TWO SITES OF COENZYME Q IN MITOCHONDRIA OF SACCHAROMYCES CEREVISIAE

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Received December 20, 1968

Coenzyme Q is now recognized as an essential component in the electron transfer chain (1) of mitochondria for many forms of life. At the time of the discovery of the presence and activity of CoQ_{10} in systems from beef heart mitochondria, and for several years afterwards, the equations showing the presence of CoQ in the chain depicted a shared or single site for the functionality of CoQ. In our recent study (2) of the organic structural specificity of CoQ in the succinoxidase and DPNH oxidase systems of beef heart mitochondria, we utilized the entire series from CoQ_1 to CoQ_{10}; it was clearly recognized for the first time that the site of CoQ for succinoxidase is different from the site of CoQ for DPNH oxidase. The evidence for the presence of two sites was based upon differences in the organic structural specificities for coenzyme Q in the two sites. Although there were several organic chemical comparisons showing the difference, perhaps the most significant structural difference from the biological viewpoint is the comparable activity of CoQ_2 through CoQ_{10} for succinoxidase and the diminishing activity of the CoQ group below CoQ_7 - CoQ_{10} for DPNH oxidase. Consequently, we proposed two different sites for CoQ in DPNH-CoQ reductase (complex I) and succinate-CoQ reductase (complex II). This proposal was subsequently supported by similar *in vitro* data on CoQ_{12} (3).

In studying further the structural requirements of the two sites for CoQ, it seemed that we should first explore a possible relationship between the lesser specificity of CoQ for succinoxidase and the known absence of a phosphorylation site in the region of complex II. Since yeast lacks the first phosphorylation site(4), which is associated with complex I, the specificity of the isoprenoid side chain of CoQ in the DPNH oxidase

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system of yeast was appropriate for elucidation. However, in comparing the specificity of the isoprenoid side chain of CoQ for beef heart and yeast systems, it is necessary to take into account that the dominant CoQ in beef heart is CoQ_{10} and that in yeast is CoQ_6 (5). Also, it must be borne in mind that CoQ_{10} has almost absolute dominance in beef heart mitochondria, but in microbiological systems such as those of yeast, the dominance of CoQ_6 is only relative since all of the lower homologs from CoQ_5 down to CoQ_1 are also apparently present, but in diminishing amounts (6).

We have extracted CoQ_6 (7) from lyophilized mitochondria from S. cerevisiae, and assayed the succinoxidase and DPNH oxidase activities in the presence of the homologs, coenzyme Q_1 to coenzyme Q_{10}. The experimental conditions were those described (2).

The need for CoQ in the DPNH oxidase system from lyophilized beef heart mitochondria was demonstrated by Szarkowska (8) when pentane was used for extraction of the CoQ_{10}. Table I shows the DPNH oxidase and succinoxidase activities in yeast mitochondrial systems which have been extracted with pentane, and restored by addition of a CoQ.

Although the level of succinoxidase is comparatively low in the mitochondria of yeast, it is evident in table I that there is no specificity for the isoprenoid side chain of Q since all of the members of the group from CoQ_1 to CoQ_{10} restored activity of the pentane-extracted system to a level comparable with that of CoQ_6 which occurs naturally in this yeast. The sites for CoQ_6 in succinoxidase of yeast and CoQ_{10} for succinoxidase of beef heart mitochondria are similar in that the length of the side chain may vary from two to ten isoprenoid units without changing the electron transfer of the quinone.

The data in table I show that the restoration of DPNH oxidase of extracted yeast mitochondria was more effective for CoQ_2, Q_3, Q_4, and Q_5 than it was for the other members of the group below and above these CoQ's in molecular weight. Although, the greatest restoration of activity was not observed with CoQ_6 which is the dominant CoQ of yeast, it is clear that the lower molecular weight homologs of CoQ are more effective in restoring the activity of DPNH oxidase from yeast; these relative activities probably bear a relationship to the presence to the lower molecular weight homolog, CoQ_6 in yeast. By contrast, the higher homologs from CoQ_7 to CoQ_{10} are the most effective in restoring DPNH oxidase activity of beef heart mitochondria and these relative activities probably bear a relationship to the presence of the higher molecular weight homolog, CoQ_{10}, in beef heart.

Table 1

Restoration of DPNH Oxidase and Succinoxidase Activities by CoQ Homologs in Pentane-Extracted Mitochondria from S. cerevisiae

Homolog	DPNH Oxidase	Succinoxidase
	μatoms O_2/min/mg protein	
---	0.050	0.010
CoQ_1	0.441	0.051
CoQ_2	0.823	0.065
CoQ_3	0.918	0.044
CoQ_4	1.127	0.078
CoQ_5	0.568	0.069
CoQ_6	0.365	0.077
CoQ_7	0.412	0.087
CoQ_8	0.341	0.072
CoQ_9	0.332	0.072
CoQ_{10}	0.401	0.070

Our finding that CoQ_4 rather than the naturally occuring dominant CoQ_6 was more effective in restoring the DPNH oxidase activity of the yeast system may reflect experimental difficulties of handling the relatively insoluble CoQ's and the in vitro systems rather than the real difference in the relative intrinsic activities of CoQ_4 and CoQ_6. This real difference might be clarified by additional experimentation. However, the following relationships are clear: (A) the higher molecular weight homologs of the CoQ group are not the most active for DPNH oxidase of yeast as they are for DPNH oxidase of beef heart mitochondria; (B) there is specificity of the isoprenoid side chain of CoQ for DPNH oxidase of both yeast and beef heart; (C) the lack of activity (3) of CoQ_{12} in the DPNH oxidase system of beef heart reflects that excessive isoprenoid chain length and geometric isomerism are not acceptable, and may correlate with the data of table I showing that isoprenoid chain length greater than that of the dominant CoQ_6 of yeast is less acceptable to the site for DPNH oxidase.

The DPNH oxidase in mitochondria from S. cerevisiae is sensitive to antimicin A, but insensitive to rotenone for CoQ_1, Q_2, and Q_{10}. These responses are in agreement with data of Estabrook, et al. (4), and Ernster, et al. (7).

Table 2

Effect of Inhibitors on the DPNH Oxidase Activity of Pentane-Extracted Yeast Mitochondria

CoQ	No Inhibitor	Antimycin A (6.7 γ/mg protein)	Rotenone (6.7 γ/mg protein)
		μatoms O_2/min/mg protein	
CoQ_1	0.620	0.000	0.569
CoQ_2	1.356	0.036	1.234
CoQ_{10}	0.285	0.018	0.370

The results of this study show that in yeast mitochondria there are two sites for coenzyme Q_6 as there are two sites for coenzyme Q_{10} in beef heart mitochondria. In both cases, the organic structural specificity of the site for CoQ in DPNH oxidase is relatively rigid, and probably reflects redox, structural and steric features not only of the molecule of CoQ itself, but also its intramolecular binding to adjoining macromolecular structures which are the environment of the site. There is no organic structural specificity for the isoprenoid side chain of CoQ for the site for succinoxidase for both yeast and beef heart mitochondria, at least from CoQ_2 to CoQ_{10}.

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