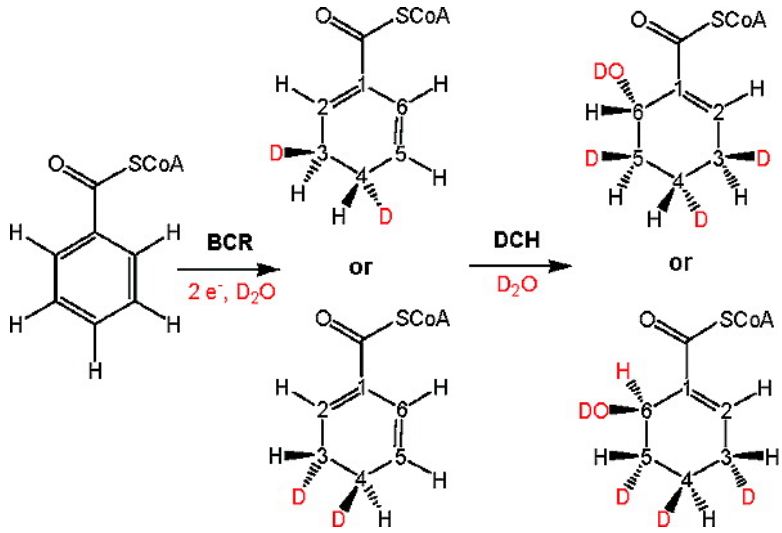
Communication

Mechanism of Enzymatic Birch Reduction: Stereochemical Course and Exchange Reactions of Benzoyl-CoA Reductase

Ba#rbel Thiele, Oliver Rieder, Bernard T. Golding, Michael Mu#ller, and Matthias Boll

J. Am. Chem. Soc., **2008**, 130 (43), 14050-14051 • DOI: 10.1021/ja805091w • Publication Date (Web): 01 October 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/01/2008

Mechanism of Enzymatic Birch Reduction: Stereochemical Course and Exchange Reactions of Benzoyl-CoA Reductase

Bärbel Thiele,[†] Oliver Rieder,[‡] Bernard T. Golding,[§] Michael Müller,[‡] and Matthias Boll*[,†]

Institute of Biochemistry, University of Leipzig, 04103 Leipzig, Germany, Institute of Pharmaceutical and Medical Chemistry, University of Freiburg, 79104 Freiburg, Germany, and School of Chemistry, Newcastle University, NE1 7RU Newcastle, U.K.

Received July 2, 2008; E-mail: boll@uni-leipzig.de

The Birch reduction is a widely used synthetic tool in organic chemistry that achieves 1,4-dihydro additions to benzenoid and other aromatic compounds. The reaction proceeds by alternate electron transfer and protonation steps to the aromatic ring and requires solvated electrons, which are usually generated by dissolving an alkali metal in liquid ammonia.[1] Considering these nonphysiological conditions it is remarkable that a similar reaction exists in biology: the dearomatizing benzoyl-coenzyme A reductase (BCR) plays a key role in the anaerobic degradation of aromatic compounds.[2−4]

In anaerobic bacteria many low-molecular aromatic growth substrates are converted to the central intermediate benzoyl-CoA (BCoA), which serves as substrate for BCR.[5] The enzyme catalyzes the reduction of BCoA (**1**) to cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA, **2**) rather than the kinetically favored 2,5-dienoyl-CoA isomer (Scheme 1).[6] A mechanism similar to the classical Birch reduction has been suggested in which the rate limiting first electron transfer yields a radical anion.[7,8] The CoA ester moiety is considered to stabilize this intermediate by formation of a relatively stable thioester ketyl radical. Remarkably, BCR couples electron transfer to the aromatic ring from the donor reduced ferredoxin (Fd) to a stoichiometric ATP hydrolysis, a reaction that has long been considered as an exclusive feature of nitrogenase.[2,9] The oxygen sensitive BCR has so far only been isolated from the facultatively anaerobic bacterium *Thauera aromatica*.[2]

Previous attempts to elucidate the stereochemistry of the BCR reaction (syn or anti dihydro addition) by NMR analysis of the dienoyl-CoA product were not successful due to ambiguous data obtained from the protons at C-4. To overcome these problems we aimed to determine the stereochemistry at the level of the 1,4-hydration product of dienoyl-CoA, 6-hydroxycyclohex-1-ene-1-carboxyl-CoA (6-OH-enoyl-CoA, **3**), which is formed by the subsequent enzyme in the pathway, dienoyl-CoA hydratase (DCH)[10] (Scheme 1). We present results obtained from reacting BCoA in H_2O/D_2O, and d_5-BCoA in H_2O with BCR and DCH.

The copurification of BCR/DCH was conducted as described with the expected specific activities (for BCR 0.25 μmol min mg^{-1}); reversed phase HPLC analysis confirmed that BCoA was converted to dienoyl-CoA and 6-OH-enoyl-CoA in a 1:1 ratio.[6] The CoA esters were synthesized as described.[6] Enzymatic conversion of 1 mM CoA esters was carried out under a N_2-atmosphere in 10 mL of the established assay buffer using 7.5 mM Ti(III)-citrate as electron donor.[2] The reaction products were isolated by preparative reversed phase HPLC and freeze-dried.

Assignment of NMR Data Obtained from 6-OH-enoyl-CoA. The ^{1}H NMR spectrum of nondeuterated **3** exhibited signals at 1.5, 1.7, 2.03, 2.18, 4.51 and 7.1 ppm accounting for eight protons as described.[6] To elucidate the relative stereochemistry for the additions to **1**, further NMR experiments were conducted. The results of H-H COSY and NOESY experiments are summarized in Table 1.

Scheme 1. Reaction Catalyzed by BCR and DCH in D_2O

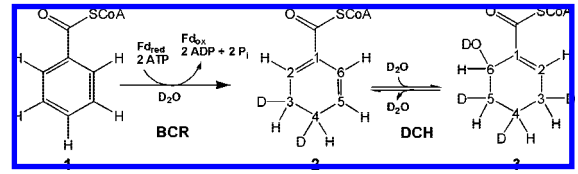
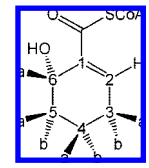


Table 1. Signal Couplings in H-H COSY and NOESY NMR Experiments; the Relative Integrals of 6-OH-enoyl-CoA **3** Formed from BCoA in H_2O (a), d_5-BCoA in H_2O (b), and BCoA in D_2O (c) Are Shown

signal (ppm)	assignment	H−H−COSY	NOESY	a	b	c
1.5	H-4;5	1.7; 2.03; 2.18; 4.51	1.7; 2.03; 2.18; 4.51	3	<2	>2
1.7	H-5	1.5	1.5	1	1	<0.1
2.03	H-3	1.5; 2.18; 7.1	1.5; 2.18; 7.1	1	0.7	1
2.18	H-3	1.5; 2.03; 7.1	1.5; 2.03; 7.1	1	0.9	0.26
4.51	H-6	1.5	1.5	1	0.65	1
7.1	H-2	2.03; 2.18	2.03; 2.18	1	0.28	1

Table 2. Assignment of the Proton Signals of 6S-6-OH-enoyl-CoA (3)



label	proton signal (ppm)	calculated	label	proton signal (ppm)	calculated
2a	7.10	6.95	4b	1.50	1.81
3a	2.18	2.37	5a	1.70	2.05
3b	2.03	2.17	5b	1.50	1.89
4a	1.50	1.81	6a	4.51	4.89

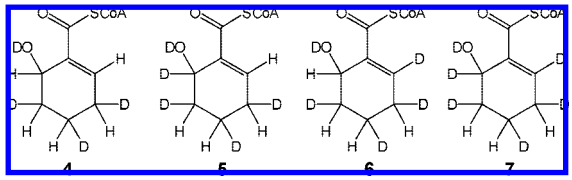
The signal at 1.5 ppm showed a relative integral of three protons, which derive from the protons on more than one carbon atom. The downfield signal at 7.1 ppm is assigned to the alkene proton (H-2), whereas the signal at 4.51 belongs to the carbinol proton (H-6). The signals at 2.03 and 2.18 showed COSY crosspeak signals with the alkene proton and are therefore assigned to the H-3 protons. The signal at 1.5 ppm showed COSY crosspeaks to all other protons except to that assigned to the 7.1 ppm signal. Thus the protons accounting for this signal are at C-4 and C-5. The 1.7 ppm signal showed only crosspeaks with the 1.5 ppm signal, which points to a proton at C-5. The NOESY experiments support these findings. The data were additionally refined by calculating the ^{1}H NMR shifts using the Perch-NMR-Predictor 1.3 software (Table 2).

If formed in H_2O, compound **3** should carry three additional hydrogens from the solvent at C-3, C-4, and C-5 (Scheme 1 and Table

[†] University of Leipzig.
[‡] University of Freiburg.
[§] Newcastle University.

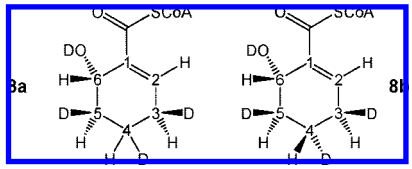
10.1021/ja805091w CCC: $40.75

Scheme 2. Differentially Labelled 6-OH-enoyl-CoA Species Formed from BCoA in D_2O[a]



[a] Relative abundance: **4**, 25−30%; **5**, 55−60%; **6** and **7**, 5−10% each; calculations were derived from ^{1}H-NMR peak integrals, an overalying signal from a CoA-derived proton was subtracted.

Scheme 3



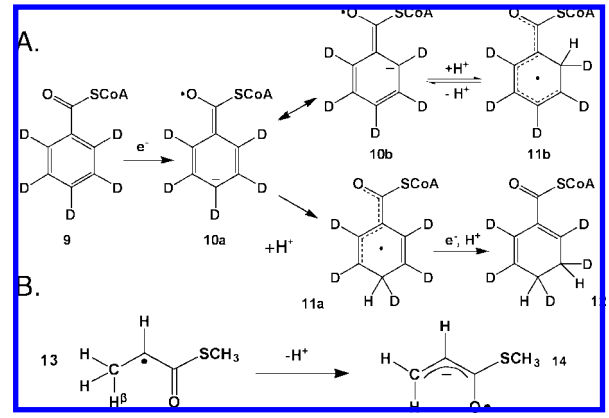
2). However, NMR analysis of **3** in D_2O or from d_5-BCoA in H_2O revealed four different products with additional deuterium/hydrogen incorporations at C-2 and C-6. Thus, in addition to the expected trideuterated (**4**), tetradeuterated (**5**, **6**) and pentadeuterated (**7**) compounds were also identified, indicating unexpected exchange reactions at C-2 and C-6 (Scheme 2). HSQC-NMR analysis revealed that no carbon carries more than one incorporated H or D from the solvent; each of the carbons in position 3−5 carry one hydrogen and one deuterium in all compounds.

Stereochemistry of DCH Reaction. For easier presentation of the data obtained *R* and *S* are henceforth used. Here they are arbitrarily chosen as only the relative stereochemistry could be solved (e.g., 6*S*- or 6*R*-6-OH-enoyl-CoA). Notably, the ring nomenclature inverts during the two-step formation of 6-OH-enoyl-CoA by BCR and DCH (Scheme 1). ^{1}H NMR of **3** shows no signal at 1.7 ppm (Table 1). Thus, the position 5a carries a deuterium and position 5b is hydrogenated after the two-step reaction process (Table 2). The integral of the signal at 2.18 ppm was much lower than that of the signal at 2.03 ppm, which suggests that the deuteration mainly occurred at position 3a. These findings yield the structure **8a**. The second step introduces a deuterium at C-3 (previously C-5) and a deuteroxy group at C-6 (C-2). C-3 and C-6 are *S* configured. Consequently the hydration step occurred as a 1,4-anti addition, which is consistent with the mechanism of 1,2-syn-additions to 2-monoenoyl-CoA compounds such as crotonyl-CoA.[11]

Stereochemistry of BCR Reaction. The stereocenters created by the BCR reaction are located at C-4 and C-5 in **8**. From the considerations made above the C-3-*S* and the C-5-*R* configurations are established; these hydrogen atoms/deuterons are cis configured. However, it was not possible to deduce the configuration of C-4 directly via correlations with C-3 or C-5.

Calculations using MOPAC and Austin Model 1 theory revealed that the distance between H-3b and H-4b (*cis*) is 2.41 Å. The distance between H-3b and H-2 is 2.62 Å, whereas the distance between H-3b and H-4a (trans) is 3.09 Å (Supporting Information). We therefore applied quantitative NOESY techniques and monitored the reflexes at 1.5 ppm (H-4a and H-4b) and at 7.1 ppm (H-2) when irradiating the proton at 2.03 ppm (H-3b). If the proton at C-4 is located at 4b, a stronger quantitative response than observed at 7.1 ppm is expected; if located at 4a, a much smaller response should be observed. Only a clear response at 7.1 ppm was detected but none at 1.5 ppm, while in the unlabeled compound a response at 1.5 ppm is visible (Table 1). Therefore we assigned the proton to the 4a position completing the stereochemistry of both reactions (**8b**). Accordingly, the BCR catalyzed reduction is trans selective.

Scheme 4. (A) Proposal for Birch Reduction Mechanism of BCR and (B) Comparison with the β Deprotonation of the Methyl Thiopropionate Radical **13** to the Radical Anion **14**



Mechanism of BCR. The unusual proton exchanges at C-2 and C-6 during the two-step formation of 6-OH-enoyl-CoA cannot be explained by DCH reaction. Instead, exchanges on the level of a radical intermediate during BCR catalysis appear to be most plausible (Scheme 4). After the first electron transfer, the highest electron densities of the resulting radical anion are at the C-2, C-4, and C-6 positions with **10a** and **10b** representing resonance structures.[7] Protonation and further conversion of **10a** yield **11a** and **12**, protonation of **10b** yields **11b**. The alternative protonations are both plausible but require a sterically flexible donor (e.g., H_2O). The pK_a of enoyl thioester radicals, such as **11b**, are remarkably low (pK_a = 14 for the β deprotonation of **13** to **14**).[12] The resonance-based stability of **10b** and the proposed partial protonation of the carbonyl oxygen by the enzyme should shift the pK_a to a value below 10, which could explain the observed exchanges. As the free radical **11b** is more stable than **11a**, the latter should be reduced more readily. Moreover BCR may govern selective electron transfer to **11a** but not to **11b**.

The formation of a ketyl radical intermediate has recently been demonstrated during the mechanistically sophisticated dehydration of an α-hydroxyacyl-CoA compound.[13] The corresponding dehydratases are the only enzymes that are homologous to BCR, and similar mechanisms via radical intermediates have been proposed many years ago.[8]

Conclusions. The results obtained provide the first evidence that BCR reaction stereoselectively yields the *trans*-dienoyl-CoA product. Moreover, the unexpected exchanges at C-2 and C-6 provide evidence for the proposed Birch reduction mechanism. They can be explained by the low pK_a of a radical intermediate, and are in perfect agreement with the established radical mechanism of homologous α-hydroxyacyl-CoA dehydratases.

Supporting Information Available: Full NMR spectra and results from MOPAC calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Birch, A. J.; Hinde, A. L.; Radom, L. *J. Am. Chem. Soc.* **1981**, *103*, 284–9.
(2) Boll, M.; Fuchs, G. *Eur. J. Biochem.* **1995**, *234*, 921–33.
(3) Boll, M. *J. Mol. Microbiol. Biotechnol.* **2005**, *10*, 132–42.
(4) Boll, M. *Biochim. Biophys. Acta* **2005**, *1707*, 34–50.
(5) Gibson, J.; Harwood, C. S. *Annu. Rev. Microbiol.* **2002**, *56*, 345–69.
(6) Boll, M.; Laempe, D.; Eisenreich, W.; Bacher, A.; Mittelberger, T.; Heinze, J.; Fuchs, G. *J. Biol. Chem.* **2000**, *275*, 21889–95.
(7) Möbitz, H.; Boll, M. *Biochemistry* **2002**, *41*, 1752–8.
(8) Buckel, W.; Keese, R. *Angew. Chem., Int. Ed.* **1995**, *34*, 1502–6.
(9) Unciuleac, M.; Boll, M. *Proc. Natl. Acad. Sci.U.S.A.* **2001**, *98*, 13619–24.
(10) Laempe, D.; Eisenreich, W.; Bacher, A.; Fuchs, G. *Eur. J. Biochem.* **1998**, *255*, 618–27.
(11) Willadsen, P.; Eggerer, H. *Eur. J. Biochem.* **1975**, *54*, 247–52.
(12) Smith, D. M.; Buckel, W.; Zipse, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 1867–1870.
(13) Kim, J.; Darley, D. J.; Buckel, W.; Pierik, A. J. *Nature* **2008**, *452*, 239–42.

JA805091W