Structural Specificity of Inducers of Protocatechuic Acid Oxidase Synthesis in Neurospora

Although many compounds are known to induce the biosynthesis of specific enzymes in microorganisms (*1, 2*), little information is available on the structural specificity of inducing compounds. This note is concerned with the induction of protocatechuic acid oxidase synthesis by various analogs of protocatechuic acid (3,4-dihydroxybenzoic acid), the only known substrate for this enzyme.

Protocatechuic acid oxidase catalyzes the oxidative rupture of the aromatic ring of protocatechuic acid, yielding a tricarboxylic acid—*cis, cis*-β-carboxymuconic acid (*3, 4*). The enzyme is absent in extracts of wild-type strains of *Neurospora crassa* when they are grown on a mineral medium containing biotin with glucose or sucrose as the carbon source. Synthesis of the enzyme is induced by some of the compounds listed in Table 1 when they are added to the growth medium at a concentration of 100 to 200 μg/ml. The enzyme was assayed in extracts of the mycelia by the spectrophotometric method described elsewhere (*4*).

The metabolism of both *p*-hydroxybenzoic acid and *m*-hydroxybenzoic acid has been demonstrated to proceed via protocatechuic acid in other microorganisms (*5*). *Neurospora* will grow to some extent on *m*-hydroxybenzoic acid and *p*-aminobenzoic acid. It is presumed that the induced synthesis of protocatechuic acid oxidase results at least partly from the endogenous production of the inducer-substrate protocatechuic acid from *m*-hydroxybenzoic, *p*-aminobenzoic, and *p*-hydroxybenzoic acids. Definitive evidence for this will depend on the demonstration of enzymatic conversion of these compounds to protocatechuic acid *in vitro*, which thus far has been unsuccessful.

The other compounds tested show a rather specific structural dependency with regard to induced enzyme synthesis. An aromatic ring with a carboxyl group or a group easily oxidized to a carboxyl is required. Furthermore, introduction of a hydroxyl group at the 2-position results in complete inactivity. Substituting methoxy groups for both hydroxyl groups of protocatechuic acid completely inactivates the molecule, indicating a requirement for at least one free hydroxyl group in either the meta or para position. Since vanillic acid is about 10 times more effective than isovanillic acid as an inducer, there seems to be a high degree of specificity for the hydroxyl group para to the carboxyl group.

Vanillic acid, the inducer most extensively examined thus far, is not metabolized by the intact organism, is not attacked by protocatechuic acid oxidase, and does not inhibit enzyme activity. 2,4-dihydroxybenzoic acid, although not an inducer, competitively inhibits the oxidation of protocatechuic acid. The specificity of the enzyme-synthesizing mechanism therefore seems different from the specificity of the enzyme synthesized, as has been observed in other inducible systems (*1, 2*).

Table 1. Compounds tested as inducers of protocatechuic acid oxidase synthesis. Response: +, maximal enzyme synthesis; ±, less than one-tenth maximal enzyme synthesis; −, no enzyme synthesized.

Compound	Substitution						Response
	1	2	3	4	5	6	
Salicylic acid	COOH	OH					−
Anthranilic acid	COOH	NH_2					−
2,3-Dihydroxybenzoic acid	COOH	OH	OH				−
2,4-Dihydroxybenzoic acid	COOH	OH		OH			−
Gentisic acid	COOH	OH			OH		−
2,6-Dihydroxybenzoic acid	COOH	OH				OH	−
Pyrogallol carboxylic acid	COOH	OH	OH	OH			−
m-Hydroxybenzoic acid	COOH		OH				+
Protocatechuic acid	COOH		OH	OH			+
Protocatechuic ethyl ester	$COOC_2H_5$		OH	OH			−
Vanillic acid	COOH		OCH_3	OH			+
Vanillin	CHO		OCH_3	OH			±
Isovanillic acid	COOH		OH	OCH_3			±
Isovanillin	CHO		OH	OCH_3			−
Veratric acid	COOH		OCH_3	OCH_3			−
3,4-Dimethoxyphenylacetic acid	C—COOH		OCH_3	OCH_3			−
Piperonylic acid	COOH		$O{-}CH_2{-}O$				−
Piperonal	CHO		$O{-}CH_2{-}O$				−
Gallic acid	COOH		OH	OH	OH		+
3,5-Dihydroxybenzoic acid	COOH		OH		OH		−
Tyrosine	C—CNH_2—COOH			OH			−
p-Hydroxybenzoic acid	COOH			OH			+
Anisic acid	COOH			OCH_3			−
p-Aminobenzoic acid	COOH			NH_2			±
Benzoic acid	COOH						*
Phenylalanine	C—CNH_2—COOH						−
Catechol			OH	OH			−
Shikimic acid (cyclohexene ring)	COOH		OH	OH	OH		−

* Growth inhibited.

S. R. Gross*
E. L. Tatum

Department of Biological Sciences,
Stanford University, California

References and Notes

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* Public health postdoctorate research fellow of the National Cancer Institute.

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> *Man Thinking; him Nature solicits with all her placid, all her monitory pictures; him the past instructs; him the future invites. Is not indeed every man a student, and do not all things exist for the student's behoof? And, finally, is not the true scholar the only true master? But the old oracle said, "All things have two handles: beware of the wrong one."*
> —R. W. Emerson, *The American Scholar.*