STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, XLVIII, 1, 2003

HORSERADISH PEROXIDASE - CATALYZED OXIDATION OF SOME BENZYL-TYPE ALCOHOLS

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ABSTRACT. Horseradish peroxidase catalyzed the selective oxidation of some hydroxymethyl-furan derivatives to the corresponding aldehydes only in the presence of a thiol, manganous ions, sodium malonate and a co-solvent.

INTRODUCTION

Horseradish peroxidase (HRP) is a haemoprotein known to catalyze the oxidation of a wide range of compounds. Its normal catalytic cycle involves the oxidation by hydrogen peroxide of the native state of the enzyme (ferric) to an intermediate two oxidation equivalents above (termed Compound I), which contains a porphyrin radical cation and a ferryl group. Compound I then interacts with the substrate, yielding Compound II (one oxidation equivalent above the native state) and substrate radical cation. Compound II similarly reacts with another substrate molecule to yield the ferric state of the enzyme and a substrate radical cation [1].

While the oxidation of typical substrates (phenols and aromatic amines) presents low selectivity and often proceeds towards rather complex reaction mixtures, a few more selective reactions are also performed by HRP [1]. Among these is the oxidation of benzyl-type alcohols to the respective aldehydes. The earliest example in that respect was provided by Saunders [2], while oxidizing mesitol with HRP and H_2O_2 (Fig. 1).

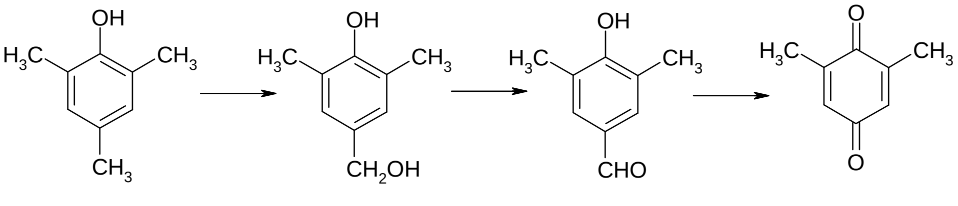


Fig. 1. Mesitol oxidation as performed by HRP/H_2O_2. All of the above compunds were recovered from the reaction mixture [1].

However, this has remained for a long time an isolated observation, although other peroxidases are known to perform the same reaction on a larger number of substrates.

Soybean peroxidase (SBP) has been shown to oxidize benzyl-type alcohols at extremely acid pH's [3]. Although the SBP reactivity is rather similar to that of HRP, SBP is characterized by stronger resistance to external factors (either temperature or pH). Thus, although HRP was eventually shown to oxidize the hydroxymethyl group, the enzyme was rapidly inactivated at the respective pH [3].

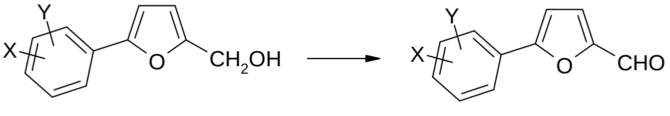
Lignin peroxidase and manganese peroxidase have veratryl alcohol as a natural substrate (while employing manganous ions as cofactors). Although the veratryl alcohol redox potential is outside the range of HRP substrates, the selective oxidation of veratryl and benzyl alcohols (mediated by thiols and Mn^{2+}) to the respective aldehydes has been surprisingly reported [4]. O_2 is consumed and H_2O_2 is not required in the process. An organic chelator (such as malonate) was also required. Aerobically generated thiyl radicals were found to have a key role both in initiating the reaction and in providing a complex with the manganous ion that would eventually interact with the substrate and generate substrate radical cations. Only two substrates were tested (veratryl alcohol and benzyl alcohol) [4].

While the influence of all components of the reaction mixture has been examined in the previous study [4], little attention was paid to the possibility of using non water-soluble substrates. However, performing the reaction in organic media would greatly enhance the range of prospective substrates.

Our interest in the chemistry of heterocycles has recently led to the synthesis of some hydroxymethyl-substituted furan derivatives as well as of the respective aldehydes [5-8]. In order to perform the HRP-catalyzed oxidation of the former to the latter, the problem of substrate insolubility in water had to be overcome.

RESULTS AND DISCUSSION

The oxidation of the hydroxymethyl substituted furan derivatives 1A-8A to the corresponding aldehydes 1B-8B (Fig. 2) was performed in conditions



1: X=H, Y=4-F
2: X=H, Y=4-I
3: X=H, Y=3-Br
4: X=H, Y=2-Cl
5: X=H, Y=2-Br
6: X=H, Y=2-Y
7: X=3-Me, Y=5-NO2
8: X=4-Me, Y= 3-NO2

Fig. 2. The selective oxidation of some furan derivatives.

identical to those already reported, with the significant exception of the presence of a large quantity of co-solvent (its concentration was chosen in the range of 40-70% in order to provide a homogenous reaction mixture). Of the co-solvents tested, the better yields were obtained with methanol. Although no side reactions were observed (significantly, no carboxylic acid could be detected), the yields were not very high (20-60%, depending mainly on the co-solvent used).

Control experiments were run with the furan derivative 1 (see EXPERIMENTAL) using HRP and hydrogen peroxide as oxidizing agents in water/ methanol mixtures, in the pH range 2-8. No aldehyde could be detected over a 24h period. Longer exposure brought about non-enzymatic degradation of the substrate. On the other hand furfurol was easily oxidized to furoic acid under the same conditions. Control experiments with the aldehyde counterpart of 1 as substrate showed that on exposure to either HRP/H_2O_2 or HRP/ thiol/Mn^{2+} over more than 24 hrs gradual consumption of the aldehyde occurred. This was expected since carbonyl derivatives (especially isobutyraldehyde and acetophenone) are known peroxidase substrates [1].

The reaction mechanism suggested for this oxidation is illustrated in Fig. 3.

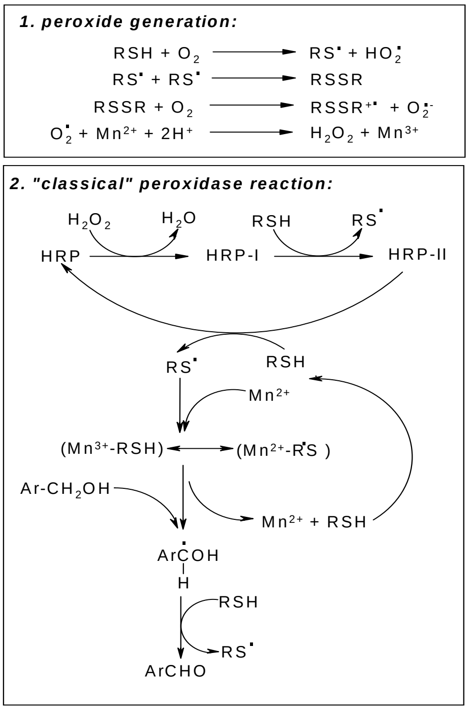


Fig. 3. The proposed mechanism for the oxidation of benzyl-type alcohols [4]. HRP-I and HRP-II are the peroxidase Compounds I and II, respectively. The mechanism implies two distinct steps: (1) peroxide generation from molecular oxygen (initiated by thiols), and (2), a peroxidasic reaction sustained by peroxide generated in (1).

Thus, the method described proves to be an interesting synthetic route towards aldehydes, and is of use for both water soluble and non-water soluble substrates.

EXPERIMENTAL

Horseradish peroxidase was an aqueous preparation [9]. A single stock solution was used and the enzyme concentration (ε = 98000 at 408 nm), as well as its RZ and activity [10] were routinely checked by known procedures. All other reagents were of analytical grade. 1A-8A were prepared as previously described [6,7].

A typical reaction mixture included a thiol (glutathion or cysteine, 16 mM), $MnCl_2$ (1 mM), sodium malonate (10 mM) and the substrate (1 mM) in acetate buffer (0,1M, pH=4)/organic solvent (methanol, DMSO or dioxane). The reaction was started by adding the enzyme (1 μM). After stirring for 60-90 min the mixture was extracted with ether; the organic solvent was evaporated and the residue recrystalyzed from petroleum ether, thus providing the aldehyde (yields 20-60%; the best yield was obtained with 1 using methanol as a co-solvent).

The ^{1}H-NMR spectra were recorded on a Varian Gemini 300 Spectrometer operating at 300MHz, in $CDCl_3$ solution and TMS as internal standard. IR spectra were obtainded in KBr pellets on a Nicolet FT205 spectrometer and are reported in wavenumbers (cm^{-1}). The mass spectra were recorded on Double focusing Varian Matt 311 Spectrometer, with an electronic impact source at 70eV and 300mA.

The melting points and the spectral data of 1B-8B have identical values with those previously described [8,9] confirming the reaction products.

1B: **5-(4-fluorophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 69;
IR (KBr) 1650 (CHO);
^{1}H-NMR ($CDCl_3$) δ 6.8(d,1H) 7.34 (d,1H) 7.80 (d,2H) 7.82 (d,2H) 9.64 (s,1H);
MS m/e: 190(92)M, 171(5), 162(21), 144(2), 133(100).
2B: **5-(4-iodophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 144^{0}C;
IR (KBr) 1680(CHO);
^{1}H-NMR ($CDCl_3$) δ 6.87(d,1H) 7.34(d,1H) 7.55(d,2H) 7.90(d,2H) 9.65(s,1H);
MS m/e: 298(100)M, 270(13), 241(28), 171(3).
3B: **5-(3-bromophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 107^{0}C;
IR (KBr) 1700(CHO);
^{1}H-NMR ($CDCl_3$) δ 6.87(d,1H) 7.34(d,1H) 7.33(d,1H) 7.53(d,1H) 7.75(d,1H) 7.98(s,1H) 9.67(s,1H);
MS m/e: 250, 252 (100)M, 222, 224(8), 193, 195(33).
4B: **5-(2-chlorophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 77^{0}C;
IR (KBr) 1673(CHO);

^{1}H-NMR ($CDCl_3$) δ 7.30-8,05(m,6H) 9.69(s,1H);
5B: **5-(2-bromophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 57^{0}C;
IR (KBr) 1683(CHO);
^{1}H-NMR ($CDCl_3$) δ 7.37-7.97(m, 6H) 9.69(s,1H);
MS m/e: 250, 252(100)M, 222, 224(10), 193, 195(33).
6B: **5-(2-iodophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 64^{0}C;
IR (KBr) 1675(CHO);
^{1}H-NMR ($CDCl_3$) δ 6.87(d,1H) 7.34(d,1H) 7.55-7.93(m,4H) 9.65(s,1H);
MS m/e: 298(100)M, 270(13), 241(32), 171(2).
7B: **5-(3-methyl-5-nitrophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 113^{0}C;
IR (KBr) 1680(CHO);
^{1}H-NMR ($CDCl_3$) δ 2.68(s,3H) 6.96(d,1H) 7.41(d,1H) 8.03 (d,2H) 8,14(d,2H) 8.18(s,1H) 9.75(s,1H),
MS m/e: 231(100)M, 205(3)M-28, 201(13)M-30, 185(7), 128(43)
8B: **5-(4-methyl-3-nitrophenyl)-furan-2-carboxaldehyde**: white crystals, mp: 143^{0}C;
IR (KBr) 1675(CHO);
^{1}H-NMR ($CDCl_3$) δ 2.65(s,3H) 6.95(d,1H) 7.37(d,1H) 7.45 (d,1H) 7.95(d,1H) 9.39(s,1H) 9.69 (s,1H);
MS m/e: 231(100)M, 205(3)M-28, 201(13)M-30, 185(7), 128(43).

ACKNOWLEDGEMENT. We are grateful to Mr. R. Dumitru for his help on the enzyme preparations.

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