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The DOPA Ephemera: A Recurrent Motif in Invertebrates

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Abstract. 3,4-Dihydroxyphenylalanine (DOPA) occurs transiently in nature as a free or peptide-bound amino acid. It is probably universally distributed in tissues and fluids of invertebrates. DOPA is a highly versatile metabolite, participating in neuroendocrine, immune, and reproductive functions, as well as in the formation of such products as bioadhesives, silks, integuments, and pigments. The mechanism by which DOPA is formed from tyrosine or peptidyl tyrosine remains to be determined in most cases. Future advances in DOPA chemistry may lead to a better understanding of the resonance between structural and sensory functions in animals.

Introduction

3,4-Dihydroxyphenyl-L-alanine (DOPA) is a biochemical ephemera in the sense that it is rarely an end product and is a highly transient intermediate to boot. Its presence in living matter was established at the beginning of the 1900s (Bloch, 1917) and is, in the minds of most, intimately associated with the biosynthesis of neurotransmitters, hormones, and pigments in higher organisms (Nagatsu, 1973). In the blissfully arcane literature on invertebrates, however, DOPA finds a much more extensive functional distribution. Its metabolism affects everything from egg capsules to silks, integuments, immunity, and feeding. DOPA, the free amino acid, is invariably derived from tyrosine by a number of pathways that will be briefly discussed below. But there is another form, which I shall refer to as peptidyl-DOPA, that is widely distributed in nonarthropod invertebrates and generally associated with the formation of sclerotized structures (Waite, 1990). There is but a single known exception to its functional association with sclerotization, and that is the apparent role of peptidyl-DOPA in the biosynthesis of quinoprotein enzyme cofactors in eukaryotes (Janes *et al.*, 1990). In this case, a redox cofactor, peptidyl 6-hydroxy-DOPA, serves in the catalytic oxidation of amines.

Are tyrosine and peptidyl-tyrosine hydroxylated to their DOPA counterparts by the same suite of enzymes? Do they share similar functions? Do any of their functions overlap? Although there has been some speculation on these subjects, there are no clear answers yet. The aims of the present essay are: to describe the known enzymic origins of DOPA; to set out the phyletic distribution of DOPA and related metabolites; and to offer some thoughts on the significance of DOPA-containing pathways.

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Origins of DOPA

To date, there are three known types of enzymes that form DOPA from tyrosine. Enzymes of the first type (type I) are found in the adrenal medulla of the kidney and sympathetic ganglia of the nervous system (or their equivalents) in higher animals (Nagatsu, 1973). The key enzyme here is tyrosine hydroxylase (TH; E.C. 1.14.16.2). TH is an iron-containing mixed-function oxidase that catalyzes the specific *o*-hydroxylation of tyrosine in the presence of pteridine cofactors:

$$\text{I. tyrosine} + O_2 + \text{tetrahydrobiopterin} \rightarrow \text{DOPA} + H_2O + \text{dihydrobiopterin.}$$

Dihydrobiopterin (DHP) is recycled as tetrahydrobiopterin by a NADPH-coupled DHP reductase (E.C. 1.6.99.7). TH is important as the rate-limiting step in catecholaminergic pathways (Nagatsu, 1973). The enzyme appears to be a tetramer of identical subunits weighing 60 kD each. Recent molecular studies suggest that, although a single gene encodes TH, several tissue-specific forms of TH mRNA may be produced by alternative splicing (Grima *et al.*, 1987). The role of heterogeneity in translated products is not known. The DOPA formed by

this enzyme is strictly transient and goes on to become dopamine followed by noradrenaline or adrenaline (adrenal medulla). The enzymatic hydroxylation of tyrosine in insect hemolymph is enhanced by the addition of tetrahydropteridine, a close relative of tetrahydrobiopterin (Pau and Kelly, 1975), but no one has yet suggested this enzyme activity to be that of TH. Although the enzyme is presumed to be ubiquitous, the actual distribution of TH in animals other than a few vertebrates and fruit flies, has not been rigorously demonstrated. TH is not capable of hydroxylating peptidyl-tyrosine (Waite and Marumo, unpublished studies).

The second enzymatic pathway, involving DOPA (type II), must be presented cautiously to avoid confusion. Indeed, if the multiplicity of names for the reaction pathway is any indication (*i.e.*, monophenolase, phenolase, phenoloxidase, tyrosinase, *o*-diphenolase, *o*-diphenoloxidase, polyphenoloxidase, DOPA oxidase, catecholase, or catecholoxidase), confusion already reigns. Two points can be made about type II enzymes: (1) enzyme-substrate specificity in various organisms, and even in tissues from the same organism, is highly diverse; and (2) these enzymes are capable, in principle, of catalyzing two distinct but not necessarily punctuated reactions. The first reaction is the *o*-hydroxylation of monophenols (such as tyrosine) to diphenols or catechols (such as DOPA). The recommended name for this activity is monophenol monooxygenase (E.C. 1.14.18.1). Note that the reaction must be primed by a catechol (Hearing *et al.*, 1978). Reaction stoichiometry for the conversion of the monophenol, tyrosine, is shown below[1]:

IIA. tyrosine + DOPA + O_2 → DOPA

+ DOPA-quinone + H_2O.

The second activity is the oxidative dehydrogenation of a catechol (such as DOPA) to an *o*-quinone. The Nomenclature Committee of the International Union of Biochemistry (1984) recommends catechol oxidase (E.C. 1.10.3.1) as the most appropriate name for this step:

IIB. DOPA + ½ O_2 → DOPA-quinone + H_2O.

Few, if any, of the enzymes in this class catalyze the two reactions equally well. The two rates are roughly comparable in the frog and human skin enzymes (Barisas and McGuire, 1974; Nishioka, 1978), while in fungal and microbial enzymes, IIB has a turnover number 10–20 times greater than IIA (Lerch and Ettlinger, 1972; Vanni and Gastaldi, 1990). Enzymes in insect cuticle rarely have detectable monophenol monoxygenase activity. This is also characteristic of enzymes involved in the tanning of various other sclerotized structures (Table I). Moreover, in a number of invertebrate structures, DOPA-derived metabolites may actually be oxidized by an enzyme activity more akin to laccase (E.C. 1.10.3.2), in which there is removal of only one electron at a time:

IIC. 4 DOPA + O_2 → 4 DOPA-semiquinone + $2H_2O$.

This can have the effect of shifting chemical reactivity from the ring to the sidechain of DOPA or related metabolites (Sugumaran, 1988; Hopkins and Kramer, 1992).

Considering the diversity of forms and roles that this class of enzymes encompasses, it is pointless to attempt to generalize its molecular properties. Thus far, the enzymes appear to contain binuclear copper in which the metals are often ferromagnetically silent (Jolley *et al.*, 1974). Sequence homologies around the active site of the enzymes appear to be high, especially with respect to histidines (Watters *et al.*, 1992; Müller *et al.*, 1988). In other respects, such as molecular weight, amino acid composition, and isoelectric point, enzymes from different organisms, even different tissues, vary greatly. Some of the fungal enzymes have demonstrated activity towards peptidyl-tyrosine (Marumo and Waite, 1986; Lissitzky and Rolland, 1962), but to the best of my knowledge, invertebrate catechol oxidases that have any effect on proteins prefer peptidyl-DOPA (Watters *et al.*, 1992; Waite, 1985).

The third enzyme-catalyzed pathway (type III) for producing DOPA from tyrosine involves peroxidase, but in an untypical setting. Peroxidases (from horseradish or milk) hydroxylate tyrosine to DOPA, *in vitro*, at temperatures near 0°C, when supplied with an electron donor such as dihydroxyfumarate (Klibanov *et al.*, 1981):

III. tyrosine + O_2 + dihydroxyfumarate → DOPA

+ oxalooxalate + H_2O.

This reaction is intriguing in that it requires oxygen instead of peroxide. Yields of DOPA can be as high as 70%, although at temperatures above 0°C, many byproducts are formed. It is unknown whether the peroxidase formation of DOPA has any biological basis, or whether peptidyl tyrosine can be converted to peptidyl-DOPA.

Distribution of DOPA Pathways

Reaction pathways involving DOPA and related products are widely distributed throughout the Invertebrata. The composite creature in Figure 1 represents an integration of all the DOPA pathways with known functions. Probably no single organism possesses all of these pathways, although certain arthropods may come close. Tyrosine and DOPA are involved in the cephalopod ink sac where an enzyme of category II (with both monophenol monoxygenase and catecholoxidase activities) converts the two amino acids to melanin (Prota *et al.*, 1981). When ejected into the environment, the black ink serves as a defensive olfactory and visual cloaking device. Aphids se-

[1] N.B. When tyrosine is the only substrate, the enzyme is termed a tyrosinase.

Table I

A functional menagerie of DOPA related pathways in invertebrate animals. Enzyme activity is subdivided into monophenol monoxygenase (IIA) and catecholoxidase (IIB)

Tissue (Organism)	Enzyme	Native substrate	Refs
Protoctista			
Sporogenesis *Haplosporidia*	IIB	?	1
Ctenophora			
Nervous system	II?	DOPA/cysteinylDOPA	2
Cnidaria			
Nervous system *Coelenterate hydrozoa*	II?	DOPA/tyrosine	3
Gorgonin *Sea fans*	?	Peptidyl-DOPA	4
Perisarc *Hydroids*	IIB	Dopamine (?)	5
Platyhelminthes			
Eggshell *Trematodes, turbellarians*	IIA & B	Peptidyl-DOPA	6
Metacercarial cyst *Trematodes*	IIB	?	7
Annelida			
Cocoon *Leech*	IIB	Peptidyl-DOPA	8
Coelomic fluid *Earthworm*	IIB	?	9
Cement *Sabellariid polychaetes*	IIB	Peptidyl-DOPA	10
Mollusca			
Ink sac *Various cephalopods*	IIA & B	D-DOPA/tyrosine	11
Byssus *Mussels*	IIB	Peptidyl-DOPA	12
Hemolymph *Various bivalves*	IIB	?	13
Periostracum *Mussels*	IIB	Peptidyl-DOPA	14
Shell Matrix *Clams, oysters, mussels*	IIA & B	Peptidyl-DOPA	15
Arthropods			
Silk *Silkworm moths*	IIB	N-3,4-dihydroxyphenyllactyl DOPA	16
Saliva *Aphids, wasps*	IIB	DOPA; catechin	17
Cuticle *Various insects*	IIA, B & C	N-acetyldopamine	18
Blood cells *Crayfish, cockroaches*	I, IIA & B	Tyrosine, DOPA ?	19
Ootheca *Cockroach, mantids*	IIB	Protocatechuic acid; N-acyldopamine	20
Wing pigment *Butterflies*	IIB	N-β-alanyldopamine, L-kynurenine	21
Echinodermata			
Brown bodies *Sea cucumbers*	IIB	DOPA ?	22
Urochordata			
Blood cells *Ascidians*	IIB	Peptidyl-DOPA	23

[1]Varndell (1981); [2]Carlberg (1988); [3]Carlberg and Elofsson (1987); [4]Tidball (1982); Holl *et al.* (1992); [5]Knight (1968); [6]Waite and Rice-Ficht (1987); Smyth and Clegg (1959); Nollen (1971); [7]Campbell (1960); [8]Knight and Hunt (1974); [9]Valembois *et al.* (1988); [10]Vovelle (1965); Jensen and Morse (1988); [11]Prota *et al.* (1985), [12]Waite (1985); Rzepecki *et al.* (1991); [13] Belyayeva (1981); [14]Waite and Wilbur (1976); Waite and Andersen (1978); [15]Wheeler *et al.* (1988); Samata *et al.* (1980); [16]Kawasaki and Sato (1985); Przibram and Schmalfuss (1927); Kramer *et al.* (1989); [17]Peng and Miles (1988); [18]Hopkins and Kramer (1992); [19]Johansson and Söderhäll (1989); [20]Kramer *et al.* (1989); Kawasaki and Yagi (1983); Whitehead *et al.* (1960); Yago *et al.* (1990); [21]Yago (1989); [22]Canicatti and Seymour (1991); [23]Azumi *et al.* (1990); Watters *et al.* (1992); Chaga (1980); Dorsett *et al.* (1987); Smith *et al.* (1991).

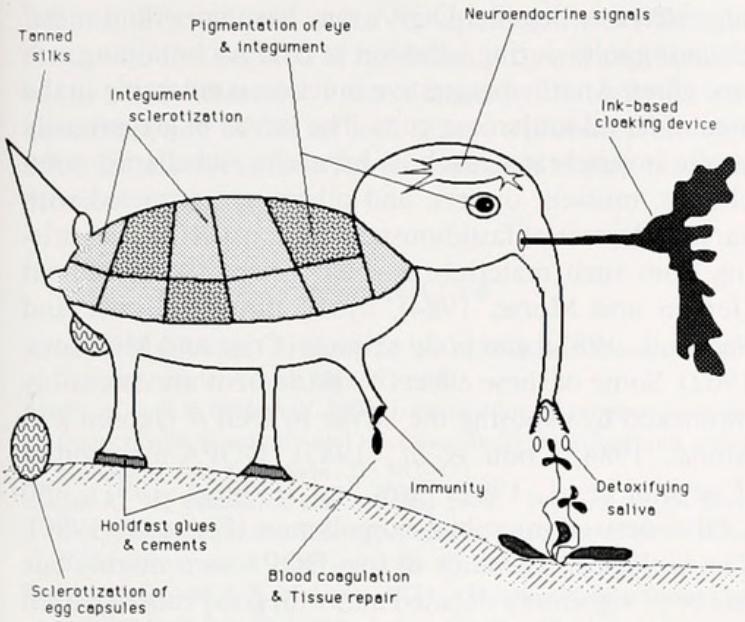

Figure 1. DOPA and enzymes that metabolize DOPA are phylogenetically and organismically scattered throughout the invertebrates. This illustration attempts to integrate all reported functions of DOPA chemistry in a single fantastical chimaera. See text and Table I for specific details.

crete a catechol oxidase into their meals to detoxify plant tissues rich in phenols (Peng and Miles, 1988). DOPA and related metabolites in plants and seeds pose an effective chemical barrier against many herbivores lacking in such salivary activity (Rehr *et al.*, 1973). Insects (Nappi *et al.*, 1991), crustaceans (Johansson and Söderhäll, 1988), holothuroids (Canicatti and Seymour, 1991), molluscs (Belyayeva, 1981), annelids (Valembois *et al.*, 1988), and tunicates (Watters *et al.*, 1992) have type II enzyme activity in body fluids, where it functions in a cell-mediated immunity leading to the encapsulation of foreign material and often the repair of injured tissue. In arthropods, the enzyme is present as a zymogen and activated by a pathogen-sensitive protease (Aspan and Söderhäll, 1991). Both monophenol monoxygenase and catechol oxidase activities are present in arthropods, while in ascidians and holothuroids only catechol oxidase is detectable (Canicatti and Seymour, 1991; Watters *et al.*, 1992). There are very few data on the molecular kinetics of these enzymes, and fewer still on the identity of the physiological substrates of such enzymes. Nappi *et al.* (1991) observed that blood tyrosine levels increased during parasite infection of *Drosophila melanogaster,* and tyrosine would be an appropriate substrate if melanin production were the chief aim of the enzyme. Perhaps the DOPA-containing peptides of morula cells (Dorsett *et al.*, 1987; Azumi *et al.*, 1990; Smith *et al.*, 1991) serve as substrates for catecholoxidase in ascidians.

Quinone-tanned adhesives and cements are especially common among marine mussels and sabellariid polychaetes. Precursors of these materials include catechol oxidases and DOPA-containing proteins, many of which have been sequenced (Vovelle, 1965; Waite, 1985; Jensen and Morse, 1988; Rzepecki *et al.*, 1991). Catechol oxidase activity has been histochemically detected in barnacle cement, but the native substrate acted upon is unknown (Yule and Walker, 1987). DOPA-containing proteins and catecholoxidases are also implicated in the sclerotization of trematode and turbellarian egg capsules and cysts (Smyth and Clegg, 1959; Nollen, 1971; Ishida and Teshirogi, 1986; Waite and Rice-Ficht, 1987). In insect ootheca, the DOPA derivatives are not protein-bound, but rather DOPA-derived metabolites of low molecular weight; *e.g.*, protocatechuic acid in cockroach ootheca (Whitehead *et al.*, 1960), and N-acyldopamines in mantid ootheca (Kawaski and Yago, 1983). The same situation seems pertinent in many insect silks, which are tanned by the action of DOPA metabolites and catechol oxidase (Przibram and Schmalfuss, 1927; Kawasaki and Sato, 1985; Kramer *et al.*, 1989).

The most extensive exploitation of DOPA chemistry has undoubtedly occurred in the development of exoskeletal integuments. This includes the ubiquitous cuticles of insects (Hopkins and Kramer, 1992), molluscan shells (Degens *et al.*, 1967), coelenterate exoskeletons such as gorgonin and perisarc (Knight, 1968; Holl *et al.*, 1992), tunicates (Robinson *et al.*, 1986) and, possibly, some of the infinitely varied packaging strategies of creatures like tardigrades, loricates, bryozoans, *etc.* (Waite, 1990). Sclerotization of insect cuticles has come to mean at least two different pathways involving oxidases and DOPA-derived metabolites (Brunet, 1980; Sugumaran, 1988). In β-sclerotization, protein cross-linking activity is localized on the N-acetylethyl side chain of N-acetyldopamine (NADA) (Andersen, 1974). The cross-linked products are colorless *in vivo* but as yet chemically uncharacterized. The more classical pathway dubbed "quinone-tanning" (which probably doubles in melanization) involves a direct attack on the aromatic ring by nucleophilic groups, presumably from the protein-chitin components (Hopkins and Kramer, 1992). Details about the chemical transformations occuring during sclerotization are still controversial (Sugumaran, 1988) and beyond the scope of this article. Crustaceans and insects may share many of the same features of cuticle sclerotization, but this is not the case for other arthropods such as arachnids, chelicerates, and chilopodes, nor for other invertebrates such as mollusks and coelenterates (especially gorgonians). In at least some of these instances, sclerotization, although still ostensibly based on quinone-tanning, appears to involve the action of catecholoxidase on DOPA-containing proteins. How widespread this strategy really is remains unclear (Waite, 1990).

The function of pigmentation by melanin formation is still debated (Hill, 1992). It may protect underlying tissues from ultraviolet light, to scavenge radicals, or to provide

adaptive colorations, *e.g.*, camouflage or adornment. In contrast to vertebrates, in which pigmentation is largely an intracellular event localized in skin, eyes, and hair bulb cells, invertebrates commonly secrete the granular organelles containing enzymes and substrate precursors directly into acellular structures (Riley, 1977; Hiruma and Riddiford, 1988). The two most common melanins, eumelanin and pheomelanin, are formed by the catalytic oxidation and polymerization of DOPA, or DOPA and cysteine, respectively (Prota and Thompson, 1976). Insect pigments such as papilliochrome (Table I), derived from other DOPA-derived co-reactants, are also possible (Yago, 1989) and probably common.

In the neuroendocrine systems of higher organisms, DOPA is formed from tyrosine by tyrosine hydroxylase (type I). Not until its further transformation to dopamine, noradrenaline or adrenaline, however, is the *o*-diphenol endowed with neuroendocrine function. Subsequent transformations, involving *O*-methylation, sulfation, or oxidation to pigments, may just be ways of terminating the signal function (Nagatsu, 1973). That dopamine and noradrenaline are *not* universally employed as neurotransmitters is suggested by the discovery that only DOPA and related metabolites (5-hydroxyDOPA and cysteinylDOPA) occur in the nervous systems of some cnidarians and ctenophores (Carlberg and Elofsson, 1987; Carlberg, 1988). If a type II enzyme is responsible for the formation of DOPA and cysteinyl-DOPA in these organisms, then it would be intriguing to know how melanins are prevented from forming. Indeed, perhaps there is a suggestion here that neurotransmitters and pigments-sclerotins may have had a common evolutionary origin in the ectoderm of primitive organisms.

Significance of DOPA

Table I is, alas, a bitter pill for those wishing to discover the origin of DOPA in invertebrates. With the exception of the neuroendocrine catecholamines and melanization pathways (inks, host encapsulation of pathogens, and pigmentation), pathetically little is known about the formation of DOPA and peptidyl-DOPA. Is the hydroxylation brought about by a tetrahydropterin-linked tyrosine hydroxylase, a type IIA monophenol monoxygenase, or even a prolyl hydroxylase (E.C.1.14.11.2)-like activity directed instead toward peptidyl-tyrosyl residues? Nothing at present detracts from or advocates any of these. Many organisms go to considerable lengths to separate, even compartmentalize, the tyrosine-to-DOPA and DOPA-to-quinone steps into discrete, non-continuous biochemical transformations. Why should this be, when a bifunctional type II enzyme could have done it in one fell swoop? The answer may be that transient DOPA has unique functions that cannot be properly discharged when the tyrosine to quinone conversion is unpunctuated. Waite (1987) has suggested that peptidyl-DOPA may have important metal chelating roles during adhesion in DOPA-containing marine glues. Another suggestive function is emerging in the area of larval settlement cues. The larvae of gregariously sessile invertebrates, such as barnacles, sabellariid polychaetes, mussels, oysters, and others, are attracted with varying degrees of fastidiousness by conspecific cues arising from such materials as shell (Crisp, 1967), cement (Jensen and Morse, 1984), byssal threads (Eyster and Pechenik, 1987), and body extracts (Crisp and Meadows, 1962). Some of these effects on settlement are ostensibly mimicked by exposing the larvae to DOPA (Jensen and Morse, 1984; Coon *et al.*, 1985), DOPA-metabolites (Chevolot *et al.*, 1991; Pires and Hadfield, 1991), and DOPA-derived microbial exopolymers (Fitt *et al.*, 1990). The biological relevance of free DOPA as a marine cue has been vigorously debated and with good cause (Jensen and Morse, 1990; Pawlik, 1990; Johnson *et al.*, 1991). DOPA is notoriously unstable in seawater. Moreover, the *specific* appeal of many cue-containing materials would seem to militate against their reliance on metabolites as common as free DOPA. Crisp (1974) first proposed that, because of the nature of lamellar-turbulent flow, effective settlement cues were only to be found following physical contact with the surface. This suggests that larvae have sensors (receptors) for immobilized cues present on settlement surfaces. Perhaps then peptidyl-DOPA (or one of its metabolites) present in natural settlement-inducing materials, such as shell, byssus, or cement, is the cue. This would satisfy two observations: that free DOPA can mimic settlement cues to some extent, and that settlement cues are highly selective. The latter would be fulfilled, for example, if larvae sensed some form of DOPA, as well as primary flanking sequences in the protein.

Postscript

The potential number and diversity of DOPA-producing and oxidizing enzymes in invertebrates casts a pall on strategies that involve their purification and characterization after the whole organism has been disrupted. Although such studies are still commonplace (*e.g.*, Oshima and Nagayama, 1980; Simpson *et al.*, 1987; Maruyama *et al.*, 1991), they will have very little to offer biology until at last they address the functions and distribution of the enzymes *in vivo*. The DOPA ephemera is unique in that it seems to be pivotal in the seesaw between structural and sensory biochemistry. A better understanding of the evolutionary and physiological resonance between these two would undoubtedly scintillate any number of sclerotized minds, including mine.

Acknowledgments

There are too many researchers to whom I owe a debt of gratitude for advancing or assiduously working the field.

I thank them all and make my apologies in advance to those who feel slighted at not being properly represented in the cited literature. I never intended to write an exhaustive review of this subject, simply a probing overview. Bill Carr deserves credit for tempting me to step into this morass.

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