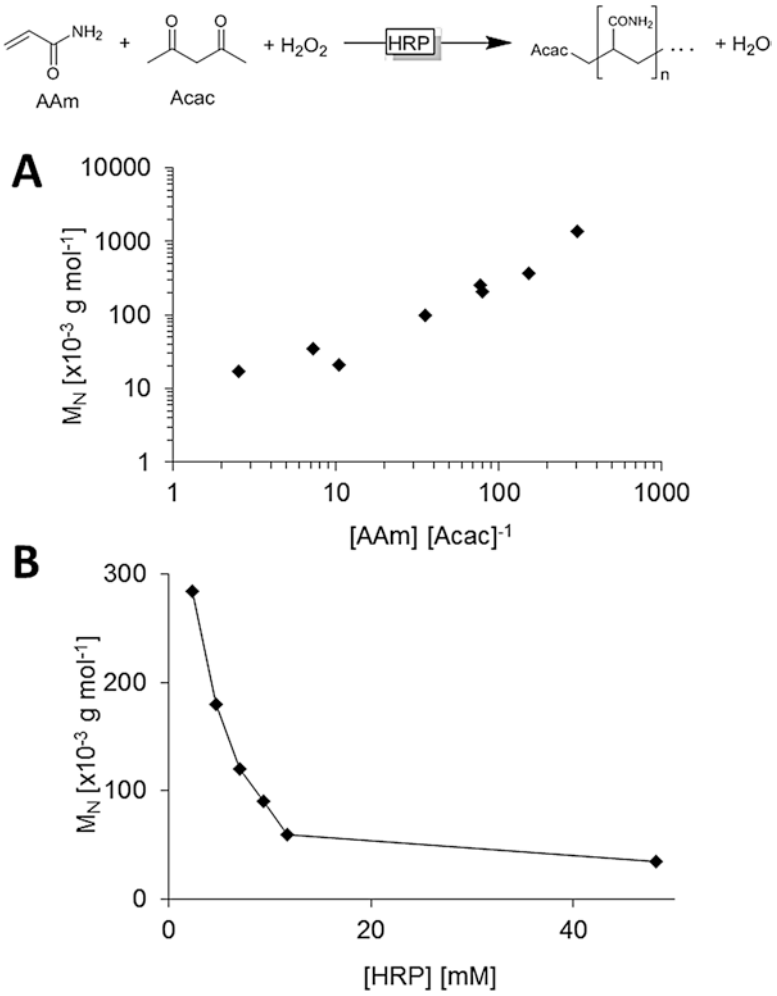
Therefore, H_2O_2 often is added several times in small portions to minimise H_2O_2-caused inactivation. More elegantly, some in situ H_2O_2 generation systems have been developed in the past years, which may be applicable to use peroxidases more efficiently in polymerisation processes [12–20].

For laccases, O_2 serves as oxidant to initiate the polymerisation reaction. The issue with O_2 is that it also is an efficient radical scavenger inhibiting the polymerisation reaction. Therefore, also in the case of laccases (though not for enzyme stability reasons), the oxidant concentration needs to be carefully controlled [10]. This is also true for peroxidase reactions as also here trace O_2 amounts can significantly impair the polymerisation reaction. Very recently an efficient measure to reduce the O_2 content simply by adding glucose/glucose-oxidase to the reaction mixture was proposed by Stevens and coworkers [21, 22] (Scheme 11.7).

It is generally assumed that the enol form of the β-diketo compound (more phenol-like) represents the actual substrate for the laccase- or peroxidase-catalysed H-atom abstraction [23]. Hence, factors influencing the keto-enol equilibrium will influence the in situ concentration of the actual enol substrate. Using more alkaline pH values is a double-edged measure; on the one hand, higher pH values favour higher enol concentrations, while on the other hand, the pH optima of laccases and peroxidases are more in the slightly acidic range [24]. Another possibility is to engineer the β-diketo compound itself and favour the enol content through steric and/or electronic variations. It should, however, be kept in mind that both factors may interfere with the acceptance of the β-diketo compound by the enzyme (especially in case of sterically demanding starting materials) or with the polymer-initiation activity of the resulting radical (particularly in case of using electronegative substituents to increase the enol content). Kaplan and coworkers systematically investigated the influence of the initiator molecule on the polymer properties for the horseradish peroxidase (HRP)-catalysed polymerisation of styrene [25] and acrylamide [26] (Table 11.1) impressively demonstrating the influence the initiator can have on the conversion as well as on polymer properties such as molecular weight (M_W) and polydispersity (PD).

Ideally, the initiator molecule would be circumvented at all. This would not only eliminate its cost contribution but would also be favourable from an enzyme activity point of view (many initiators exhibit solvent-like properties and can – in too high concentrations – inactivate the biocatalyst). Early reports claiming initiator-free enzyme-initiated polymerisation [27, 28] could not be reproduced by others [10, 26, 29].

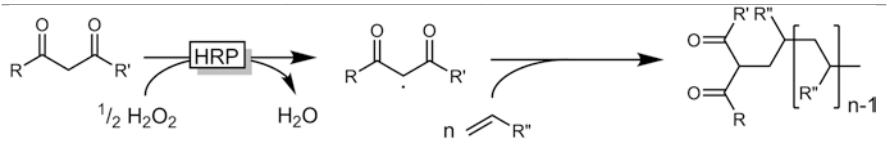
Finally, also the solvent can have a significant influence on the polymerisation reaction. Especially if hydrophobic monomers are used, their solubility in the mostly aqueous reaction mixtures can be an issue. Polar organic solvents can be used to increase the monomer solubility [25, 30]. But frequently the presence of water-mixable cosolvents impairs the stability of the biocatalyst used. An alternative to increasing the water solubility of the monomers is to use a biphasic reaction

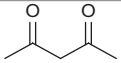
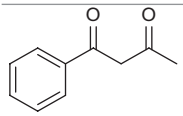
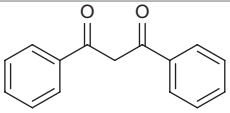
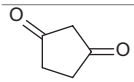
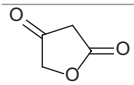
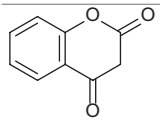
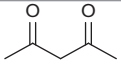
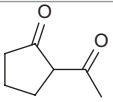
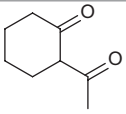
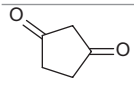
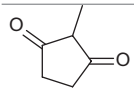


Scheme 11.7 Influence of initiator (Acac, **a**) and enzyme (HRP, **b**) concentration on the polymer weight of the HRP-initiated polymerisation of acrylamide

mixture containing an aqueous reaction mixture with the biocatalyst and a hydrophobic organic phase composed of the monomer in high concentrations (ideally neat). Such emulsion polymerisations have been investigated especially for styrenes [29, 31, 32]. Even better than a biphasic system would be to use neat reaction conditions without any cosolvent whatsoever. For this, immobilised preparations of the biocatalyst are required [33–41]. Another interesting approach is to solubilise the hydrophilic enzymes in organic media by coating them with surfactants [42].

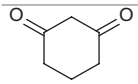
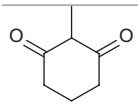
Table 11.1 Influence of the initiator molecule on the performance of HRP-initiated polymerisation reactions



Initiator	Yield [%]	M_W [g mol^{-1}]	PD [–]
Styrene polymerisation			
	16.7	26,900	2.07
	14.1	80,100	1.96
	14.4	96,500	2.16
	59.4	67,600	1.98
	41.1	50,900	2.22
	14.5	57,200	1.64
Acrylamide polymerisation			
	93	124,000	2.5
	76	5100	4.4
	84	56,300	2.9
	78	84,500	2.7
	38	10,500	3.9

(continued)

Table 11.1 (continued)

	72	27,000	3.3
	86	9800	3.9

11.3 Selected Examples

In recent years the number of reported examples for enzyme-initiated vinyl polymerisations has been growing steadily. Scheme 11.8 gives a representative overview over some of the literature examples.

Graft polymerisation is receiving increasing attention especially using HRP as catalyst. For example modifying starch with (poly)acrylamide [55], (poly) methyl acrylate [56] or (poly)butyl acrylate [57] has been reported (Scheme 11.9) [58]. As grafting mechanism, H-atom abstraction from a starch-OH-group by HRP-generated Acac has been proposed.

Another interesting grafting approach has been reported with silica surfaces using laccases [59] or HRP [60]. In the latter case, for example, SiO_2 particles were first covered with the initiator (Acac) followed by HRP-initiated grafting of acrylamide onto the SiO_2 particle (Scheme 11.10).

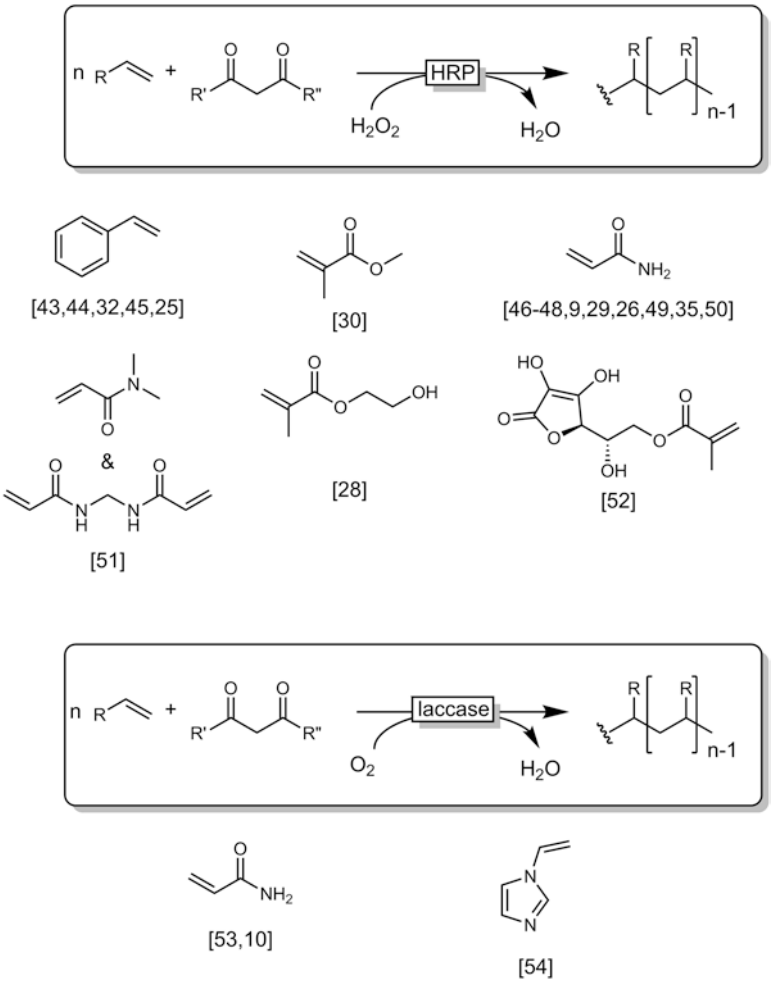
Also lignin represents an attractive target to graft polymers onto. Interestingly, this appears to be a laccase domain [61–67].

Cross-linking of chitosan using laccases was used to self-immobilise the enzyme [68].

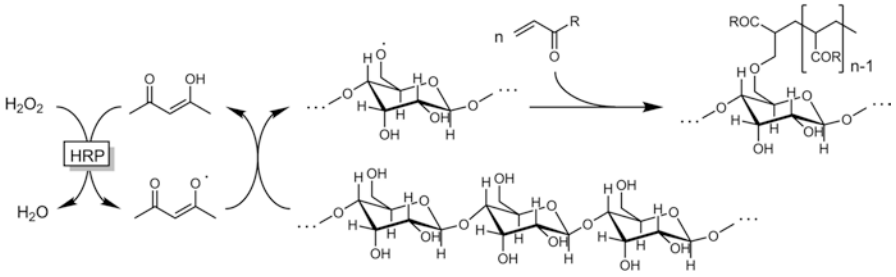
In polymer chemistry, the so-called reversible deactivation radical polymerisation (RDRP) is very much in focus now due to its power to control the molecular weight and the polydispersity of the polymer products. Also in enzyme-initiated polymerisations, RDRP is being used more frequently [54, 69, 70].

11.4 Conclusions

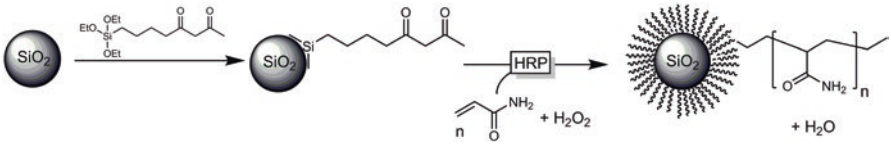
The use of enzymes to initiate radical polymerisation reactions is enjoying steadily growing interest. Partially, this may be due to the fact that enzymatic reactions are generally perceived to be more environmentally benign than ‘chemical’ reactions. A quantitative study comparing the environmental impact of both, however, is lacking so far. It should be kept in mind that not only the actual reaction (conditions) determines the environmental impact but also factors such as catalyst’s preparation and downstream processing to obtain the desired product. Hence, perceived advantages such as mild reaction conditions or the use of water as solvent may well turn out to be less important than thought or maybe even counterproductive.



Scheme 11.8 Selected examples of horseradish peroxidase- (HRP) or laccase-initiated vinyl polymerisations



Scheme 11.9 Proposed mechanism for the HRP-Acac-initiated acrylate grafting on starch



Scheme 11.10 Surface-initiated enzymatic polymerisation using HRP

Nevertheless, enzyme-initiated polymerisation remains an active and dynamic field of research, and some exciting new developments may be expected in the future.

Today, the peroxidase from horseradish is by far the most popular biocatalyst in use, which is somewhat astonishing considering that the number of available peroxidases/peroxygenases and laccases is steadily increasing [71]. Though it is not expected that new enzymes will have a significant impact on the polymer structure, it may well be that increased activity and/or stability may contribute to the economic feasibility of these processes.

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