The Microbiological Degradation of Aromatic Compounds

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The breakdown of aromatic compounds by ring cleavage is an essential biochemical step in Nature’s ‘carbon’ cycle and is performed by several kinds of microorganism. Bacteria are the most versatile in this respect, but several yeasts and fungi are able to degrade a more limited range of benzenoid structures (see list of Reviews on p. 188).

Among the eubacteria, representatives of the families Coccaceae, Mycobacteriaceae, Pseudomonadaceae, Spirillaceae, Bacteriaceae and Bacillaceae are active in this respect. The yeasts Oospora, Candida, Debaromyces, Pichia and Saccharomyces grow in media with catechol as sole carbon source. Certain higher fungi, e.g. Aspergillus, Penicillium and Neurospora attack aromatics and a variety of soil and wood-rotting fungi dissimilate the aromatic polymer lignin, as well as other plant phenolics. In all the above cases of aromatic ring metabolism, molecular oxygen is an obligatory oxidant.

The photosynthetic bacteria Rhodopseudomonas and some Rhodospirillum strains utilize benzoate under strictly anaerobic conditions in the light. Finally, there exists an anaerobic type of aromatic ring metabolism—e.g. the so-called methane fermentation of benzoate, although it is doubtful whether these methane bacteria are in pure culture.

These microbes produce, mostly as a result of induction, a whole sequence of enzymes which convert aromatic substrates into an ortho or para dihydroxyphenol derivative, followed by cleavage of the ring to aliphatic acids; these ring fission products are then funnelled into the Krebs cycle through a variety of pathways, depending on the organism and cultural conditions.

The distinctive biochemical step is ring cleavage. There are many instances of microbes being able to modify aromatic compounds, either by hydroxylation, or elimination of substituent groups, without necessarily causing fission. In the case of the aromatic amino acids, phenylalanine, tyrosine and tryptophan, pathways of metabolism exist that have much in common in all forms of life. It is only possible to sketch the salient features of microbial aromatic ring metabolism here, since the topic has, of recent years, greatly increased in scope.

(1) Aromatic hydroxylating enzymes

Aromatic hydrocarbons undergo a perhydroxylation to the trans-dihydro-arenediols, which are then dehydrogenated to the o-dihydroxyphenol prior to ring fission, thus:

\[ \begin{array}{cccc}
\text{H} & \text{H} & \xrightarrow{\text{En...Fe}^{2+}\text{O}} & \text{H} & \text{OH} \\
\text{C} & \text{C} & \xrightarrow{\text{H}_2\text{O}} & \text{C} & \text{OH} \\
\text{I} & \xrightarrow{\text{II}} & \text{OH} & \text{H} \\
\text{III} & \xrightarrow{\text{IV}} & \text{OH} & \text{OH} & \text{Ring fission}
\end{array} \]
A similar system exists in liver microsomes. The bacterial enzymes are extremely labile and difficult to separate; parts of this scheme are therefore still hypothetical. The corresponding ‘diol’ intermediate has been isolated from cultures of naphthalene (Walker & Wiltshire, 1958) and phenanthrene (Colla, Fiecchi & Treccani, 1959); evidence for its formation also exists in the case of benzene (Marr & Stone, 1959, 1961) and anthracene (Colla et al. 1959). Ayengar, Hayaishi, Nakajima & Tomida (1959) studied reaction III, using sonic extracts of Aerobacter aerogenes known to contain a specific myoinositol dehydrogenase, but conveniently lacking the enzymes catalysing reactions I, II, and IV. With trans-3,5-cyclohexadiene-1,2-diol as substrate, catechol was formed; NAD and a regenerating system were necessary for maximal activity.

The perhydroxylation step is Fe²⁺ dependent, and to account for the invariant formation of trans-dihydrodiols, the enzyme + oxygen + substrate complex is visualized as being of the epoxide type; second-order nucleophilic attack by a hydroxyl anion would yield the trans-isomer.

Polynuclear aromatic hydrocarbons attach to the relevant bacterial enzymes at C–C bonds of high electron density (the K regions of Pullman & Pullman, 1955). If they have a linear configuration, e.g. naphthalene, anthracene, attachment and ring splitting takes place on the same ring. Angular aromatic compounds such as phenanthrene afford attachments to an enzyme at a bond in a ring other than the one containing the ring-splitting site (Rogoff, 1962).

Benzoate, salicylate and anthranilate are common intermediates in degradative pathways of more complex aromatic compounds. These acids are converted to catechol by bacterial oxidases before ring fission; recently much light has been shed on these particular enzymes. Ichihara, Adachi, Hosokawa & Takeda (1962) prepared active cell-free systems which oxidatively decarboxylated these acids and caused ring cleavage of the resulting catechol. If meta-substituted benzoates were used as substrates, the oxidase preparations converted them to the relevant catechols only, since the specificity of the fission enzymes prevented them from being metabolized further; in this way they were able to study the initial enzyme reactions. Another successful approach has been the purification of the relevant enzyme systems. Thus Hosokawa, Nakagawa & Takeda (1961) fractionated crude anthranilate oxidase preparations into two indispensable protein components. Katagiri, Yamamoto & Hayaishi (1962) separated salicylate hydroxylase activity from the catechol cleavage enzyme. Results show these enzymes to be Fe³⁺-dependent requiring NADH or NADPH (and regenerating systems, e.g. ethanol and alcohol dehydrogenase). In the case of salicylate hydroxylase, an absolute requirement for flavin adenine dinucleotide (FAD) was demonstrated in addition.

\[
\begin{align*}
 & \text{O}_2 + \text{NADH} \\
 & \text{CO}_2 + \text{RH}
\end{align*}
\]

The mode of action of reduced pyridinenucleotides is not yet clear, but as suggested by Mason (1957) for a mixed function oxidase, one atom of oxygen might be
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used to hydroxylate the substrate, and the second be reduced by the NADH (or NADPH). A variety of electron donors function in different hydroxylating systems, e.g. NADH, NADPH, FADH₂ in the case of the bacterial enzymes; ascorbic acid with phenolases; tetrahydropteridine in the phenylalanine hydroxylase of liver, although NADPH serves as a reductant indirectly to reduce the oxidized pteridine derivative.

Brief mention must be made at this point of the fate of the other monohydroxybenzoates. Yano & Arima (1958) succeeded in extracting from some bacteria hydroxylase A, which converted m-hydroxybenzoate to protocatechuate, and from other organisms a hydroxylase B, which gave gentisate. Both hydroxylases required NADPH as co-factor. The subsequent pathways of these two dihydroxybenzoates differ (Figs. 1, 2). p-Hydroxybenzoate gives protocatechuate in all organisms studied.

![Diagram of oxidative fission of o-dihydroxyphenols by micro-organisms.]

Fig. 1. Oxidative fission of o-dihydroxyphenols by micro-organisms.

(2) Aromatic ring-cleavage enzymes*

The enzyme molecule contains Fe²⁺ and sulphhydryl groups, the integrity of both being essential for activity; molecular oxygen is obligatory.

In the case of o-dihydroxyphenol derivatives, two distinct cleavage enzymes are known. Their action may be illustrated with respect to catechol and protocatechuic acid, two central aromatic intermediates (Fig. 1).

(a) Pyrocatechase (catechol 1,2-oxygenase) and protocatechuic acid oxidase (protocatechuic 3,4-oxygenase) open the C–C bond between the two hydroxyls.  

* Dagley, Evans & Ribbons (1960).
The ring fission product, a cis-cis muconic acid, is then lactonized by a Mn$^{2+}$-dependent enzyme; hydrolytic cleavage of this to $\beta$-oxoadipate is achieved by a delactonizing enzyme. An enzymic C4–C2 split of this oxo-acid in the presence of coenzyme A and catalytic amounts of succinyl-coenzyme A provides the substrates for the tricarboxylic acid cycle, thus:

$$\begin{align*}
\beta\text{-oxoadipate} & \xrightarrow{\text{succinyl-CoA. thiolase}} \text{thiophorase} \xrightarrow{\text{acetyl-CoA.}} \\
\text{succinate} & \xrightarrow{\text{thiophorase}} \text{thiophorase} \xrightarrow{\text{acetol-CoA.}}
\end{align*}$$

(b) Catechol 2,3-oxygenase (metapyrocatechase) and protocatechuic 4,5-oxygenase open the C–C bond adjacent to a hydroxyl. The muconic semialdehyde so formed can undergo a non-enzymic reaction with NH$_4^+$ ions to give a pyridine nucleus, or be metabolized to Krebs cycle acids, one of which is certainly pyruvate. In their announcement of the new catechol pathway, Dagley & Stopher (1959) suggested a route of metabolism of $\alpha$-hydroxymuconic semialdehyde to pyruvate without gaseous exchange; subsequently, Nishizuka, Ichiyama, Nakamura & Hayashi (1962) have suggested the following route:

1. $\text{catechol} \xrightarrow{\text{O}} \text{OHC-CH=CH-CH=O(OH)-CO}_2\text{H}$
2. $\text{NAD} \xrightarrow{\text{H}} \text{HO}_2\text{C-CH=CH-CH}_2\text{-CO-}_2\text{H}$
3. $\text{H}_2\text{O} \xrightarrow{\text{NAD}} \text{CO}_2+\text{CH}_3\text{-CH(OH)-CH}_2\text{-CO-}_2\text{H}$
4. $\text{NAD} \xrightarrow{\text{H}} (\text{CH}_3\text{-CO-CH}_2\text{-CO-}_2\text{H})$
5. $\text{H}_2\text{O} \xrightarrow{\text{CH}_3\text{-CO}_2\text{H+CH}_3\text{-CO-}_2\text{H}}$

It is interesting to note that Gholson et al. (1962) have recently implicated $\alpha$-hydroxymuconic semialdehyde in the catabolism of tryptophan by mammalian liver. Ribbons & Evans (1962) adduced evidence in favour of pyruvate and malate as the products of protocatechuate metabolism via $\gamma$-carboxy-$\alpha$-hydroxymuconic semialdehyde.

In the case of $p$-dihydroxyphenols, rupture of the bond between the carbon atom bearing a hydroxyl and an adjacent carbon atom carrying a side chain or carboxyl, occurs. For homogentisate, this method is common to micro-organisms (Chapman & Dagley, 1962) and mammals, being the pathway of tyrosine metabolism. Both homogentisicase and gentisicase give rise initially to the aliphatic oxo-acids with the cis (maleyl) configuration, but a glutathione dependent isomerase converts them to the trans (fumaryl) isomers; a hydrolase then catalyses fission of these to fumarate and acetoacetate, and fumarate and pyruvate respectively (Fig. 2).

There are two routes of tryptophan metabolism found among Pseudomonads. The ‘anthranilate’ pathway involves oxidative fission of the pyrrol ring to formylkynurenine, elimination of the formyl group and a non-oxidative cleavage of the kynurenine to alanine and anthranilate (subsequently to be metabolized via catechol). In the ‘quinoline’ pathway, kynurenine acid is formed, which is then hydroxylated in the C7, 8, positions; fission of the aromatic nucleus apparently occurs in two ways. Hayashi & collaborators (Kuno et al. 1961) initially suggested a split between the two hydroxyls, then revised this view (Kojima, Itada & Hayashi, 1961) in favour of cleavage between C8 and the angular C atom. The substituted pyridine rings so formed give rise to glutamate in both cases.
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Mechanism of action of bacterial oxygenases. These all belong to the class of enzymes called 'oxygen transferases' by Mason (1957). A tracer study with $^{18}$O proves that the two oxygen atoms in the ring fission product are derived from molecular oxygen (Hayashi, Katagiri & Rothberg, 1957). It is thought that the first step is the formation of an oxyferro-oxygenase. Electrons have been donated to the oxygen from the iron, allowing the O—O bond to be weakened (Ingraham, 1962);

\[
\text{En...Fe}^{2+} \rightarrow \text{En...Fe}^{3+}
\]

association of the perferryl complex with the $o$-dihydroxyphenol group leads to a redistribution of electrons and fission of the C—C bond. Oxygenases are labile in oxygen; Taniuchi et al. (1962) were able to restore catechol 2,3-oxygenase (metapyrocatechase) activity with sodium borohydride (and other reducing agents to a certain extent) best under anaerobic conditions. FADH$_2$, FMN and riboflavin in the presence of NADH were also effective, and they suggest a physiological role for flavin coenzymes in reductive enzyme reactivations.

![Diagram of the mechanism of action of bacterial oxygenases](image)

(8) Fate of substituent groups on monocyclic aromatic rings before cleavage

There are obviously a great many biochemical reactions which ring-splitting microorganisms perform on substituted benzenoid structures before nuclear fission. A few of these may be illustrated.

(i) Aromatic methyl ethers are demethylated to the corresponding hydroxy-derivative. Semicarbazide traps the methoxyl C as formaldehyde; in its absence it is oxidized terminally to CO$_2$ (Woodings, 1961).

![Diagram of the oxidative fission of $p$-dihydroxyphenols](image)
(ii) Methyl substituents may be oxidized step-wise to the carboxylic acid (e.g. the cresols), but in some cases it remains intact until after ring fission (e.g. MCPA metabolism, Evans, Gaunt & Davies, 1961).

(iii) Chloro-substituents usually remain on the ring unless removal is obligatory for the introduction of hydroxyls, which are necessary for fission.

(iv) Nitro and sulphonie acid groups can be eliminated and replaced by hydroxyl.

(v) A carboxyl may be oxidatively decarboxylated; in some cases anaerobic decarboxylation occurs, e.g. of 4,5-dihydroxyphthalate to protocatechuate (Ribbons & Evans, 1960).

(vi) Aliphatic side-chains are dealt with in a variety of different ways. They may be eliminated, by \( \beta \)-oxidation (Webley, Duff & Farmer, 1955) or other mechanisms familiar to biochemistry, or even remain intact—e.g. phenylpropionic acid (Coulson & Evans, 1959; Dagley & Chapman, 1961, private communication).

(4) Metabolism of polynuclear aromatic hydrocarbons

Perhydroxylation followed by dehydrogenation produces 1,2-dihydroxynapthalene, 1,2-dihydroxyanthracene, and 3,4-dihydroxyphenanthrene from their parent hydrocarbons. The actual site of cleavage in the naphthalene nucleus is the bond between the angular C and C1 which also carries a hydroxyl (Davies & Evans, 1962). There is strong evidence that it takes place at the analogous bond in 1,2-dihydroxyanthracene and 3,4-dihydroxyphenanthrene (Fernley & Evans, 1958; Griffiths & Evans, 1963, personal communication).

The case of naphthalene will serve to illustrate this pathway: A Fe\(^{3+} \) oxygenase converts 1,2-dihydroxynaphthalene to \( \delta \)-hydroxybenzalpyruvate; a hydrolytic split converts this to salicylaldehyde and pyruvate; a NAD dependent dehydrogenase produces salicylate from the aldehyde; a salicylate hydroxylase gives catechol, which is ruptured to \( \alpha \)-hydroxymuconic semialdehyde by the catechol 2,3-oxygenase also present in the cell-free extract.

(5) Anaerobic metabolism of certain aromatic compounds

Hitherto, we have considered only the aerobic metabolism of aromatic compounds by micro-organisms. Although much less is known about them, certain derivatives of benzene are dissimilated under anaerobic conditions by some bacteria.

Photosynthetic bacteria require accessory hydrogen donors for anaerobic photosynthesis in light; although evolution of oxygen has never been observed, an oxidizing entity of sufficient positive potential to perform the peroxidation of benzoate is produced by several strains of non-sulphur photosynthetic bacteria, e.g. Rhodopseudomonas and Rhodospirillum. According to Proctor & Scher (1960) sequential induction evidence supports the following tentative scheme:

\[
\text{Benzoate} \rightarrow \text{protocatechuate} \rightarrow \text{catechol} \rightarrow \text{unidentified oxo-acids.}
\]

Clarke & Fina (1952) confirmed the utilization of benzoate by methane-producing cultures; catechol and protocatechuate could not be metabolized by these cultures, and exogenous CO\(_2\) was not reduced mainly to methane. In subsequent tracer studies (Fina & Fiskin, 1960; Roberts, 1962) it was determined that the carboxyl-C and C4 of benzoate behaved the same as exogenous CO\(_2\). They were not reduced to methane primarily; on the other hand, C1 of the ring was converted almost entirely
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to methane. Benzoate utilizing cultures did not produce methane and CO₂ from fumarate, acrylate or isobutyrate without very long adaptive lag periods. Cyclohexane carboxylic acid, butyric and propionic acids, however, produced gas readily with no lag period. Propionate, containing C₄ of the ring, was isolated from the benzoate culture liquor; it did not contain C₁ or C₇. One can thus speculate that there is a preliminary saturation of the ring followed by a rupture between C₁ and C₂ before propionate is released. No other organic acid has yet been isolated.

REVIEWS


REFERENCES


