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## Correction to Oxygen-Independent Alkane Formation by Non-Heme Iron-Dependent Cyanobacterial Aldehyde Decarbonylase: Investigation of Kinetics and Requirement for an External Electron Donor

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Oxygen independent alkane formation by non-heme iron-dependent cyanobacterial aldehyde decarbonylase: investigation of kinetics and requirement for an external electron donor

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In our original publication we characterized the decarbonylation reaction catalyzed by cAD as being independent of oxygen. Evidence to support this view derives from that fact that the reaction does not formally involve the oxidation of the substrate and that we observed a higher number of turnovers under anaerobic conditions than in aerobic buffers. Further support came from H<sub>2</sub><sup>18</sup>O-labeling experiments that indicated that the oxygen in formate was derived from water, rather than molecular oxygen, and the fact that the formation of alkanes did not appear to consume NADH, which would be necessary for an oxidative reaction. However, subsequent to publication, further experiments have led us to conclude that we cannot exclude the possibility that the activity we observed was due to trace amounts of oxygen in the reaction buffer.

The difficulties in establishing the dependence of the reaction on molecular oxygen stem, in part, from the very low activity of the enzyme under either aerobic or anaerobic conditions. We have found that oxygen scrubbing systems that are routinely employed to scavenge oxygen from biochemical reactions, such as sodium dithionite, glucose oxidase/glucose and protocatechuate dioxygenase/protocatechuate, still result in high levels of activity using the assay conditions we describe, even when included in large excess. Although these observations support our initial assertion that oxygen was not involved, when the assays were performed in an anaerobic chamber capable of maintaining oxygen concentrations at very low concentrations, i.e. below 0.5 ppm, (which was not available to us in our original investigations) we observed very little activity.

Although the <sup>18</sup>O-labeling experiment should have identified the involvement of molecular oxygen, analysis of the data was complicated by a background rate of non-enzymatic <sup>18</sup>O exchange into the aldehyde substrate from H<sub>2</sub><sup>18</sup>O. Appropriate controls were performed to account for this background reaction, but if the enzyme itself significantly increased the rate of <sup>18</sup>O exchange into the aldehyde prior to reaction, the conclusions drawn from the experiment may not be valid. We are currently conducting further experiments to clarify the requirement for oxygen and reducing equivalents in the reaction to resolve the discrepancy in these results. In the meantime, given this discrepancy, we conclude that we cannot unambiguously rule out the involvement of molecular oxygen in the cAD-catalyzed reaction. If molecular oxygen is required, the enzyme must have a very low apparent *K<sub>m</sub>* for O<sub>2</sub>.