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Enzyme-mediated radical initiation of acrylamide polymerization: main characteristics of molecular weight control

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Abstract

The free-radical polymerization of acrylamide initiated by a redox system (hydrogen peroxide/β-diketone) catalyzed by horseradish peroxidase is studied with emphasis on the control of the molecular weight of the polymer. The case where 2,4-pentanedione (Acac) is used as the β-diketone is particularly examined. It is shown that the concentration of Acac and that of the enzyme readily control the molecular weight of the polymer. The concentration of hydrogen peroxide does not influence the molecular weight to a significant extent except at extreme values for which enzyme degradation or side reactions interfere with the normal enzymatic cycle. The variations of the molecular weight induced by changes in the chemical structure of the β-diketone are attributed to the reactivity toward the enzymatic cycle. The experimental results are rationalized by the use of classical kinetic equations for free-radical polymerization and including the specific expressions of enzymatic catalysis. The tendencies indicated by the theoretical expressions account for the evolutions given by the experimental results.

Keywords: Oxidoreductase; Horseradish peroxidase; Free-radical polymerization

1. Introduction

Horseradish peroxidase (HRP) is an enzyme that catalyzes the oxidation of several organic substances (phenols, anilines,…) by hydrogen peroxide (H_2O_2) and a few hydroperoxides [1]. The generally accepted mechanism involves the production of free radicals [2]. This property has widely been used in the case where phenol and aromatic amines are the reducing species [3–6]; in addition, the catalysis has been tested in non-aqueous and interfacial systems [7,8]. Nevertheless, other kinds of molecules can be used as substrates for HRP like carbonyl compounds [9,10]. In that case, it has been established that the real substrate of HRP is the enol form of the carbonyl compound [10]. The radicals produced in this way can initiate free-radical polymerization converting the ternary mixture (HRP, H_2O_2, substrate) into an 'enzymatically catalyzed redox system'. The HRP-mediated polymerization of vinyl monomers was first reported by Derango et al. with acrylic monomers like acrylamide (AAm) and hydroxyethylmethacrylate [11]. The precise mechanism was not investigated and the authors assumed the oxidized form of the enzyme to be the initiator.

Since 1995 our laboratory has been investigating the polymerization of AAm initiated by a redox system using β-diketones as reducing reactants and catalyzed by HRP [12–15]. The case where the β-diketone is 2,4-pentanedione (acetylacetone, Acac) has been particularly studied in our laboratory but also elsewhere [16,17]. Recently, HRP was used to catalyze the initiation of methyl methacrylate polymerization in water miscible cosolvents at room temperature [18].

It is now largely accepted that the redox system relies on the generation of free-radicals through the HRP-catalyzed oxidation of Acac by H_2O_2 even if the radical Acac has not yet been shown to be the true initiating species. The ternary system (HRP, H_2O_2, Acac) was shown to readily initiate AAm polymerization [12]. In addition, it has shown that changing the β-diketone could have dramatic consequences on the results of the polymerization (yield and molecular weight of the polymer) [13]. For instance, the use of 2-acetylcyclopentanone instead of Acac brought about a 15-fold reduction of the molecular weight. This result could be explained by several reasons such as different reactivity in the enzymatic cycle, chain transfer reactions, keto–enol equilibria… So, although this redox system is, in

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its principle, similar to other classical ones such as ammonium persulfate–sodium metabisulfite [19] or persulfate–thiosulfate [20], it exhibits original characteristics mainly linked to the enzymatic catalysis involved in its mechanism. Moreover, it offers the possibility of changing the chemical structure of the reducing species with a rather wide choice.

The knowledge of the main kinds of reactions involved in the enzyme-catalyzed redox system was a prerequisite to start a study about the great features of this new initiating system as for the control of the characteristics of the polymer. The aim of this work was to investigate the influence of each component (HRP, H_2O_2 and Acac) on the molecular weight of the polymer and to relate this influence to its role in the polymerization mechanism. Moreover, we wanted to clarify which processes were controlling the molecular weight (i.e. chain transfer reactions) and in which way the enzymatic catalysis was involved.

2. Experimental

2.1. Materials

AAm (Aldrich, 99% +, electrophoresis grade), HRP (Sigma, 87 purpurogallin units mg^{-1}, type I), hydrogen peroxide (H_2O_2, Prolabo, 30 wt% stabilized aqueous solution), 2,4-pentanedione (Acac, Aldrich) and methanol (Prolabo, analytical grade) were used without further purification. The commercial solution of hydrogen peroxide was titrated volumetrically and it was found to have a concentration of 8.1 mol l^{-1} in fairly good agreement with the manufacturer's indications. We checked that the concentration of this solution did not varied significantly between the various experiments.

2.2. Polymerization

A typical procedure for polymerization experiments can be described as follows. To 15 ml of an AAm solution (reagent grade water with a resistivity of 18 MΩ cm^{-1}, prepared with Millipore Milli-Q system) introduced into a flask, the required amount of Acac is added. The mixture is stirred by a magnetic stirrer and degassed during 30 min. A volume of 0.5 ml of a concentrated HRP solution and the required volume of the H_2O_2 solution are successively added under stirring. Because of the small volumes involved, the H_2O_2 solution is added by means of a microsyringe. After 24 h, the reaction mixture is added dropwise to a large excess of methanol to precipitate polyacrylamide (PAAm). The precipited PAAm is filtered off, washed with methanol and dried under vacuum.

2.3. Measurements

Viscosimetry: The polymers were mainly characterized by viscometry using the value of the reduced viscosity at a given concentration in water (0.17 g dl^{-1}) and at 30°C and in some cases, of the intrinsic viscosity from which the number average molecular weight was calculated thanks to a relation given by Shawki and Hamielec [21].

Size exclusion chromatography: These characterizations were performed at the Laboratoire de Physico-chimie Macromoléculaire, ESPCI (Paris). The apparatus was composed of three columns Shodex OHpack SB-806M HQ coupled with a differential refractometer Waters RI410 and a home made differential viscosimetric detector. The eluant was water containing 0.5 mol l^{-1} of $LiNO_3$.

3. Results and discussion

3.1. Influence of hydrogen peroxide initial concentration

In a previous paper, the reactions involved in the ternary system were examined and roughly identified [15]. It was found that the numerous chemical processes involved in the ternary system should be separated into two families: 'initiation processes' and 'degradation process'. The role of H_2O_2 was complex since, in the explored range of concentration, it was involved in three processes: (1) the catalytic oxidation of Acac; (2) degradation reactions of HRP through the formation of the so-called compound III; and (3) chemical oxidation of Acac to give a cyclic peroxide, a reaction that we will call here 'cycloperoxidation'. As a matter of fact, the choice of adequate concentrations for H_2O_2 was shown to be a good way to limit the undesirable reactions (2) and (3).

Several polymerizations were carried out at room temperature varying the initial concentration of hydrogen peroxide (Table 1). The polymerization occurs only in a limited range of concentration which lies between 10^{-4} and 10^{-2} mol l^{-1} for $[HRP]_0 = 1.9$ g l^{-1}. The upper limit is due to enzyme degradation by H_2O_2 as previously mentioned [15]. As for the lower limit, it probably comes from the total consumption of H_2O_2 by side reactions (inactivation of HRP, Acac oxidation into cycloperoxide) or from an excessively slow initiation. The variation of the molecular weight can be approximated by that of the reduced

Table 1
Yield and reduced viscosity of PAAm obtained with various initial concentrations of hydrogen peroxide ($[AAm]_0 = 1.22$ mol l^{-1}; $[Acac]_0 = 0.061$ mol l^{-1}; $[HRP]_0 = 1.9$ g l^{-1}; room temperature)

Entry	$[H_2O_2]_0$ (mol l^{-1})	Yield[a] (%)	η_{red}[b] (ml g^{-1})
1	0.124	0	–
2	0.011	97	292
3	0.003	97	177
4	0.001	94	212
5	7.0×10^{-4}	89	204
6	2.0×10^{-4}	75	578
7	9.0×10^{-5}	28	344
8	5.0×10^{-5}	0	–

[a] The yield corresponds to the weight ratio of dry polymer recovered after a 24 h reaction with acrylamide introduced initially.
[b] Determined at [PAAm] = 0.17 g dl^{-1} in water at 30°C.

Table 2
Influence of the initial concentrations of Acac and enzyme on the yield and characteristics of the PAAm obtained

Entry	$[AAm]_0$ (mol l^{-1})	$[Acac]_0 \times 10^2$ (mol l^{-1})	$[H_2O_2]_0 \times 10^3$ (mol l^{-1})	$[HRP]_0$ (g l^{-1})	Yield[a] (%)	η_{red}[b] (ml g^{-1})	$M_n \times 10^{-3}$ [c] (g mol^{-1})	I_p
9	0.64	0.8	11.0	1.90	90	248	208	–
10	0.64	1.8	11.0	1.90	100	164	99	–
11	0.64	6.1	11.0	1.90	72	50	21	–
12	0.64	25.1	11.0	1.90	100	44	17	–
13	1.23	17.0	11.0	1.90	96	74	35	–
14	1.23	1.6	11.0	1.90	95	294	252	–
15	1.23	0.8	11.0	1.90	92	408	367	–
16	1.23	0.4	11.0	1.90	10	1350	1391	–
17	1.23	0.2	11.0	1.90	0	–	–	–
18	0.64	2.0	0.7	0.06	93	299	283	2.2
19	0.64	2.0	0.7	0.10	100	230	177	2.4
20	0.64	2.0	0.7	0.19	96	170	116	2.5
21	0.64	2.0	0.7	0.34	96	137	89	2.7
22	0.64	2.0	0.7	0.49	92	118	61	2.8
23	0.64	2.0	0.7	2.03	79	72	34	3.1
24	0.64	2.0	0.7	5.98	56	78	–[d]	–
25	0.64	2.0	0.7	12.02	58	74	–[d]	–

[a] The yield corresponds to the weight ratio of dry polymer recovered after a 24 h reaction with acrylamide introduced initially.
[b] Determined at [PAAm] = 0.17 g dl^{-1} in water at 30°C.
[c] The intrinsic viscosities were determined and the number-average molecular weights were deduced thanks to the experimental relation established by Shawki and Hamielec: $[\eta] = 6.8 \times 10^{-4} M_n^{0.66}$ ($[\eta]$ is expressed in dl g^{-1}) [21].
[d] Not determined.

viscosity (at a given concentration). It seems that H_2O_2 concentration does not have a great influence on the molecular weight except when it is close to the upper and lower limits. For these values, the reduced viscosity becomes much higher indicating an increase of the molecular weight.

3.2. Influence of acetylacetone initial concentration

The influence of Acac initial concentration was checked at two different monomer concentrations (Table 2, [AAm] = 0.64 and 1.23 mol l^{-1} for entries 9–12 and 13–17, respectively). It is clear that there is a lower limit for Acac concentration since for concentrations lower than 4 mmol l^{-1} no polymer can be recovered. On the contrary no upper limit can be evidenced as in the case of H_2O_2. This is an indication of the distinct roles of these two components. Acac does not take part into degradation reactions and is only involved in the production of radicals (even if it is not the true substrate of HRP but its enol form). As a result, only a lower limit is conceivable, for which the initiation rate is too low to give rise to significant polymerization. From the values of the reduced viscosity, it is clear that Acac concentration influences the molecular weight: the higher the Acac concentration, the lower the reduced viscosity. For further investigation, the intrinsic viscosities of these polymers were determined and the number-average molecular weights were deduced (see experimental section). The results are depicted in Fig. 1 where M_n is plotted against the ratio of monomer to Acac initial concentrations. Previous results from Lalot and coworkers [13] are also added for comparison. The molecular weight is conveniently correlated to the AAm to Acac initial concentrations ratio which indicates that this is the relevant parameter for controlling the polymer molecular weight. When the $[AAm]_0/[Acac]_0$ ratio is varied from about 1 to 10^3, M_n increases from 10^4 to 10^6 g mol^{-1}. As already indicated in a previous paper [12], the polydispersity of the polymers (I_p) is close to what is usually obtained with radical polymerization, that is to say, $I_p = 2$–3.

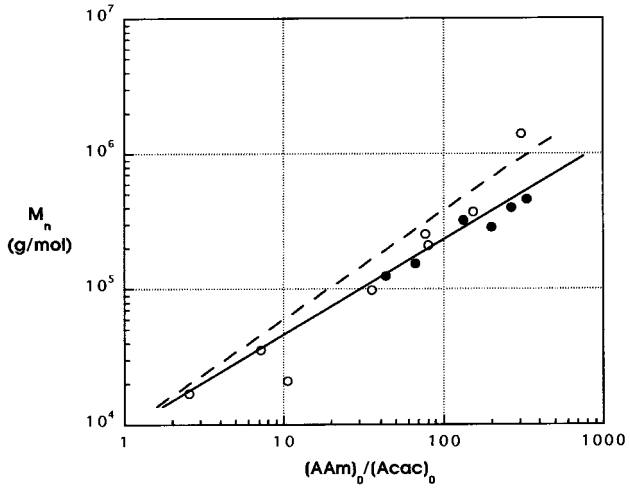

Fig. 1. Variation of the molecular weight of the polymer as a function of the ratio of the initial concentration of acrylamide to that of acetylacetone (○). The values obtained by Teixeira et al. [13] with the same initiating system are also plotted for comparson (●). The continuous line (–) represents the fit of the experimental points by a power-law (exponent = 0.8). The dashed line (– – –) indicates the tendency of the calculated values.

3.3. Influence of enzyme concentration

Several experiments were carried out with enzyme concentrations ranging from 0.06 up to 12 g l^{-1} (Table 1, entries 18–25). For these experiments, H_2O_2 concentration was kept at a low value (7×10^{-4} mol l^{-1}) in order to maintain a H_2O_2 to HRP ratio favorable to polymerization for all enzyme concentrations [15]. The polymerization yield is always around 95% except that it drops down to about 60% for the two highest concentrations. The values of the reduced viscosity clearly indicate that HRP concentration influences the molecular weight. The higher the enzyme concentration, the lower the reduced viscosity and the molecular weight as well. We can notice that for enzyme concentrations higher than 0.49 g l^{-1} no significant variation of the reduced viscosity of the polymer can be evidenced. This would mean that the molecular weight does not vary significantly in that range of concentration. We should notice that the yield is much lower for the two higher enzyme concentrations used than for the other experiments. It could mean that a significant part of the polymer is lost in the precipitation step and especially the low-molecular-weight-products. As a result, the viscosimetric measurements made with the corresponding products does not account only for the polymerization initial conditions but also for the fractionation during the precipitation. So, at the present time, it is not possible to decide if these two experiments indicate a real tendency or are simply due to the experimental procedure. Further experiments must be carried out to clarify this point.

3.4. Analysis with kinetic equations from free-radical polymerization and enzyme catalysis

The enzyme-mediated polymerization is assumed to follow the mechanism depicted in Scheme 1, where M is the monomer, P the polymer chain, K the equilibrium constant, and k the rate constant. The rate of the enzymatic cycle can be derived under the form of Eq. (1), with the assumption that $k_2 \gg k_3$ which is a rather general result [22]. Furthermore, since $k_1 \approx 10^7$ l mol^{-1} s^{-1}, the limiting step will be the production of Acac$^{\cdot}$ from HRP-II for all the experimental conditions used in this study. Provided that the initiator efficiency equals 1, Eq. (1) finally reduces to Eq. (2). The instantaneous degree of polymerization can be written as shown by Eq. (3), where C_M, $C_{H_2O_2}$, C_{Acac} and C_{enol} are the transfer constants to monomer, hydrogen peroxide, Acac ketone and enol forms, respectively [23,24]. The chain transfer to the cyclic peroxide is assumed to be negligible. The termination is primarily be disproportionation [25]. The last two terms of this equation can be unified using the equilibrium constant of enolization K_e and defining an 'overall transfer constant' (Eq. (4)). Then Eq. (3) becomes Eq. (5), where [Acac] = [keto] + [enol form] represents the total concentration of Acac in the polymerization medium either under the keto form or under the enol form.

Equilibria involving the initating species

Acac = enol	K_e
Acac + H_2O_2 = PIV	K_{ox}

Initiation

HRP + H_2O_2 → HRP-I + H_2O	k_1
HRP-I + enol → HRP-II + Acac$^{\cdot}$	k_2
HRP-II + enol → HRP + Acac$^{\cdot}$ + H_2O	k_3
M + Acac$^{\cdot}$ → Acac-M$^{\cdot}$	k_i

Propagation

Acac-$M_n^{\cdot}$ + M → Acac-$M_{n+1}^{\cdot}$	k_p

Termination

Acac-$M_n^{\cdot}$ + Acac-$M_p^{\cdot}$ → P_n + P_p	k_t

Scheme 1.

$$r = \frac{[\mathrm{HRP}]_0}{\dfrac{1}{k_1[\mathrm{H_2O_2}]_0} + \dfrac{1}{k_3[\mathrm{enol}]_0}} \tag{1}$$

$$R_i = 2r = 2k_3[\mathrm{HRP}]_0[\mathrm{enol}]_0 \tag{2}$$

$$\frac{1}{\mathrm{DP_n}} = \frac{1}{\mathrm{DP_0}} + C_M + C_{H_2O_2}\frac{[H_2O_2]}{[AAm]} + C_{Acac}\frac{[\mathrm{Acac(ketoform)}]}{[AAm]} + C_{enol}\frac{[enol]}{[AAm]} \tag{3}$$

$$\mathrm{DP_0} = \frac{k_p}{\sqrt{k_t k_3}}\frac{[AAm]}{\sqrt{2[enol][HRP]}} \tag{4}$$

$$\frac{1}{\mathrm{DP_n}} = \frac{1}{\mathrm{DP_0}} + C_M + C_{H_2O_2}\frac{[H_2O_2]}{[AAm]} + C_A\frac{[Acac]}{[AAm]} \tag{5}$$

The following values are given in the literature for the polymerization of AAm at 25°C in water: $C_M = 0.12 \times 10^{-4}$, $k_p = 18,000$ l mol^{-1} s^{-1} and $k_t = 14.5 \times 10^6$ l mol^{-1} s^{-1} [26]. As for k_3 since no value is available for Acac to the best of our knowledge, we use the value of k_2 determined for malonaldehyde by Mac Donald and Dunford [27] and assume that $k_3 \approx 0.1k_2$. As a result we take $k_3 = 10$ l mol^{-1} s^{-1}, a value that is not intended to be more

Table 3
Polymerization of acrylamide involving various β-diketones and initiated either by the enzyme-catalyzed redox system at 25°C or by 4,4′-azobiscyanovaleric acid (ACV) at 60°C. Yield and molecular weight of the polymer

β-Diketone	Enol content[a] (%)	HRP-catalyzed redox system[b]		ACV[c]	
		Yield[d] (%)	$Mn \times 10^{-3}$ [e] (g mol^{-1})	Yield[f] (%)	$Mn \times 10^{-3}$ [g] (g mol^{-1})
–	–	–	–	99	590
2,4-pentanedione	16	93	124	99	484
2-acetylcyclopentanone	30	76	5.1	99	472
2-methylcyclohexane-1,3-dione	100	86	9.8	99	275
2-methylcyclopentane-1,3-dione	100	38	10.5	99	430

[a] In water at 25°C (data from Ref. 20).
[b] $[AAm]_0 = 0.66$ mol l^{-1}; $[\beta\text{-diketone}]_0 = 0.017$ mol l^{-1}; $[HRP]_0 = 2.0$ g l^{-1}; 25°C (from Ref. 13).
[c] $[AAm]_0 = 0.70$ mol l^{-1}; $[\beta\text{-diketone}]_0 = 0.010$ mol l^{-1}; $[ACV]_0 = 1.5 \times 10^{-3}$ mol l^{-1}; 25°C.
[d] After a 180 min reaction.
[e] Number-average molecular weight determined by intrinsic viscosity measurements.
[f] After a 75 min reaction.
[g] Number-average molecular weight determined by size exclusion chromatography.

than an order of magnitude. For the chain transfer to hydrogen peroxide with AAm, it can be found that: $C_{H_2O_2} = 5 \times 10^{-4}$ at 25°C [26]. As for the transfer to Acac (without differentiating keto and enol forms) no results are available for AAm but some data can be found for other monomers [26].

The concentration of enol is calculated from that of Acac and H_2O_2 by taking into account the two equilibria already mentioned: enolization of Acac and its cycloperoxidation by H_2O_2. The numerical data of Milas et al. are used [28], assuming that the concentration range did not change the cycloperoxidation equilibrium composition. This latter assumption was checked in a previous paper [15]. If we consider, for instance, the conditions described in entry 12 (Table 2), we find: $(DP_{n,0})^{-1} = 32 \times 10^{-4}$, $C_{H_2O_2}([H_2O_2]/[AAm]) = 0.086 \times 10^{-4}$ and C_A $([Acac]/[AAm]) = 3.9 \times 10^{-4}$ (with $C_A = 10^{-3}$). Thus, it is clear that only the first term of Eq. (5) is relevant for all the experimental conditions used in this study. The various chain transfer processes that have been enumerated do not influence significantly the molecular weight of the polymer. So the variation of the molecular weight as a function of the initial concentration of Acac (Fig. 1) comes from the variation of the concentration of enol that is controlled through the keto–enol equilibrium and the cycloperoxidation equilibrium.

We can try to go a little bit further by comparing the molecular weights determined experimentally to the values given by Eq. (4) and estimating the concentration of enol in the solution as described above. We should underline here that Eq. (4) is an instantaneous degree of polymerization. As a result, if this equation is used with the initial concentrations of the reactants, it would give the molecular weight of the PAAm chains produced at the very beginning of the polymerization. On the contrary, the molecular weights experimentally determined and reported in Fig. 1 are average values obtained after the polymerization has completed, that is to say, cumulative MW. So, it is not intended to predict quantitatively MW, nor to check the values of the kinetic constants, but only to verify if Eq. (4) gives a correct tendency for the variation of M_n with the concentration of Acac. This could be reasonable provided that the MW distributions of the various PAAm examined are similar. The results obtained are compared to the number-average MW determined by viscosimetry (Fig. 1). Apart from the fairly good agreement between the experimental values and the calculated ones, we can see that the calculated values give the good tendency for the influence of the AAm to Acac ratio on M_n. So, the control of the molecular weight of the polymer by the concentration of Acac can be depicted by Eq. (4) without considering any chain transfer reaction. Finally, we must notice that in these experimental conditions $[H_2O_2]_0/[HRP]_0 = 250$. There is probably a fraction of the enzyme which is degraded in side reactions (it will be denoted δ with $\delta < 1$) and, consequently, is not involved in the initiating reactions [15]. A rough way to account for this fact would be to use an 'effective enzyme concentration' given by $[HRP]_{eff} = (1 - \delta)[HRP]_0$ instead of $[HRP]_0$ in Eq. (4). Nevertheless, since $[HRP]_0$ and $[H_2O_2]_0$ are kept constant in all these experiments, this is not important for the comparison made.

Starting from these results, we can reconsider previous experiments where the chemical structure of the β-diketone was varied, as was reported in a previous paper [13]. The use of some cyclic β-diketones gave rise to dramatic changes in the molecular weight of the polymer. In order to check if these variations came from chain transfer, additional experiments were carried out using 4,4′-azobiscyanovaleric acid (ACV) as initiator, in the presence of the different β-diketones. The molecular weights of the PAAm obtained are given in Table 3 as well as those of the PAAm synthesized with the HRP catalyzed redox systems involving

the same β-diketones. First, it is obvious that the presence of the β-diketone brings about a decrease of the molecular weight. This could be interpreted as the result of chain transfer to the β-diketone and it is worth mentioning that all the β-diketones tested cause a decrease of the molecular weight. If we now turn to the results obtained with the enzyme catalyzed redox system, we see that two main differences are evidenced. The variation of the molecular weight is much wider compared to the case of the ACV-initiated polymerizations. Furthermore, the yield remains close to 100% with the ACV-initiated polymerization while it varies between 38 and 93% for the HRP-mediated redox system. These results demonstrate that the influence of the β-diketone structure in the HRP catalyzed redox system is not a consequence of the chain transfer activity but rather comes from their reactivity in the enzyme catalyzed radical generation. More precisely, we should say that, even if chain transfer to the β-diketone takes place, it is not predominant and cannot account for the variations of the molecular weight observed with the enzyme-catalyzed redox systems. According to the results described above for Acac, three aspects related to the β-diketone structure have to be considered: the enol content, the reactivity toward the 'chemical' oxidation by H_2O_2 and the substrate affinity for HRP (i.e. the value of k_3). If we consider the enol content at 25°C of the various β-diketones tested (Table 3), we get aware that this is probably not the only determining factor even if it can partly account for the relatively high molecular weights obtained with Acac. We can try to make a more quantitative comparison based on Eq. (4) since it was shown to be relevant for the polymerization with Acac and the preceding results indicate that chain transfer is not the main reason for the variations observed. In the case of Acac (1) and 2-acetylcyclopentanone (2), if we assume that the concentration of enol is responsible for the variation of the molecular weight, this would involve: $[\text{enol}(2)] = 600[\text{enol}(1)]$. This seems clearly unrealistic when considering the data on keto–enol equilibrium even if the cycloperoxidation equilibrium can vary to a certain extent from one β-diketone to another. Now, if we suppose that the enol content of 2-acetylcyclopentanone in the polymerization medium is that given by the equilibrium data (i.e. no cyclic peroxide is formed) we have $[\text{enol}(2)] = 4[\text{enol}(1)]$. As a result, to account for the difference in the molecular weights, we should have $k_3(2) = 150k_3(1)$. So, from the polymerization results, we can estimate that the rate constant k_3 for 2-acetylcyclopentanone should be two orders of magnitude higher than that of Acac.

Other studies have to be done to determine the sensitivity of these β-diketones to H_2O_2 oxidation and to investigate the kinetics of their reaction with HRP. This information would allow to select the best β-diketone for a given polymer synthesis. Changing the β-diketone allows one to adjust the range of the molecular weight of the polymer (either oligomers or long chains can be obtained in the same conditions of concentration) and can also allow one to insert defined functional groups at the end of the chains (with specific β-diketones carrying desired substituent groups).

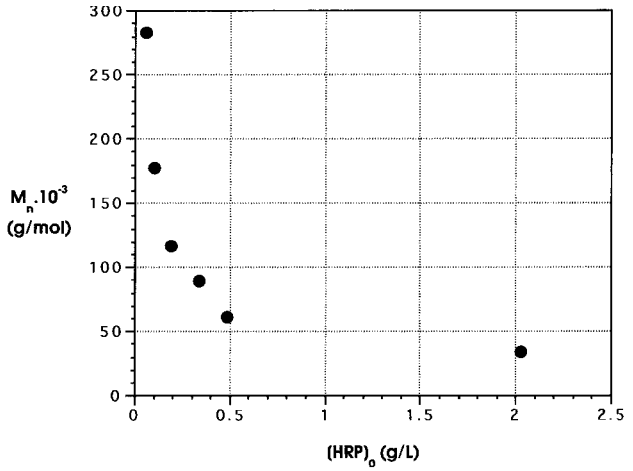

Fig. 2. Variation of the molecular weight of the polymer as a function of the initial concentration of horseradish peroxidase. $[AAm]_0 = 0.64$ mol l^{-1}; $[Acac]_0 = 0.02$ mol l^{-1}; $[H_2O_2]_0 = 7 \times 10^{-4}$ mol l^{-1}.

As for the effect of the concentration of hydrogen peroxide (see Section 3.1), Eq. (5) shows that, since the term of chain transfer to H_2O_2 is negligible, the concentration of hydrogen peroxide does not influence the molecular weight in our conditions. This comes from the fact that the step involving H_2O_2 in the enzymatic cycle is not the kinetically limiting one. Nevertheless, for extreme values, the polymerization is disturbed either due to enzyme degradation (high concentrations) or to a total consumption of H_2O_2 in side reactions (low concentrations). Obviously, this is not taken into account by the preceeding equations. The partial degradation of HRP by H_2O_2, can be included in Eq. (4) by using an effective enzyme concentration as mentioned above. Nevertheless, this requires quantitative data about the degradation of HRP by H_2O_2, similar to what has been done for lactoperoxidase [29]. As a conclusion, no satisfactory control of the molecular weight can be achieved by varying the concentration of H_2O_2.

When the concentration of HRP was varied between 0.06 and 2 g l^{-1} (Fig. 2), a regular decrease of the molecular weight was evidenced through the evolution of the reduced viscosity. This is predicted by Eq. (4) with an inverse dependence on the squareroot of HRP concentration. For higher concentrations of HRP (6 and 12 g l^{-1}), the molecular weight does not vary anymore. The preceding equations cannot account for this leveling down of the molecular weight which was attributed to a fractionation of the polymer during the precipitation step.

4. Conclusions

The molecular weights of several PAAm synthesized by radical polymerization initiated by an HRP-catalyzed redox system were measured and correlated to the initial concentrations of reactants. It was demonstrated that the two substrates of HRP involved in the redox system: H_2O_2 and enol, do not have the same influence on the molecular weight of the polymer. While H_2O_2 does not seem to influence the molecular weight except at extreme values, Acac is shown to readily control the length of the chains, any other concentrations being equal. Playing with the concentration of Acac, it is then possible to vary the molecular weight over two decades. The concentration of enzyme also appears to influence the molecular weight but to a lesser extent. These variations were rationalized using the characteristic expressions of enzymatic catalysis and the classical kinetic equations of free-radical polymerization. Despite the lack of kinetic constants for Acac, which does not allow a quantitative comparison, it was shown that the tendencies indicated by the theoretical expressions are able to account for the experimental results. Particularly, it was demonstrated that no chain transfer process was significant in the conditions chosen when Acac is used. Moreover, the strong influence of the chemical nature of the β-diketone on the molecular weight was shown to come at least partly from its reactivity toward HRP compounds I and II. As a result, general guidelines are provided in order to apply these enzymatically catalyzed redox systems to the synthesis of polymers with controlled molecular weights and defined end-groups.

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