BINDING OF ^{14}C-LABELED DOPA BY MYCOBACTERIUM LEPRAE IN VITRO

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o-Diphenoloxidase which converts dopa and other phenolic compounds to quinones has been established to be a specific metabolic property of Mycobacterium leprae (Table 1). We reported previously the binding of tritium-labeled dopa by the leprosy bacilli. The present report deals with the uptake of ^{14}C-labeled dopa by M. leprae separated from infected armadillo tissues. Initially, the armadillo bacteria were characterized by studying properties of o-diphenoloxidase in the bacilli.

Table 1. Oxidation of dopa by various mycobacteria

Organisms	Dopa Oxidation
M. leprae (human skin)	+
M. leprae (human spleen)	+
M. leprae (human testes)	+
M. leprae (mouse foot-pad)	+
M. leprae (armadillo skin)	+
M. leprae (armadillo spleen)	+
M. leprae (armadillo liver)	+
M. lepraemurium (mouse spleen or "leproma")*	-
M. tuberculosis H_{37} Rv*	-
M. tuberculosis H_{37} Ra	-
M. bovis BCG	-
M. balnei	-
M. phlei	-
M. smegmatis	-
M. ulcerans	-
M. microti	-
M. sp. 607	-
"Bacillus lepraé" cultures, 10 "species" (ATCC)	-
"ICRC" bacillus	-
Kedrowsky's bacillus	-

\+ Positive - Negative

*Originally tested in Dr. R. J. W. Rees' laboratory.

The organisms were separated from homogenates of the infected tissues (usually spleen or liver) by differential and density-gradient centrifugations in inert solutions such as those of sucrose and KCl (Table 2). The armadillo bacilli oxidized both D- and L-dopa at the same rate as well as derivatives of dopa like epinephrine and norepinephrine (Table 3). In substrate-specificity, these bacilli show the same properties as M. leprae from human sources. Tyrosinase occurring in melanocytes oxidized only L-dopa. Both mammalian and plant tyrosinases produced dopachrome (absorbance maximum 480 nm) from dopa, whereas the leprosy organisms convert dopa to indole-5,6-quinone (absorbance maximum 540 nm).

We have shown earlier that the enzyme in M. leprae from human tissues is resistant to reducing agents (like ascorbic acid) and metal chelators (like cyanide). The o-diphenoloxidase of armadillo bacteria show similar properties (Tables 4 and 5). The copper chelator diethyl dithiocarbamate is the only compound which completely inhibits the enzyme in the leprosy bacilli. The other metal chelators and the reducing agents produced total inhibition of both mammalian and plant tyrosinases, while showing no effect on the bacterial enzyme. These preliminary results indicate that in M. leprae, o-diphenoloxidase is a constitutive enzyme which remains unchanged in the passage of the organisms from the human to the animal host.

To study the uptake of the radioactive substrate, the bacilli were incubated with ^{14}C-labeled dopa. After incubation, TCA was added and the reaction mixture was centrifuged. The sediment was washed four times with deionized glass-distilled water to remove all unbound dopa. The final wash showed little activity above background levels. The washed sediment was suspended in water and the radioactivity due to the bound dopa was assayed in a liquid scintillation counter. Heat-inactivated bacilli (100°C, 30 min.) served as control. The reaction system is given in Table 6. For comparison, a cultivable mycobacterium—M. phlei and a few vertebrate cell lines—melanocytes, mouse foot-pad fibroblasts, turtle heart cells, and human (skin and muscle) cells were used.

The data on the uptake of ^{14}C-dopa by M. leprae, M. phlei and the vertebrate cell lines are presented in Table 7. M. leprae and melanocytes readily take up the labeled substrate, whereas M. phlei and the other vertebrate cells do not. Both M. leprae and melanoma cells contain o-diphenoloxidase which converts dopa to quinone. Tyrosinase is a copper protein, and the copper chelator, diethyl-dithiocarbamate inhibits the uptake of dopa by M. leprae and melanoma cells (Table 8).

Table 2. Fractionation Scheme for Separation of M. leprae from Lepromatous Tissue

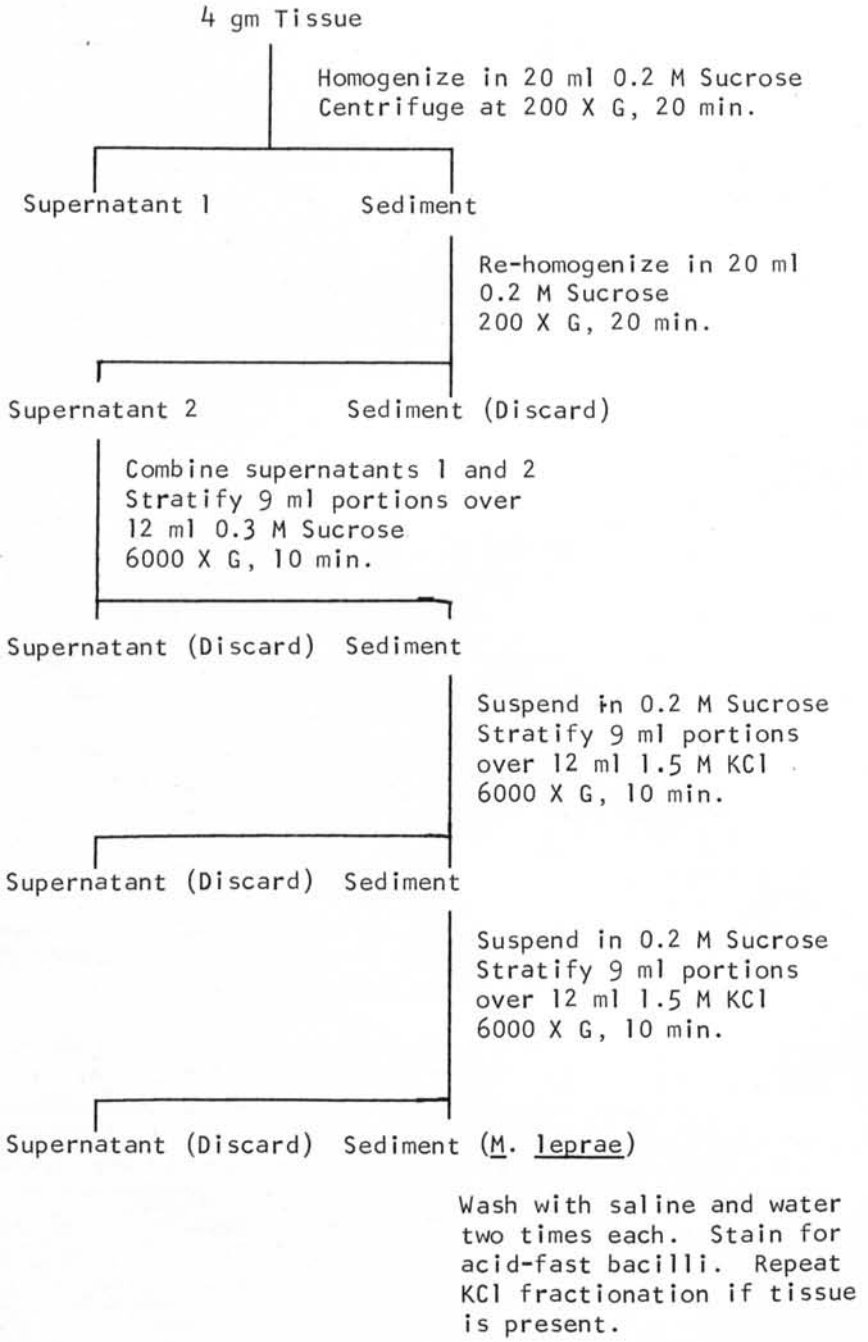

Table 3. Oxidation of dopa and its derivatives by M. leprae and by plant and mammalian tyrosinase: increase in absorbance

Substrate	M. leprae	Mushroom tyrosinase	Melanocyte culture
L-Dopa	0.100(540)[a]	0.095(480)	0.160(480)
D-Dopa	0.099(540)	0.100(480)	0
L-Epinephrine	0.100(480)	0.240(480)	0
DL-Norepinephrine	0.060(480)	0.140(480)	0

[a]Values in parentheses indicate absorbance maxima in nm.

Table 4. Effect of reducing agents on o-diphenoloxidase from different sources

Reaction system[a]	Absorbance 540 nm	Absorbance 480 nm	
	M. leprae	Mushroom tyrosinase	Melanocyte culture
Enzyme + dopa	0.108	0.120	0.140
Enzyme + dopa + ascorbate	0.098	0	0
Enzyme + dopa + GSH	0.080	0	0
Enzyme + dopa + cysteine	0.095	0	0

[a]Abbreviation: GSH, reduced glutathione.

Table 5. Effect of metal chelators on o-diphenoloxidase from different sources

Reaction system[a]	Absorbance 540 nm	Absorbance 480 nm	
	M. leprae	Mushroom tyrosinase	Melanocyte culture
Enzyme + dopa	0.099	0.130	0.140
Enzyme + dopa + NaCN	0.095	0	0
Enzyme + dopa + penicillamine	0.100	0	0
Enzyme + dopa + DDC	0	0	0

[a]Abbreviations: DDC, diethyldithiocarbamate; NaCN, sodium cyanide.

Table 6. Assay system for the uptake of ^{14}C-dopa by mycobacteria and by cell cultures

^{14}C-dopa (51 mCi/mmole)	1 μCi
Unlabeled DL-dopa	1 μmole
Na_2HPO_4-KH_2PO_4 Buffer, pH 6.8	0.1 M
Bacilli	10^9 or more
Cell cultures	0.4-1.7 mg protein
Volume	2 ml
Temperature	37°C
Time	30 min. or 120 min.

Table 7. Uptake of ^{14}C-dopa by mycobacteria and cell cultures: p moles ^{14}C-dopa

Cells	Unheated	Heated
M. leprae	343.2	66.8
M. phlei	40.8	42.3
Melanocytes	338.9	87.0
Mouse foot-pad cells	52.4	58.1
Human (skin and muscle) cells	30.5	30.6
Turtle heart cells	53.0	48.8

Table 8. Inhibition of dopa uptake by diethyldithiocarbamate (DDC): p moles ^{14}C-dopa

Cells	- DDC	+ DDC
M. leprae	107.7	25.7
Melanocytes	484.6	45.9

These data suggest that the binding of dopa is an enzymatic process and not a nonspecific uptake of the substrate. It may be mentioned that the metabolism of dopa, being an enzymatic process, will take place so long as the enzyme protein is not denatured, as in organisms stored in the frozen state; the bacilli need not be viable.

The oxidation of dopa by the bacteria and the cell cultures is shown in Table 9. When tritiated dopa is used as substrate, the ^{3}H is oxidized to water; with ^{14}C-dopa, the label is ultimately incorporated into the melanin pigment formed. Table 10 gives a comparison of the uptake of ^{3}H- and ^{14}C-dopa by M. leprae; it may be seen that the bacilli bind ^{14}C-dopa more efficiently than the tritiated substrate. The results provide additional evidence that the leprosy bacilli possess specific sites for the enzymatic binding and subsequent metabolism of 3,4-dihydroxyphenylalanine.

Table 9. Oxidation of ^{3}H-dopa by mycobacteria and cell cultures: p moles ^{3}H-dopa

Cells	Unheated	Heated
M. leprae	180.14	24.26
M. phlei	4.83	12.17
Melanocytes	139.4	0
Turtle heart cells	0	0
Mouse foot-pad cells	0	0
Human (skin and muscle) cells	0	0

Table 10. Comparison of the uptake of ^{14}C- and ^{3}H-dopa by M. leprae and by M. phlei: % of substrate bound

Bacilli	^{14}C-dopa	^{3}H-dopa
M. leprae		
Unheated	7.65	2.61
Heated	1.85	0.40
M. phlei		
Unheated	0.25	0.08
Heated	2.18	0.35