Reducing Agent-Mediated Nonenzymatic Conversion of 2-Oxoglutarate to Succinate: Implications for Oxygenase Assays

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Figure S1. Stabilities of 2OG and L-Asc in Tris-D11 buffer.
(a) Overlay of $^1$H NMR spectra of a freshly prepared mixture of 2OG in aqueous Tris-D11 buffer (bottom) compared with the same mixture after 10 hours (top). (b) Overlay of $^1$H NMR spectra of a freshly prepared mixture of L-Asc in aqueous Tris-D11 buffer (bottom) compared with the same mixture after 10 hours (top). Concentrations used: 2OG 200 µM, L-Asc 500 µM in 50 mM aqueous Tris-D11 at pH 7.5.
Figure S2. $^1$H NMR time course analysis of L-Asc mediated 2OG conversion to succinate.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of an L-Asc/2OG/Tris-D$_{11}$ buffer mixture for the shown times. (b) Plot showing L-Asc degradation and simultaneous 2OG reaction to give succinate over time. Concentrations used: 500 µM L-Asc, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ at pH 7.5. DHA: Dehydroascorbate. Error bars represent standard deviations from the mean (n =3) of three separate measurements.
**Figure S3. Dehydroascorbate (DHA) mediated conversion of 2OG to succinate.**

Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of dehydroascorbate (DHA) and 2OG in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Signals marked with asterisks are likely due to DHA degradation products. Concentrations used: 500 μM DHA, 200 μM 2OG in 50 mM aqueous Tris-D$_{11}$, pH 7.5.
Figure S4. The effect of catalase on L-Asc mediated conversion of 2OG to succinate.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of L-Asc/2OG/catalase/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). In the presence of catalase, no 2OG conversion to succinate is observed. (b) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of 2OG/H$_2$O$_2$/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (c) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of 2OG/H$_2$O$_2$/catalase/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). In the presence of catalase, H$_2$O$_2$ mediated 2OG conversion to succinate is not observed. Concentrations used: 500 µM L-Asc, 200 µM 2OG, 500 µM H$_2$O$_2$, 1735 units catalase in 50 mM aqueous Tris-D$_{11}$ at pH 7.5.
Figure S5. H$_2$O$_2$-mediated 2OG conversion to succinate.
(a) $^1$H NMR time course analysis of the reaction of 2OG with H$_2$O$_2$ to form succinate. (b) Plot of 2OG conversion to succinate vs time (1-10 hours). Concentrations used: 500 µM H$_2$O$_2$, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ at pH 7.5. Error bars represent standard deviations from the mean (n =3) of three separate measurements.
Figure S6. The effect of oxygen on the L-Asc/2OG/Tris-D_{11} buffer incubation assay.

(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of L-Asc/2OG/Tris-D_{11} buffer under anaerobic conditions (bottom) compared with the same mixture after 10 hours (top).

(b) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of L-Asc in Tris-D_{11} buffer under anaerobic conditions (bottom) compared with the same mixture after 10 hours (top). These results imply that under anaerobic conditions, L-Asc does not undergo oxidation, hence, does not generate $H_2O_2$, and no conversion of 2OG to succinate occurs. Concentrations used: 500 µM L-Asc, 200 µM 2OG in 50 mM aqueous Tris-D_{11} at pH 7.5.
Figure S7. The effect of Fe(II) on H$_2$O$_2$-mediated 2OG conversion to succinate.

(a) $^1$H NMR time course analysis of H$_2$O$_2$-mediated conversion of 2OG to succinate in the absence (blue curve) and presence (brown curve) of Fe(II). Concentrations used: 200 µM 2OG, 500 µM H$_2$O$_2$, 100 µM Fe(II) in 50 mM aqueous Tris-D$_{11}$ at pH 7.5. Error bars represent standard deviations from the mean (n =3) of three separate measurements.
Figure S8. The effect of different metal ions on the L-Asc mediated 2OG conversion to succinate.

Chart showing the extent of 2OG conversion to succinate on incubating a mixture of L-Asc/2OG/metal ion/aqueous Tris-D11 buffer for 10 hours. Concentrations used: 500 µM L-Asc, 200 µM 2OG, 100 µM metal ion in 50 mM aqueous Tris-D11 buffer at pH 7.5. Error bars represent standard deviations from the mean (n = 3) of three separate measurements.
Figure S9. The effect of Zn(II) on L-Asc mediated 2OG conversion to succinate.

(a) Overlay of \(^1\)H NMR spectra of a 10 hour incubation mixture of L-Asc/2OG/aqueous Tris-D11 buffer without Zn(II) (bottom) compared with the same mixture with Zn(II) (top). In the presence of Zn(II), L-Asc undergoes efficient oxidation enabling succinate formation. (b) The effect of different Zn(II) concentrations on the reaction of L-Asc. Concentrations used: Zn(II); 25 µM, 100 µM, 500 µM, 2.5 mM, 5 mM, L-Asc; 500 µM & 1.5 mM, and 2OG 200 µM in 50 mM aqueous Tris-D11 buffer at pH 7.5. Error bars represent standard deviations from the mean (n = 3) of three separate measurements.
Figure S10. The effect of different buffers on L-Asc mediated 2OG conversion to succinate.

Chart showing the extent of 2OG conversion to succinate on incubating a mixture of L-Asc/2OG/aqueous buffer for 10 hours. Buffers used: sodium phosphate, Tris-D11, tricine, TES, HEPES, PIPES and MOPS. Concentrations used: 500 µM L-Asc, 200 µM 2OG in 50 mM aqueous buffer at pH 7.5. Error bars represent standard deviations from the mean (n = 3) of three separate measurements.
(a) 4-HPA
4-HPA
4-HPA
4-HPA
4-HPA
4-HPA
4-HPA

L-Asc
DHA
L-Asc

7.2 7.0 6.8
5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 ppm

After 10 hours

Freshly prepared mixture

(b) 4-HPP

4-HPP
4-HPP
4-HPP

7.1 7.0 6.9 6.8 6.7
3.9 3.8 3.7 3.6 3.5 3.4 3.3 ppm

After 10 hours

4-HPP/Tris-D_{11}

Freshly prepared mixture

(Figure continues)
Figure S11. L-Asc mediated reaction of 4-hydroxyphenyl pyruvate (4-HPP) to give 4-hydroxyphenyl acetic acid (4-HPA).

(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of L-Asc/4-hydroxyphenyl pyruvate (4-HPP)/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Stability of 4-HPP in buffer: overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of 4-HPP in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (c) H$_2$O$_2$-mediated conversion of 4-HPP to 4-HPA: overlay of $^1$H NMR spectra (partial spectra shown for clarity) of a freshly prepared mixture of 4-HPP in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture with H$_2$O$_2$ after 25 minutes (top). Concentrations used: 500 µM 4-HPP, 500 µM H$_2$O$_2$ in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S12. L-Asc-mediated conversion of pyruvate to acetate.
(a) Overlay of $^1$H NMR spectra of a freshly prepared mixture of L-Asc/pyruvate/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Stability of pyruvate in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of pyruvate in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (c) H$_2$O$_2$-mediated oxidative decarboxylation of pyruvate to give acetate: overlay of $^1$H NMR spectra of a freshly prepared mixture of pyruvate in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture with H$_2$O$_2$ after 10 minutes (top). Concentrations used: 500 µM L-Asc, 200 µM pyruvate, 500 µM H$_2$O$_2$ in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S13. L-Asc mediated conversion of oxaloacetate to malonate and acetate.
(a) Overlay of $^1$H NMR spectra of a freshly prepared mixture of L-Asc/oxaloacetate/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Peaks marked with asterisks are likely due to impurities. The formation of acetate can occur by non-oxidative decarboxylation of malonate. Note that whereas malonate undergoes facile non-oxidative decarboxylation, succinate does not undergo decarboxylation to give propionate because of the lack of the `β-carbonyl group` to promote decarboxylation. (b) Stability of oxaloacetate in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of oxaloacetate in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (c) H$_2$O$_2$-mediated conversion of oxaloacetate to malonate and acetate: overlay of $^1$H NMR spectra of a freshly prepared mixture of oxaloacetate in aqueous Tris-D$_{11}$ buffer (bottom) compared with the same mixture containing H$_2$O$_2$ after 15 minutes (middle) and 1 hour (top). Concentrations used: 500 µM L-Asc, 200 µM oxaloacetate, 500 µM H$_2$O$_2$ in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S14. The effects of Fe(II), Zn(II) and catalase on the L-Asc mediated conversion of pyruvate to acetate.

Chart showing the extent of acetate formation after incubation (10 hours) of a mixture of L-Asc/pyruvate/Tris-D11 buffer (blue); mixture of L-Asc/pyruvate/Fe(II)/Tris-D11 buffer (orange); mixture of L-Asc/pyruvate/Zn(II)/Tris-D11 buffer (green); mixture of L-Asc/pyruvate/catalase/Tris-D11 buffer (red). Concentrations used: 500 µM L-Asc, 200 µM pyruvate, 100 µM Fe(II), 100 µM Zn(II) and 1735 units catalase in 50 mM aqueous Tris-D11 buffer at pH 7.5. These observations show that the addition of either Fe(II) or catalase inhibits the L-Asc mediated reaction of pyruvate to acetate. Error bars represent standard deviations from the mean (n = 3) of three separate measurements.
Figure S15. L-Asc and citrate incubation assay.
Overlay of $^1$H NMR spectra of a freshly prepared mixture of citric acid/L-Asc/Tris-D$_{11}$ buffer compared with the same mixture after 10 hours. No new signals except those assigned to the reaction of L-Asc appear in the spectrum (i.e. there is no evidence for citrate reaction). Concentrations used: 500 µM L-Asc, 200 µM citric acid in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S16. L-Asc and DL-isocitrate incubation assay.
Overlay of $^1$H NMR spectra of a freshly prepared mixture of DL-isocitrate/L-Asc/Tris-D$_{11}$ buffer compared with the same mixture after 10 hours. No new signals except those assigned to the reaction of L-Asc appear in the spectrum (i.e there is no evidence for the reaction of DL-isocitrate). Concentrations used: 500 µM L-Asc, 200 µM DL-isocitrate in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S17. L-Asc and malate incubation assay.
Overlay of $^1$H NMR spectra (parts are shown for clarity) of a freshly prepared mixture of maleic acid/L-Asc/Tris-D$_{11}$ buffer compared with the same mixture after 10 hours. No new signals except those assigned to the reaction of L-Asc appear in the spectrum (i.e. there is no evidence for the reaction of maleic acid). Concentrations used: 500 µM L-Asc, 200 µM maleic acid in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S18. Dithiothreitol (DTT) – mediated 2OG conversion to succinate.
(a) Overlay of $^1$H NMR spectra of a freshly prepared mixture of DTT/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Oxidation of DTT with dioxygen in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of DTT in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). The broad signals marked with asterisks likely represent an oxidised (di)sulfide form of DTT. Concentrations used: 500 µM DTT, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$, pH 7.5.
Figure S19. Baicalein-mediated 2OG conversion to succinate.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of baicalein/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Oxidation of baicalein in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of baicalein in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Signals marked with asterisks are believed to represent an oxidised form of baicalein. Concentrations used: 500 µM baicalein, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S20. Propyl gallate-mediated conversion of 2OG to succinate.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of propyl gallate/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Oxidation of propyl gallate with dioxygen in buffer: overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of propyl gallate in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Signals marked with asterisks are believed to be an oxidised form of propyl gallate. Concentrations used: 500 µM propyl gallate, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S21. Protocatechuic acid (PCA)-mediated 2OG conversion to succinate.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of protocatechuic acid (PCA)/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Oxidation of PCA in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of PCA in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Signals marked with asterisks possibly represent an oxidised form of PCA. Concentrations used: 500 µM protocatechuic acid, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S22. Catechol-mediated 2OG reaction to succinate.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of catechol/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top).
(b) Oxidation of catechol with dioxygen in buffer: overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of catechol in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Signals marked with asterisks possibly represent an oxidised form of catechol. Concentrations used: 500 µM catechol, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.
Figure S23. Glutathione (GSH)-mediated 2OG conversion into succinate.
(a) Overlay of $^1$H NMR spectra of a freshly prepared mixture of glutathione (GSH)/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) Oxidation of GSH in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of GSH in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). Concentrations used: 500 µM glutathione, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5. GSSG: Disulfide-glutathione.
Figure S24. Tris(2-carboxyethyl)phosphine (TCEP) – 2OG incubation assay.
(a) Overlay of $^1$H NMR spectra (partial spectra are shown for clarity) of a freshly prepared mixture of TCEP/2OG/Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). (b) TCEP stability in buffer: overlay of $^1$H NMR spectra of a freshly prepared mixture of TCEP in Tris-D$_{11}$ buffer (bottom) compared with the same mixture after 10 hours (top). The peaks labelled with asterisks may be due to impurities in the TCEP-buffer solution. Concentrations used: 500 µM TCEP, 200 µM 2OG in 50 mM aqueous Tris-D$_{11}$ buffer at pH 7.5.