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A novel method for the conversion of benzyl alcohols to benzaldehydes by laccase-catalyzed oxidation

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Abstract

A new catalytic method for the oxidation of substituted benzyl alcohols to the corresponding benzaldehydes by molecular oxygen using the enzyme/cofactor system laccase/2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) is presented. The reaction proceeds under very mild conditions giving the product in quantitative yields. The enzyme requires at least one free *ortho*-position in the substrate molecule for the reaction to proceed.

Keywords: Enzymatic catalysis; Oxidation; Benzyl alcohols; Laccase; ABTS

1. Introduction

The enzyme laccase (benzenediol: oxygen oxidoreductase; EC 1.10.3.10) is a glycoprotein containing four copper atoms per molecule in distinctive environments [1]. It occurs widely in trees and fungi [2,3], and is probably involved in the biosynthesis of lignin [4–7]. Laccase has been intensively investigated because of its capability to degrade lignin [8], and it has already found industrial application [9–11]. We are focusing our interest on the utilization of laccase in organic syntheses [12] in response to increasing demands for reactions under mild or even physiological conditions.

Laccase catalyzes the four-electron reduction of molecular oxygen to water and thereby oxidizes the substrate. In this process, phenols react via H atom abstraction, i.e., one proton and one electron, producing phenoxyl radicals that undergo a variety of subsequent reactions. Therefore, the reaction of laccase with phenols is not important in high-yield syntheses. While reactions of laccase with phenols have been comprehensively studied [13], there are very few reports on reactions of non-phenolic compounds with this enzyme [14–16]. The reactions described in the literature so far were exclusively related to the degradation of lignin model compounds, but not to synthesis applications. Since native laccase alone has insufficient reactivity towards non-phenolic substrates, it is commonly applied with a cofactor, often referred to as a

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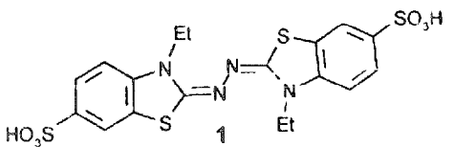
mediator. It has been demonstrated that 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) (1) and its salts are appropriate mediators for laccase [17]. The diammonium salt ABTS-$(NH_4)_2$ was used throughout this work.



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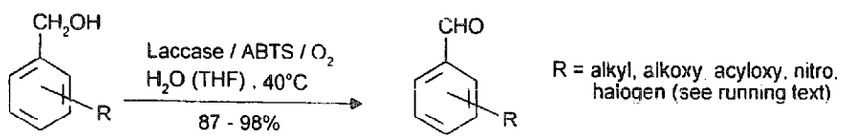
Catalyst component 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS (1)

In this paper, we discuss a new general method for the oxidation of non-phenolic benzyl alcohols to corresponding benzaldehydes by molecular oxygen using laccase/ABTS as a catalyst. The reaction proceeds under physiological conditions giving overall yields about 90%, thereby proving the high selectivity of the enzyme catalyst used. No acids or other by-products are formed. The overall reaction is given in Scheme 1.

The reaction mechanism and the kinetics of the reaction are still being investigated in our group. However, preliminary conclusions can be drawn from the results obtained so far. Native 'resting' laccase contains four Cu(II) atoms. The mediator acts as an activator of the enzyme by transferring electrons, and is converted into the very stable ABTS cation radical. This initial step enables the enzyme to start the catalytic cycle of transferring electrons from the substrate to oxygen in two-electron steps. Both the oxygen as the oxidant [18,19] and the benzyl alcohol as the substrate [22] are temporarily attached to the enzyme during this process. The binding between the substrate and the enzyme is unambiguously proven by the fact that the oxidation of the benzyl alcohol stops at the aldehyde stage. The exclusive occurrence of the aldehydes with no further formation of acids cannot be explained if the oxidation of the substrate is not accomplished by the enzyme, but is a matter of electrochemical potentials in the solution. In this hypothetical case, the aldehyde formed must undergo immediate further oxidation to the acid because of the higher reduction potential of the aldehydes compared to the corresponding alcohols. This, however, is contradictory to the observed formation of benzaldehydes in very high yields.

The cation radical of ABTS — formed by the one-electron transfer to the enzyme — is stable and does not contribute further to the reaction. The theory of this radical being the actual oxidant [20,21] was disproved by several experiments carried out in our group [22]. The most convincing evidence is the inertia of the ABTS cation radical — produced in the absence of laccase by other means, e.g., ultrasound or removal of the enzyme by ultrafiltration — towards non-phenolic benzyl alcohols in the absence of laccase. Hence, the action of the enzyme in the presence of molecular oxygen is not confined to the generation of a radical species, but is also involved in the accomplishment of the substrate oxidation in two-electron processes.

There are very few substituents and structures on the aromatic ring which interfere with the reaction, implying its broad applicability in organic synthesis. Phenolic OH groups (also NH_2 groups) must be protected since laccase oxidizes compounds carrying unprotected phenolic OH groups by electron (H atom) abstrac-



Scheme 1. Oxidation of substituted benzyl alcohols by oxygen, catalyzed by laccase/ABTS.

tion, producing resonance-stabilized aroxyl radicals, as described above. It is worth mentioning that aliphatic OH and NH_2 groups, as investigated so far, are not affected by the laccase/ABTS-catalyzed oxidation of the benzylic OH group. Therefore, these groups may remain unprotected during the reaction. This demonstrates once more the high specificity of the catalyst. Except aromatic methyl groups [12], alkyl substituents do not impair the reaction. Alkoxy and acyloxy substituents are chemically inert towards the catalyst system, rendering the reaction potentially useful for the oxidation of α-hydroxymethylphenols to corresponding benzaldehydes after protection of the phenolic OH group by etherification or esterification. Moreover, the reaction system applied does not affect other common substituents on aromatic rings, such as nitro groups or halogens.

During our investigations it became obvious that laccase does not accept 2,6-disubstituted benzyl alcohols, such as 2,6-dichlorobenzyl alcohol, as substrates. There are two possible reasons for this behaviour. The enzyme might impose strong steric requirements so that ortho-substituents in the substrate obstruct the free access to the active site of the enzyme. On the other hand, free aromatic hydrogens at ortho-positions, i.e., no ortho-substituents, might be crucial for the enzymatic reaction to proceed. Since fluorine shows a steric effect which is comparable to hydrogen, 2,6-difluorobenzyl alcohol was used to decide upon whether the mere absence of bulky ortho-substituents or the presence of ortho-hydrogens are substantial prerequisites. No reaction product was observed when this compound was subjected to the enzymatically catalyzed oxidation. The lack of reactivity clearly demonstrates the necessity of at least one aromatic hydrogen at the ortho-positions to the hydroxymethyl substituent. Consequently, the above described system of laccase and ABTS cannot be employed to oxidize 2,6-disubstituted benzyl alcohols, whereas 2-monosubstituted benzyl alcohols are converted into the corresponding benzaldehydes. The application of the reaction system to vinylogous benzyl alcohols, i.e., cinnamyl alcohols, yields a mixture of products depending on the reaction time and the substitution pattern. Therefore, the presented enzymatic oxidation as a synthetically useful method can currently not be extended to substituted cinnamyl alcohols.

The procedure presented was tested with numerous substituted benzyl alcohols, and should therefore be applicable to a great variety of compounds having similar aromatic structures. Selected examples of benzyl alcohols that were oxidized by oxygen with the laccase/ABTS couple are listed in Table 1, including the products obtained and the yields after work-up.

The advantages of the new method are the selectivity of the enzymatic catalyst system resulting in the formation of products in quantitative yields, the very simple work-up of the reaction mixture requiring no purification of the products in most cases, and the use of the 'clean' oxidant O_2. Further advantages include the physiological conditions applied during the reaction, and the simplicity of the process. Con-

Table 1
Selected examples of benzyl alcohols oxidized by O_2 employing the laccase/ABTS catalyst

Starting material	Product obtained	Overall yield (%)
Benzyl alcohol	benzaldehyde	94
3-Methoxy-4-acetoxybenzylalcohol	3-methoxy-4-acetoxybenzaldehyde	92
o-Chlorobenzylalcohol	*o*-chlorobenzaldehyde	90
p-Nitrobenzylalcohol	*p*-nitrobenzylaldehyde	98
p-Ethylbenzylalcohol	*p*-ethylbenzaldehyde	90

sidering the small quantities of the enzyme employed, the procedure turns out to be surprisingly effective, even though the two components of the catalyst, laccase and ABTS, cannot yet be counted among common chemicals. The only drawback of the procedure are the prolonged reaction times required to achieve quantitative conversion of the starting material, and the high cost of ABTS.

The standardized procedure for the enzymatically-catalyzed oxidation of benzyl alcohols is given in the Experimental Section. Nevertheless, some interesting aspects of the reaction shall be discussed here: the reaction proceeds in aqueous media with the pH maintained by acetate buffer at approximately 4.5 during the reaction. If the starting compound is water-insoluble, freshly distilled THF or dioxane is added to the mixture until all organic material is dissolved. The addition of organic solvent does not affect the course of the reaction. The molecular oxygen needed for the reaction can be provided by bubbling a stream of oxygen through the reaction mixture. However, this approach has the disadvantage of converting a small amount of the resulting aldehyde to the corresponding acid by excessive oxygen through autoxidation. It is recommended, therefore, that the reactants and solvents be placed in a reaction vessel of sufficient size, which is flushed with oxygen once before addition of the catalyst. The reaction vessel is then tightly closed, and the reaction mixture is vigorously stirred with a magnetic stirrer or shaken until the reaction is completed. Since the reaction is heterogeneous, a thorough mixing, i.e., extensive contact between the liquid and the gaseous phase, is crucial to obtain quantitative yields. The course of the reaction can conveniently be monitored by means of GC or GCMS. For this purpose, a small sample of the reaction mixture is taken, extracted with organic solvent, and analyzed by GC. The reaction is completed when only the product is detected. After completion of the reaction, the products are extracted with dichloromethane or another appropriate organic solvent. The enzyme as well as the mediator remain in the aqueous phase during this procedure ensuring the high purity of the benzaldehydes extracted. The products usually do not require further purification. However, recrystallization of the benzaldehydes is recommended.

The method described in this paper represents a generally applicable synthesis procedure using the laccase/mediator catalyst. It is an alternative to the commonly used methods to obtain benzaldehydes by oxidation of the corresponding benzyl alcohols. Moreover, it is superior to those methods by giving high yields, proceeding under physiological conditions and, therefore, impairing almost none of commonly occurring substituents on aromatic rings. However, due to special requirements of the enzyme, 2,6-disubstituted benzyl alcohols cannot be used as substrates. Further applicability of the laccase/ABTS couple to problems in synthesis is currently being investigated in our group. We believe, however, that laccase/ABTS can already be ranked among the synthetically useful enzymatic catalyst systems.

2. Experimental

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt [ABTS-$(NH_4)_2$] was purchased from Aldrich. All other chemicals were of reagent grade from commercial sources or synthesized according to known procedures. The solvents were of HPLC grade. Laccase from *Coriolus versicolor* was purchased from Merican Corporation, Tokyo. The activity of the enzyme was determined by the *p*-hydroxymandelic acid assay [14]. The composition of reaction mixtures and the purity of the products were determined by GC (Hewlett Packard Model 5890, Series II) with a capillary column (DB-5, 30 m × 0.32 mm i.d.) and GCMS (Hewlett-Packard Model 5985B, EI, 70 eV). Low attenuation was used to ensure that no by-product in small amounts remained undetected.

2.1. General experimental procedure for the oxidation of substituted benzyl alcohols to benzaldehydes by oxygen with laccase/ABTS as the catalyst

A solution of a substituted benzyl alcohol (1 mmol), and ABTS-$(NH_4)_2$ (1.5 mmol) in 50 ml of acetate buffer pH 4.5 was placed in a 250-ml flask equipped with a magnetic stirrer, and flushed with O_2 for 1 min. In case of water insoluble substrates, such as nitro or alkyl derivatives of benzyl alcohol, THF was added until all organic material had been dissolved. 0.1 ml *Coriolus* laccase (laccase activity, 51 u/ml) was added. Laccase obtained from *Pycnoporus coccineus* could also be used. The colorless solution immediately turned into deep blue–green upon addition of the enzyme. Again, the reaction mixture was flushed with O_2 for 1 min, and the reaction vessel was closed. After 24 h, a 0.5-ml sample of the reaction mixture was taken, extracted with CH_2Cl_2, and the resulting organic solution was analyzed by GC. If the reaction was not completed, an additional 0.03 ml of the above enzyme solution was added. The reaction mixture was kept at room temperature for additional 24 h and flushed with O_2 for 30 min every 8 h. Then, the reaction mixture was extracted three times with CH_2Cl_2. The aqueous layer was discarded. The organic phase was dried over $MgSO_4$, and the solvent slowly removed under reduced pressure. The corresponding benzaldehyde obtained did not require further purification in most cases.

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References

[1] E.I. Solomon, M.J. Baldwin and M.D. Lowery, Chem. Rev., 92 (1992) 521.
[2] U.A. Germann, G. Müller, P.E. Hunziker and K. Lerch, J. Biol. Chem., 263 (1988) 885.
[3] B. Reinhammar, in R. Lonntie (Ed.), Copper Proteins and Copper Enzymes, Vol. III, Chap. 1, CRC Press, Boca Raton, FL, 1984, pp. 1–35.
[4] R. Bligney and R. Douce, Biochem. J., 209 (1983) 489.
[5] R. Sterjiades, J.E.D. Dean and K.-E.L. Eriksson, Plant Physiol., 99 (1992) 1162.
[6] W. Bao, D.M. O'Malley, R.W. Whetten and R.R. Sederoff, Science, 260 (1993) 672–674.
[7] K. Okusa, T. Miyakoshi and C.L. Chen, Holzforschung, in press.
[8] N. Morohoshi, H. Wariishi, C. Muraiso, T. Nagai and T. Haraguchi, Mokuzai Gakkaishi, 33 (1987) 218.
[9] H.P. Call, International Patent, WO 92/20857 (PCT/EP92/01086), 1992.
[10] I.D. Reid, M.G. Paice, C. Ho and L. Jurasek, Tappi, 73(8) (1990) 149.
[11] M.G. Paice, L. Jurasek, C. Ho, R. Bourbonnais and F.S. Archibald, Tappi, 72(5) (1989) 217.
[12] (a) A. Potthast, T. Rosenau, C.L. Chen and J.S. Gratzl, J. Org. Chem., 60 (14) (1995) 4320; (b) T. Rosenau, A. Potthast, C.L. Chen and J.S. Gratzl, Synth. Commun., 26 (2) (1996) in press..
[13] (a) T. Higuchi, in N.G. Lewis and M.G. Paice (Eds.), Plant Cell Wall Polymers, Biogenesis and Biodegradation, ACS Symposium Series, Vol. 399, American Chemical Society, Washington, DC, 1989, pp. 482–502; (b) S. Kawai, T. Umezawa and T. Higuchi, Arch. Biochem. Biophys., 262 (1988) 99.
[14] H. Agematu, N. Shibamoto, H. Nishida, R. Okamoto, T. Shin and S. Murao, Biosci. Biotechnol. Biochem., 57(11) (1993) 1877.
[15] R. Bourbonnais and M.G. Paice, FEBS Lett., 267 (1990) 99.
[16] A. Muheim, A. Fiechter, P.J. Harvey and H.E. Schoemaker, Holzforschung, 46 (1992) 121.
[17] R. Bourbonnais and M.G. Paice, Appl. Microbiol. Biotechnol., 36 (1992) 823.
[18] E.I. Solomon and M.D. Lowery, Science, 259 (1993) 1575.
[19] J.L. Cole, P.A. Clark and E.I. Solomon, J. Am. Chem. Soc., 112 (1990) 9534.
[20] H.P. Call, World Patent Application, WO 94/29510 (1994).
[21] L. Jurasek, F.S. Archibald, R. Bourbonnais, M.G. Paice and I.D. Reid, Biosci. Symp., 1 (1994).
[22] A. Potthast, T. Rosenau, C.L. Chen and J.S. Gratzl, in preparation.