Subcellular distribution of a biologically active naphthoquinone in *Mycobacterium phlei*

Quinones have been implicated in electron transport and coupled phosphorylation in mammalian and bacterial systems. A naturally occurring vitamin K-like napthoquinone has been isolated from *Mycobacterium phlei*[1] and it has been shown, like vitamin K_1 and closely related, 2,3-dialkyl-1,4-naphthoquinones, to restore oxidation and phosphorylation to preparations that have been inactivated by irradiation[2,3].

Coupled phosphorylation in untreated bacterial preparations of *M. phlei* requires the participation of a highly organized particulate system as well as soluble components. Of the known participants in oxidative phosphorylation the particles contain the dehydrogenases, bound DPN, and the terminal respiratory pigments[4], whereas the supernatant contains other enzymes necessary for the oxidation of DPNH and for phosphorylation[5]. Since the bacterial naphthoquinone appears to be an additional component of this system, its distribution and concentration in the subcellular fractions have been determined and compared with those of certain other electron carriers.

M. phlei cells, ATCC 354, were grown, disrupted, and fractionated by methods previously described[5]. The electron carriers analyzed were compared on a molar basis. In order to compare the relative concentrations of the carriers in the particles and the supernatant, the fractions were compared on a dry weight basis.

The total flavins were extracted with 10 % trichloroacetic acid and were measured fluorometrically[6]. The fluorescence of the extract was taken to indicate the concentration of FMN, since free riboflavin has not been found in bacteria[7]. The concentration of FAD was determined by measuring the increase in fluorescence following acid hydrolysis.

To estimate the naphthoquinone, extraction with a mixture of isopropanol–isooctane (3:1) was followed by adsorption of a petroleum ether (30–60°) solution of the material onto Permutit and elution with diethyl ether in petroleum ether. The concentration of the naphthoquinone was determined by the colorimetric procedure of SCHILLING AND DAM[8], with vitamin K_1 as a standard. The values agreed with those obtained from the absorption at 249 mμ, assuming $E^{1\%}_{1\,cm} = 630$. The identity of the naphthoquinone was confirmed by paper chromatography[1].

In order to compare the concentration of the naphthoquinone with that of terminal electron carriers, cytochrome *b*, chosen for analysis as a representative of the terminal respiratory pigments, was measured by the alkaline pyridine method of PAPPENHEIMER[9]. The difference in absorption at 560 mμ between the oxidized and reduced states was used to calculate the concentration; standard curves were obtained with hematin. Prior treatment of the particulate fraction with rattlesnake venom, a method similar to that of AMBE AND CRANE[10], was necessary to obtain preparations transparent enough for photometric measurements.

It is difficult to describe enzyme systems as particulate or soluble with most disruptive procedures commonly used. However, some degree of reliability can be attained by using as an index of structural integrity a physiological function such as

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Abbreviations: DPN, diphosphopyridine nucleotide; FMN, riboflavin monophosphate; FAD, flavin-adenine dinucleotide.

oxidative phosphorylation for which retention of organized structure is essential. This activity has been used as a measure of the degree to which the system retains the pattern of enzyme localization in the intact cell.

The distribution and concentration of the electron carriers analyzed in preparations capable of oxidative phosphorylation are shown in Table I. The supernatant fraction contained both the flavins, FMN and FAD, in greater concentrations than

TABLE I

THE RELATIONSHIP OF THE NATURAL NAPHTHOQUINONE TO THE FLAVINS AND CYTOCHROME *b* IN SUBCELLULAR FRACTIONS OF *M. phlei*

Electron carrier	*Supernatant fraction*		*Particulate fraction*	
	*μmoles/mg dry wt.**	*Relative concentration***	*μmoles/mg dry wt.**	*Relative concentration***
FAD	$2.64 \cdot 10^{-4}$	1.8	$9.85 \cdot 10^{-5}$	0.6
FMN	$5.95 \cdot 10^{-4}$	4	$5.30 \cdot 10^{-5}$	0.35
FAD + FMN	$8.59 \cdot 10^{-4}$	5.2	$1.52 \cdot 10^{-4}$	1
Naphthoquinone	$6.03 \cdot 10^{-4}$	4	$3.52 \cdot 10^{-3}$	23
Cytochrome *b*	0	0	$2.65 \cdot 10^{-4}$	2

* Each value represents the average of at least 4 determinations, except for that of the natural naphthoquinone which was the average of at least 8 determinations.

** The relative concentrations of the electron carriers are expressed as molar ratios, the total concentration of the flavins in the particulate fraction being taken as unity.

the particulate fraction. The relatively high concentration of FMN in the supernatant correlates with the observed non-phosphorylative FMN-linked pathway of oxidation catalyzed by this fraction[5].

Although a small amount of the naphthoquinone was found in the supernatant, the major portion was localized in the particulate fraction. Only one naphthoquinone, with the same R_F value shown by paper chromatography[1], was regularly obtained from both fractions.

As has been observed previously for the terminal respiratory pigments of *M. phlei*[4], only the particles contain cytochrome *b*.

The combined system of the particles and the supernatant, which is capable of coupled phosphorylation, contained the flavins, naphthoquinone and cytochrome *b* in the ratio of 3.4:13.5:1. Thus the quantity of the naturally found naphthoquinone is sufficient to account for function at at least one of the phosphorylative sites in electron transport. There is an apparent excess of the natural napthoquinone. Concentrations of quinones of the same order of magnitude as that of the napthoquinone in *M. phlei* have been found in other bacteria and in mammalian and plant tissues[11]. The fact that vitamin K is necessary for the oxidation of reduced DPN and for the reduction of the terminal respiratory pigments indicates that this coenzyme functions between these two respiratory carriers[12].

It is concluded that the biologically active napthoquinone in *M. phlei* is located in the particles. Its concentration relative to other electron carriers is sufficient to support electron transport and to account for a coenzymic role in oxidative phosphorylation.

ADDENDUM

Quinones from other microorganisms have been examined; however, not with respect to other electron carriers. LESTER AND CRANE[11] have found both a benzoquinone (Q_8) and a naphthoquinone in *Escherichia coli*; the benzoquinone was present in a high concentration only in cells grown aerobically. In the present studies three quinones have been crystallized from *E. coli*: a vitamin K_2-like napthoquinone and two benzoquinones, one with an absorption maximum at 260 mμ and the other at 270–275 mμ. The three quinones were distinguishable by chromatography and were present in cells grown aerobically. Under anaerobic conditions the concentrations of the various quinones were altered. It is difficult to quantitate the quinones because of losses during fractionation and overlapping spectra; however, it appears that under anaerobic conditions the napthoquinone and the 270 benzoquinone are diminished in concentration, while the other benzoquinone is increased. The heterogeneity of these compounds in this facultative anaerobe may provide information concerning the biological role of the various benzo- and naphthoquinones, and the effects of these compounds on oxidative phosphorylation in *E. coli* are being investigated.

This investigation was supported by a research grant (No. E-2674) from the National Microbiological Institute, National Institutes of Health, U.S. Public Health Service.

The first author is a U.S. Health Service Predoctoral Fellow, the second a Senior U.S. Public Health Service Fellow (SF-290).

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Received February 4th, 1960

Preparation and properties of glutamic dehydrogenase from human placenta

The oxidation of L-glutamic acid by human placenta has been observed[1] but the properties of the placental enzyme were not studied. The key role of this enzyme in intermediary metabolism and the evidence that its rate is under hormonal control[2] led us to compare the properties of the placental and liver enzymes.