BIOSYNTHESIS OF THE BENZOQUINONE RING OF UBIQUINONE IN TETRAHYMENA PYRIFORMIS

James E. Miller

Biological Laboratories, Amherst College,
Amherst, Massachusetts

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The biosynthesis of the aromatic moiety of ubiquinone (UQ) in all organisms in which it has been investigated proceeds through para-hydroxybenzaldehyde or para-hydroxybenzoic acid (POB). This has been established in Rhodospirillum rubrum (Rudney and Parson, 1963), Azotobaceter vinelandii and Baker's yeast (Parson and Rudney, 1964), Escherichia coli (Cox and Gibson, 1964), and in the rat (Olson, et.al., 1963; Rudney and Parson, 1964). In bacteria the benzoquinone nucleus and benzoate intermediates are derived from compounds which arise from the shikimic acid pathway (Rudney and Parson, 1963). In the rat, however, the benzoate intermediate is derived from dietary aromatic amino acids. Olson, et.al., (1965) have shown conclusively that the aromatic ring of phenylalanine is incorporated into the ring moiety of rat liver UQ. The ciliated protozoan Tetrahymena pyriformis, which has nutritional requirements similar to mammals and is incapable of synthesizing aromatic amino acids, was shown by Braun, et.al., (1963) to form the quinone ring of UQ by some pathway which does not utilize carbon from aromatic compounds in the growth medium. The purpose of the present communication is to present data obtained in further investigation of UQ biosynthesis in Tetrahymena.

Tetrahymena pyriformis strain W was grown axenically in a 3% proteose-peptone medium or in the synthetic medium of Kidder, Dewey and Heinrich (1954) from which glucose was omitted. Glucose was added to both the crude and synthetic media at 1 mg/ml. Except where otherwise indicated, isotopically labeled substrates were present throughout the growth period. Labeled POB used in one

experiment was a gift of Dr. Harry Rudney and labeled shikimate (SKA) was a gift of Dr. David B. Sprinson. In other experiments labeled POB was prepared by alkaline fusion of tyrosine by the method of Rudney and Parson (1964).

After 4 days of growth cells were harvested by centrifugation, washed twice with water, saponified in a solution of methanolic KOH and pyrogallol, and the UQ was extracted from the saponification mixture into heptane. UQ samples were purified twice by thin layer chromatography on silica gel using the solvent systems of Braun, et.al. (1963), then assayed spectrophotometrically by comparing the absorbance of the oxidized compound at 275 mμ in ethanolic solution with the absorbance following reduction by borohydride (Lester, et.al., 1959). Non-labeled UQ was added to radioactive samples and ozonolysis was carried out using a method similar to that of Bentley, et.al., (1965). By this method the ring moiety of UQ can be counted as 3,6-dimethoxy-2-methyl phenylacetic acid (DDMP).

Table 1 shows that carbon from uniformly labeled glucose is incorporated into UQ and that 19-35% of the incorporated label is in the ring moiety. Incorporation of glucose into the ring is depressed by the presence of POB or shikimate in the medium. Radioactive POB and shikimate labeled the ring fragment extensively.

Phenylalanine was incorporated into UQ but the ring fragment was not labeled significantly. This finding is in agreement with that of Braun, et.al., (1963). The effect of unlabeled SKA in diluting labeling of UQ by glucose is in direct contradiction to the results of the above authors. In the present experiment, however, the glucose concentration in the medium was lower and the SKA concentration much higher and given in multiple doses. Furthermore, they (Braun, et.al., 1963) did not carry out degredation of the UQ labeled by glucose, while the present degredation results clearly show that the ring fragment is labeled. In Table 2 some of the results shown in Table 1 are summarized.

On the basis of these observations it appears that in Tetrahymena the benzoquinone ring of UQ is derived from POB, which arises from glucose by the shikimic acid pathway. This route of synthesis is known to occur in bacteria, but in view of the animal-like nutritional require-

Table 1

Incorporation of Various Compounds into Ubiquinone

$U\text{-}C^{14}$ Substrate	Medium	Specific Activity (cpm/μ mol) Substrate	UQ	Percent Substrate	Diluted UQ	DDMP	Percent UQ	Predicted Activity of DDMP[c] A	B	C
Glucose	Synthetic	1956	-	-	38	8	(21)	38	1.8	6
"	Crude	925 908	3790 4420	(410) (488)	370	71	(19)	370	18	64
"	"	523	4980	(950)	491	171	(35)	491	25	86
"	Crude + POB, 100γ/ml	629 642	1025 752	(163) (116)	73	5.6	(8)	73	3.6	13
"	Crude + SKA, 800γ/ml[a]	925	2550	(276)	290	17	(6)	290	15	50
POB	Crude	$54X10^5$	$1.3X10^5$	(2.4)	-	-	-	-	-	-
"	"	-	$8.5X10^4$	-	3178	1847	(58)	3178	154	553
"	"	-	$12X10^4$	-	149	37	(25)	149	7.4	26
SKA[b]	"	310	133	(43)	16	4.1	(26)	16	0.8	2.8
Phenylal.	Synthetic	4169	444	(11)	-	-	-	-	-	-
"	"+ acetate at 0.05%	3740	856	(23)	40	3.9	(9.7)	40	2	7

a Multiple doses (100γ/ml) at 12 hr. intervals
b Added after 72 hrs. growth
c A - All counts assumed to be in the ring (minus methyl and methoxy carbons)
B - All counts assumed to be in the side chain (2 carbons remaining in DDMP)
C - Counts assumed to be equally distributed between ring and side chain

ments of Tetrahymena the derivation of the benzoquinone ring from aromatic amino acids, as in the rat, seemed likely. The unexpected finding of a functional shikimic acid pathway in this protozoan may represent its retention by Tetrahymena, as an evolutionary fragment for formation of ubiquinone, while the enzymes for synthesis of tryptophan and phenylalanine were lost.

Table 2

Summary: Incorporation of Various Compounds into UQ Ring

Substrate	% of UQ activity in ring fragment (DDMP)*
POB-C^{14}	42% (2)
SKA-C^{14}	26% (1)
Glucose-C^{14}	25% (3)
" + POB	8% (1)
" + SKA	6% (1)

*These values are averages taken from data shown in Table 1. The number of experiments represented in the average is shown by the figure in parentheses.

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REFERENCES

Bentley, R., Ramsey, V. G., Springer, C. M., Dialameh, G. H., and Olson, R. E., Biochemistry, 4, 166 (1965).

Braun, R., Dewey, V. C., and Kidder, G. W., Biochemistry, 2, 1070 (1963).

Cox, G. B., and Gibson, F., Biochim. Biophys. Acta, 93, 204 (1964).

Kidder, G. W., Dewey, V. C., and Heinrich, M. R., Exper. Cell Res, 7, 256 (1954).

Lester, R. L., Hatefi, Y., Widmer, C., and Crane, F. L., Biochim. Biophys. Acta, 33, 169 (1959).

Olson, R. E., Bentley, R., Aiyar, A. S., Dialameh, G. H., Gold, P. H., Ramsey, V. G., and Springer, C. M., J. Biol. Chem., 238, PC3146 (1963).

Olson, R. E., Dialameh, G. H., Bentley, R., Springer, C. M., and Ramsey, V. G., J. Biol. Chem., 240, 514 (1965).

Parson, W., and Rudney, H., Proc. Natl. Acad. Sci. U.S., 51, 444 (1964).

Rudney, H., and Parson, W., J. Biol. Chem., 238, PC3137 (1963).