Biosynthesis of Bacterial Menaquinones - Evidence for the Involvement of 2-Oxoglutaric Acid

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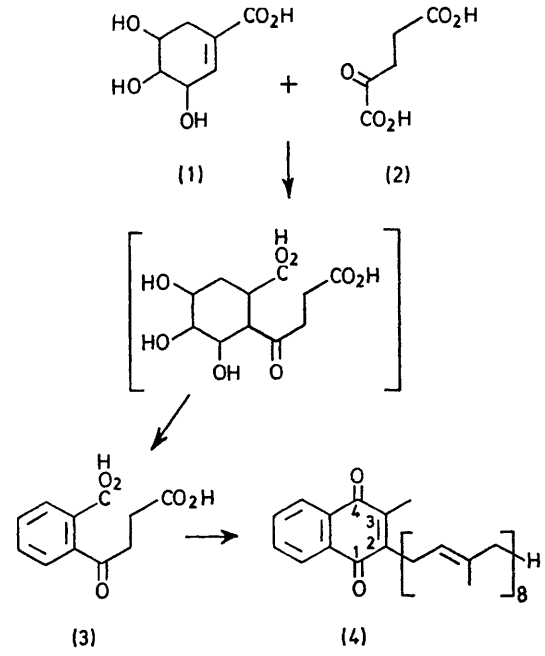
Summary Carbon atoms 2, 3, and 4 of 2-oxoglutaric acid are found to be precursors of carbon atoms 2, 3, and 4 (or 1) of the naphthalene nucleus of the menaquinone from *Escherichia coli*.

THE naphthoquinone ring system in bacterial menaquinones has a multiple biosynthetic origin. All seven carbon atoms of shikimic acid (**1**) are contributed to the aromatic ring system[1,2] while glutamic acid provides the remaining three-carbon unit.[3,4] It was postulated that naphthoquinone formation takes place by a decarboxylative addition between shikimate and 2-oxoglutarate (**2**), the transamination product of glutamate (Scheme 1). The aromatic product of the condensation (**3**) has been shown to be a good precursor of bacterial menaquinones.[2,4] We now present evidence for the direct participation of 2-oxoglutarate in the biosynthetic pathway to bacterial menaquinones.

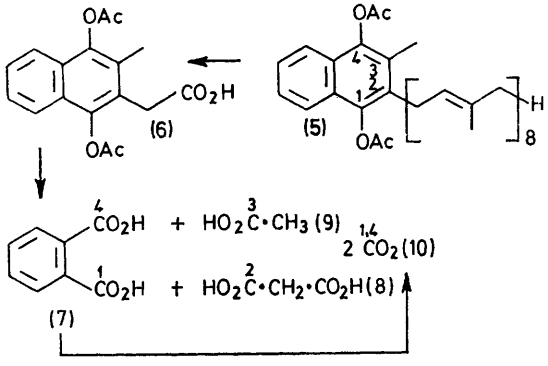
If the presumed biosynthetic hypothesis is correct (Scheme 1) C-1, C-2, and C-3 of the menaquinone (**4**) should be equally labelled by [*U*-^{14}C]-2-oxoglutarate. This compound (250 μCi, 95 mCi/mmol) was added to *Escherichia coli* cultures. The growth conditions and procedures used to purify the menaquinone-8 (**4**) have been described previously.[2] The incorporation of radioactivity was 0·011%, which agrees well with the value of 0·015% found for the incorporation of L-[*U*-^{14}C]-glutamate into menaquinone-8 in *E. coli*.[2] Since this organism produces ubiquinone-8 in addition to menaquinone-8, the measurement of the specific activity of both quinones indicates the effectiveness of a particular precursor in providing the three-carbon unit. This follows since ubiquinone biosynthesis requires shikimate, *S*-adenosyl methionine, and mevalonate,[5] while menaquinone biosynthesis involves



SCHEME 1

these precursors plus the three-carbon unit. In the present experiment, the ratio of activity in menaquinone-8 to ubiquinone-8 (24:1) is in good agreement with ratios of 35:1 and 20:1 found in experiments with L-[*U*-^{14}C]-glutamate and DL-[2-^{14}C]-glutamate in *E. coli*. With labelled acetate, a non-specific precursor, values close to unity were observed for this ratio in *E. coli*.[2]

Additional support for the direct participation of 2-oxoglutarate was obtained by determining the labelling pattern (Scheme 2) within the menaquinone-8, as previously described.[2] The results shown in the Table indicate that appreciable metabolism of the 2-oxoglutarate has occurred with formation of isoprenoid precursors. However, the proportion of activity found in the naphthalene portion (55%) is very similar to that obtained with the L-[*U*-^{14}C]-glutamate feeding experiment in *E. coli* (58%).[2]



SCHEME 2

Chemical degradation of menaquinone biosynthesized from [U-^{14}C]-*2-oxoglutarate*

Degradation compound	Specifiv activity[a] (dpm/μmol)	% of (5)
(5)	249	100
(6)	138	55
(7)	51	21
(8)	42	17
(10)	43	17

[a] The samples were counted so as to give a standard error in the net counting rate of 2%.

In addition, approximate values for the activities of the three relevant carbon atoms can be deduced from the experimental results (Table). Assuming that metabolism of the 2-oxoglutarate tends to produce randomly labelled shikimic acid and polyprenoid moiety, the specific activities (*A*) of C-2 and C-4 are given by: *A*(C-2) = A(**8**) − 2/38 [*A*(**5**) − *A*(**6**)] and *A*[C-4(1)] = *A* (**10**) − 1/6[*A*(**7**) − *A* (**10**)].† *A*(C-3) is given approximately by the specific activity of the acetic acid fragment (**9**).‡ This calculation gives values of 17·0% for C-2, 14·6% for C-3, and 16·3% for C-4 (1).

Thus, [*U*-^{14}C]-2-oxoglutarate contributes essentially equal proportions of activity to the menaquinone carbon atoms C-2, C-3, and C-4 (and/or C-1). This supports the proposed biosynthetic involvement of 2-oxoglutarate in the biosynthesis of bacterial menaquinones.

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† Because the degradation involves a symmetrical compound the two carbonyl positions of the quinone ring, C-1 and C-4, cannot be distinguished.

‡ The methyl group is known to be derived from methionine and 2-oxoglutarate is not likely to contribute activity to this moiety. Since there are difficulties in isolating the acetic acid from the oxidation mixture, the activity is obtained from A(**6**) − A(**7**) − A(**8**).

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