β-Diketones as Key Compounds in Free-Radical Polymerization by Enzyme-Mediated Initiation

Didier Teixeira, Thierry Lalot,* Maryvonne Brigodiot, and Ernest Maréchal

Laboratoire de Synthèse Macromoléculaire, UMR 7610 "Chimie des Polymères", Université Pierre et Marie Curie, 4 place Jussieu, Case 184, F-75252 Paris Cedex 05, France

Received June 2, 1998; Revised Manuscript Received October 22, 1998

ABSTRACT: A novel initiating system for free-radical polymerization at room temperature has recently been described by our laboratory. It is based on the catalytic properties of horseradish peroxidase (HRP) to generate radical species from hydrogen peroxide and 2,4-pentanedione (acetylacetone) through an oxydoreductive pathway. In the presence of an acrylic monomer (acrylamide), initiation takes place leading to a polymer. The enolic form of acetylacetone is assumed to directly intervene in the catalytic cycle so the question has been raised in order to determine if other β-diketones might play a similar role in HRP-mediated polymerization. Assumptions on the initiation mechanism are discussed in relation to the well-known HRP catalytic scheme.

Introduction

Numerous enzyme-catalyzed oxidoreductions generate free radicals as intermediates in biocatalytic pathways.[1] This thesis particularly holds true for reactions catalyzed by horseradish peroxidase (HRP). HRP is an oxidoreductase acting on H_2O_2 and a few alkyl hydroperoxides as oxidants[2] and on several natural reducing substrates (RH), such as phenol, hydroquinone, cathecol, resorcinol, pyrogallol, aniline, and *p*-aminobenzoate.[3]

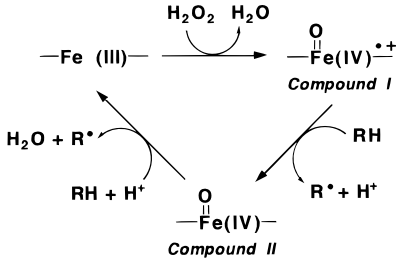
We recently introduced a new approach to HRP-catalyzed reactions based on the potential use of β-diketones instead of natural HRP substrates as initiators for free-radical polymerization. In this respect, 2,4-pentanedione (acetylacetone) was successfully tested in our preliminary studies.[4] Therefore, the question has been raised to determine whether other β-diketones could be used to increase the number of HRP-mediated polymerizations.

This note extends prior observations on the use of β-diketones[4] and outlines a few rules which could permit us to define the best RH candidate for free radical polymerization by enzyme-mediated initiation. According to the abundant literature on enols[5] and by analogy with the HRP-catalyzed oxidation of phenol and derivatives,[6] the enolic form of β-diketones was assumed to be the key structure in the biocatalytic cycle and provided insights into the initiation mechanism. The validity of our concept is also discussed in relation to the different mechanisms involved in the formation of polymers resulting from enzyme-mediated initiation.

Experimental Section

Materials. Acrylamide (Aldrich, 99%+, electrophoresis grade), horseradish peroxidase (Sigma, 87 purpurogallin units·mg^{-1}, type I), H_2O_2 (Prolabo, 30% stabilized aqueous solution), and methanol (Prolabo, analytical grade) were used without further purification. 2,4-Pentanedione (**1**) was distilled before use; 2-acetylcyclohexanone (**2**), 2-acetylcyclopentanone (**3**), 1,3-cyclohexanedione (**4**), 5,5-dimethyl-1,3-cyclohexanedione (**5**), 2-methyl-1,3-cyclohexanedione (**6**), 1,3-cyclopentanedione (**7**), 2-methyl-1,3-cyclopentanedione (**8**), 2,2,6,6-tetramethyl-3,5-heptanedione (**9**), and 2-acetyl-1,3-cyclohexanedione (**10**) with purity ≥98% were used without further purification. Compounds **1**, **8**, and **10** were purchased from Aldrich, **2**−**4**, **6**, and **7** from Lancaster, and **5** and **9** from Fluka.

Scheme 1. Commonly Accepted Biocatalytic Cycle of Horseradish Peroxidase where the Porphyrin Ring Is Schematically Drawn by the Two Horizontal Lines[6,9]



Polymerization of Acrylamide in Water. A detailed procedure has been previously described.[4] A typical experiment is however given in ref 7. For the experiments carried out with β-diketones other than 2,4-pentanedione, the same procedure was followed. The reaction medium was protected against light. Systematically, blank experiments were carried out in order to determine whether the polymerization of acrylamide is enzyme-mediated. Thus, the following mixtures were carefully prepared in the same concentration conditions and according to the same procedure: (i) acrylamide (0.66 M), H_2O_2, (11 mM) and β-diketones (17 mM); (ii) acrylamide (0.66 M), H_2O_2, (11 mM) and HRP (2.0 mg·mL^{-1}). An attempt with acetylacetone and thermally deactivated HRP was also performed. No polymer was obtained from these mixtures after 3 h of reaction.

Characterization of Polyacrylamide. The average molecular weights (MW) of polyacrylamide were determined from intrinsic viscosity measurements according to Mark−Houwink equations given in ref 8. Evaluation of [η] was performed by extrapolation of experimental data (five measures) according to Huggins and Martin methods.[9] Average [η] values were used to calculate the number- and weight-average molar masses.

Results and Discussion

Basic Considerations. Although the fundamental mechanism of HRP catalysis has not yet fully been elucidated, the enzymic production of radical intermediates is known to arise from the oxidation of the reducing substrate by compounds I and II (oxoiron(IV) porphyrin π-cation radical and oxoiron(IV) porphyrin containing enzyme, respectively) (Scheme 1).[10] In the absence of polymerizable monomers, the primary radicals spontaneously combine,[11] but the appearance of free radicals

10.1021/ma980872+ CCC: $18.00
Published on Web 12/12/1998

Table 1. Acrylamide Polymerization by HRP-Mediated Initiation at Room Temperature[a] Showing the Influence of the *β*-Diketone Structure on the Yield and the Average Molar Masses of Polyacrylamide

β-diketones	Structure	Yield (wt.-%)[b]	$\overline{M_n}$ (g.mol^{-1})[c]	Polydispersity index I_p
1		93	124000	2.5
2		84	56300	2.9
3		76	5100	4.4
4		72	27000	3.3
5		77	27500	3.3
6		86	9800	3.9
7		78	84500	2.7
8		38	10500	3.9

[a] 3 h reaction in distilled water (25 °C, pH 5.1); $[\text{acrylamide}]_0 = 0.66$ M, $[H_2O_2]_0 = 10$ mM; $[\beta\text{-diketone}]_0 = 17$ mM; $[HRP]_0 = 2.0$ g·L^{-1} when acetylacetone is used; for other *β*-diketones, $[\text{acrylamide}]_0 = 0.66$ M; $[H_2O_2]_0 = 11$ mM; $[\beta\text{-diketone}]_0 = 17$ mM; $[HRP]_0 = 2.0$ g·L^{-1}. [b] The yield corresponds to the weight of polyacrylamide recovered after precipitation in methanol (large excess), filtration and drying, to the weight of acrylamide used initially. [c] Average molar masses were determined from viscosity measurements at 25 °C in water according to the relations $[\eta] = 6.8 \times 10^4 \bar{M}_n^{0.8}$ and $[\eta] = 6.31 \times 10^5 \bar{M}_w^{0.66}$.[8]

during the second and third steps of the biocatalytic pathway is similar to the generation of the primary radical involved in conventional radical initiation.

Thus, it would appear that eqs 1 and 2 might be combined to define a novel initiating system where the first reaction (eq 1) enzymatically produces the primary

$$H_2O_2 + 2\ RH \xrightarrow{HRP} 2H_2O + 2R^\bullet \quad (1)$$

$$R^\bullet + M \rightarrow RM^\bullet \quad (2)$$

radicals R$^\bullet$ and the second reaction (eq 2) is relative to their addition to monomeric molecules leading to the formation of the first propagating radicals RM$^\bullet$; according to this analysis, RH behaves as both an enzyme substrate (eq 1) and a potential initiator (eq 2). This concept has been recently introduced in our preliminary attempts to polymerize acrylamide in water at room temperature, where several natural reducing molecules (RH) were tested.[4] Although they are all HRP substrates,[3] most of them also behave as inhibitors of free-radical polymerization and polyacrylamide was not obtained when they were used. On the other hand, polyacrylamide was formed when acetylacetone (**1** in Table 1) was used as a proton donor. It was the first example of enzymatically mediated polymerization of acrylamide carried out in the presence of RH.[4]

***β*-Diketones as Key Compounds.** The choice of acetylacetone as proton donor was initially guided by the two following observations: *β*-diketones have weakly bonded α-hydrogens, and cyclic *β*-diketones, such as 5,5-dimethyl-1,3-cyclohexanedione, are substrates for chloroperoxidases which belong to the same subsubclass of the enzyme classification as that of HRP (E.C. 1.11.1.a and 1.11.1.7, respectively).[3] By analogy with the relative acidity of phenols, the assumption that the enolic form of acetylacetone is a key intermediate in the catalytic pathway led us to consider other *β*-diketones involved in a keto−enol equilibrium. Alicyclic *endo*−*exo* (**2** and **3** in Table 1) and *endo*−*endo* *β*-diketones (**3**−**8** in Table 1) are known to favor high enol contents in water at room temperature.[5] Diketones **2**−**8** were successfully tested.

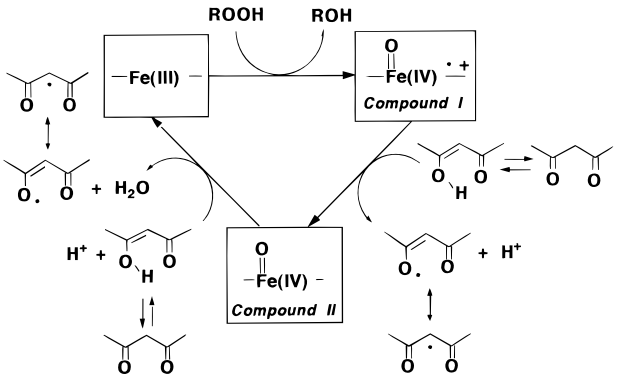
Regardless of the *β*-diketone structure (Table 1), the yield and the molecular weight (MW) of polyacrylamide are lower and the dispersity index (I_p) higher than those obtained with **1**. In addition, the higher the MW of polyacrylamide is, the lower I_p. Up to now, it has not been possible to directly correlate MW and I_p with the keto−enol equilibrium constant, which suggests that other parameters than the enol concentration must be taken into account as confirmed by several negative results. For instance, polymerization was not observed when compounds **9** and **10** were used at the same initial concentration as that of **1**. Bulky substituents on C_γ (**9**) or C_α (**10**) seem to alter the ability of these compounds to act as HRP substrates although the enol content is at least equal to that of acetylacetone. Besides, *β*-diketones may act as transfer agents, which could explain the wide range of MW produced. In summary, RH candidates suitable for HRP-mediated polymerization were found to have a *β*-diketone-type structure without steric hindrance in α and *γ* positions and to be involved in a keto−enol equilibrium.

Apparently Contradictory Observations. On one hand, the possibility that the *β*-diketone is involved in the HRP biocatalytic cycle is supported by the obvious analogy with phenol and its influence on the characteristics of polyacrylamide (Table 1). On the other hand, our results differ from those obtained by Derango and co-workers.[12] They found that polymerization could be initiated without using a proton donor and the initiating species was assumed to be the oxoiron(IV) *π*-cation radical whereas no polymer was obtained in all of our experiments when no proton donor was used. Recently, Kobayashi et al.[13] concluded that the phenolic moiety was chemoselectively reacted in the HRP-catalyzed polymerization of 2-(4-hydroxyphenyl)ethyl methacrylate. In accordance with Derango et al., the authors also obtained polymer without using a proton donor. The most remarkable point is that a very large excess of hydrogen peroxide with respect to monomer (9/1 molar ratio at least) was used in Derango's and Kobayashi's experiments whereas it is 1/66 at the most in our experiments. Derango's and Kobayashi's observations and ours are apparently contradictory; however, the corresponding experiments were carried out under completely different conditions.

Since the same catalyst is used in the three studies (refs 12 and 13 and the present work), unifying explanations taken into account for a given composition of the reaction mixture will have to be found in agreement with the HRP biocatalytic cycle.

Assumptions on HRP-Mediated Initiation. *β*-Diketone, hydrogen peroxide, and HRP must be simultaneously present in the reaction medium to achieve polymerization of acrylamide under the conditions described in this article. If one of these components is

Scheme 2. Analogy with the Horseradish Peroxidase Catalyzed Oxidation of Phenol Accounting for the Possible Role of the β-Diketone Enolic Form in the Generation of the Primary Radicals



omitted or if thermally deactivated HRP is used (blank experiments), polymerization of acrylamide does not occur. Therefore, several side reactions that might have explained polymer formation may be rejected for the systems studied.

Thus, homolytic cleavage of hydrogen peroxide does not occur significantly at room temperature as indicated by the two corresponding blank experiments (negative result in the absence of the enzyme, negative result in the absence of the β-diketone). The addition of compound I (oxoiron(IV) π-cation radical) to the monomer as the initiating reaction can be rejected as well since no polymer was obtained in the absence of RH compound (see Experimental Section). Thus, to be efficient, the initiation step requires the presence of HRP, hydrogen peroxide, and β-diketone.

According to Scheme 2, the formation of the primary radicals is believed to arise from the second and third steps of the enzymic cycle, and their formation is supported by the obvious analogy with the reaction scheme of HRP-mediated oxidation of phenols (see Scheme 1 for analogy).

The generation of keto−enoxy radicals as the first radical species in the reaction medium does not imply that they actually initiate the polymerization. Initiation may take place by their reaction on the monomer but also by radical transfer to any molecule, i.e. enzyme impurity, amino acid residue, and hydrogen peroxide itself, and the resulting radical can be a potential initiator. This thesis particularly holds for hydrogen peroxide due to its high transfer constant value. It is, therefore, of the utmost importance to characterize the polymer functionalities. Structural data are necessary to identify the initiator residue which may be incorporated in the polymer.[14]

Conclusions

The suitability of the β-diketone chosen as the HRP substrate appears to be related to the structure of the compound, the keto−enol equilibrium constant, and the degree of steric hindrance at the α and γ positions of the molecule. The mechanism of HRP-mediated polymerization has not been identified; however, all the three components (enzyme, H_2O_2, and RH) must be present to initiate the polymerization of acrylamide in the conditions described in this work. Further research is in progress to elucidate the mechanism of the polymerization initiated by this system and to determine if the RH substrate is incorporated into the polymer chain or not.

References and Notes

(1) For recent literature, see: Dunford, D. Catalysis by haem iron. In *Comprehensive Biological Catalysis*; Sinnott, M., Ed.; Academic Press: London, 1997; Vol. 3 and references cited therein.

(2) Schonbaum, G. R.; Lo, S. *J. Biol. Chem*. **1972**, *10*, 3353.

(3) Barman, T. E. *Enzyme handbook;* Springer-Verlag: New York, 1985; Vol. I, p 234.

(4) Emery, O.; Lalot, T.; Brigodiot, M.; Maréchal, E. *J. Polym. Sci.: Part A: Polym. Chem*. **1997**, *35*, 3331. Lalot, T.; Brigodiot, M.; Maréchal, E. *Polym. Int.*, submitted for publication (this paper reports on a kinetic approach of acrylamide radical polymerization by horseradish peroxidase-mediated initiation).

(5) Toullec, J. Keto−enol equilibrium constants. In *The chemistry of enols*; Rappoport, Z., Ed.; John Wiley & Sons: New York, 1990; p 353.

(6) Dixon, M.; Webb, E. C. *Enzymes*, 3rd ed.; Academic Press: New York, 1979; p 207.

(7) 10 mL of acrylamide solution (0.73 mol·L^{-1}, 7.3 mmol, 0.5183 g) prepared from reagent grade and degassed water with a resistivity of 18 MΩ.cm^{-1} were introduced into a flask. The solution was degassed over 15 min again. 0.5 mL of HRP (40 mg·mL^{-1}, 20 mg of enzyme) and of H_2O_2 (0.23 mol·L^{-1}, 0.115 mmol, 3.91 mg) solutions and 17.5 μL of distilled 2,4-pentanedione (17.1 mg, 0.17 mmol) are successively injected under stirring (250 rpm). After 3 h of reaction at room temperature, the mixture was poured into a large excess of methanol. A precipitate appeared which was filtered off, washed with methanol, and dried under vacuum at 50 °C.

(8) Shawki, S. M.; Hamielec, A. E. *J. Appl. Polym. Sci.* **1979**, *23*, 3323.

(9) Lovell, P. A. Dilute Solution Viscometry. In *Comprehensive Polymer Science*; Booth, C., Price, C., Eds.; Pergamon Press: Oxford, England 1989; Vol. 1, p 184 and references therein.

(10) Baek, H. K.; Van Waert, H. E. *J. Am. Chem. Soc.* **1992**, *114*, 718. Traylor, T. G.; Traylor, P. T. In *Active Oxygen in Biochemistry*; Valentine, J. S., Foote, C. S., Greenberg, A., Liebman, J. F., Eds.; Blackie Academic & Professional: London, 1995; p 143.

(11) The combination of phenoxy radicals and their mesomeric forms has been extensively applied to the HRP-mediated synthesis of polyphenols; for reaction schemes and relevant works, see: Kobayashi, S. *Adv. Polym. Sci.* **1995**, *121*, 1.

(12) Derango, A. R.; Chiang, L.-C.; Dowbenko, R.; Lasch, J. G. *Biotechnol. Tech*. **1992**, *6*, 523.

(13) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554.

(14) Lalot, T.; Brigodiot, M.; Maréchal, E. To be submitted for publication.

MA980872+