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CARBOXYLATION IS THE INITIAL ENZYME REACTION IN THE ANAEROBIC DEGRADATION OF THE POLYCYCLIC AROMATIC HYDROCARBON NAPHTHALENE

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Abstract

Polycyclic aromatic hydrocarbons such as naphthalene are highly recalcitrant environmental pollutants that are only slowly metabolized by microorganisms under anoxic conditions. The anaerobic biochemical attack on such extremely stable molecules is unknown but based on metabolite analyses of supernatants of enriched and pure anaerobic cultures, carboxylation or methylation have been proposed as initial naphthalene reactions. However, the extremely tedious growth of such cultures has prevented in vitro studies and the identification of the initial activation reaction, so far. Here, we provide biochemical evidence that anaerobic naphthalene degradation is initiated via direct carboxylation. Crude cell extracts of a sulfate-reducing enrichment culture N47 converted naphthalene and ^{13}C-labelled bicarbonate to 2-[*carboxyl*-^{13}C]naphthoic acid at a rate of 0.12 nmol min^{-1} mg^{-1} protein. Divalent metal ions or ATP were not required nor did avidin or EDTA inhibited the carboxylase activity. The enzyme, namely naphthalene carboxylase, catalysed a much faster exchange of ^{13}C-labelled bicarbonate with the carboxyl group of [^{12}C]2-naphthoic acid at a rate of 3.2 nmol min^{-1} mg^{-1} protein, indicating that the rate limiting step of the carboxylation reaction is the activation of the naphthalene molecule rather than the carboxylation itself. The new carboxylation reaction is unprecedented in biochemistry and opens the door to understand the anaerobic degradation of polycyclic aromatic hydrocarbons which are among the most hazardous environmental contaminants.