THE INHIBITORY EFFECTS OF QUINONES ON THE SUCCINIC OXIDASE SYSTEM OF THE RESPIRATORY CHAIN

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(Received June 19th, 1961)

SUMMARY

A number of *p*-benzoquinones have been shown to be inhibitors of the succinic oxidase system of heart-muscle preparations. Two representative quinone inhibitors were shown to act non-competitively in the succinic–methylene blue reductase system mediated by ubiquinone homologues or other active quinones. Certain relationships between the structure of a quinone and its inhibitory action were established. A new finding is that the nature of the substituent adjacent to an unsubstituted position influences the reaction of the latter with a sulphydryl group. A probable mechanism is proposed.

INTRODUCTION

Recently it has been shown that certain quinones related to ubiquinone (coenzyme Q), such as 2,3-dimethoxy-5-methyl-*p*-benzoquinone and 2,3-dimethoxy-5-methyl-6-bromo-*p*-benzoquinone, are inhibitors of succinate and DPNH oxidation in mitochondrial preparations[1,2]. It was suggested that these quinones act non-competitively by combining with essential sulphydryl groups of the enzyme systems. In the present study the inhibitory action of a number of benzoquinones has been tested. Certain relationships between structure and inhibitory action have been established. The non-competitive nature of the inhibition has been determined rigorously.

MATERIALS AND METHODS

Quinones: Ubiquinone homologues and analogues were generous gifts from Dr. O. ISLER, Hoffman-La Roche and Co., Ltd., Basel. Most of the other quinones were kindly given by Dr. F. M. DEAN, Organic Chemistry Department, University of Liverpool. The quinones were usually added to reaction mixtures as ethanolic solutions.

Heart-muscle preparations: These were obtained by a method previously described[3].

Enzyme activities: Succinic oxidase was measured at 37° as previously described[4] except in one experiment (see Table II) where it was measured polarographically at 20°. Succinic methylene blue reductase was determined by the method of REDFEARN[5].

RESULTS

Structural criteria for inhibition

A number of *p*-benzoquinones were tested as inhibitors of succinic oxidase activity. The results (Table I) suggest that certain structural requirements are necessary for inhibition to occur. Firstly, all the inhibitory quinones tested possess at least one free position in the nucleus. Secondly, the nature of the substituent adjacent to the free position appeared to determine whether or not the quinone was inhibitory. Thus,

TABLE I

INHIBITION OF SUCCINIC OXIDASE BY *p*-BENZOQUINONES

Quinone	*Inhibitory effect*
Aurantiogliocladin	—
Ubiquinone-(5)	—
2,5-Dimethoxy-6-methylbenzoquinone	—
3,5-Dimethoxy-6-methylbenzoquinone	—
2,3-Dimethoxy-5-methylbenzoquinone	+
2,3,5,6-Tetramethylbenzoquinone (duroquinone)	—
2,3,5-Trimethylbenzoquinone (cumoquinone)	+
2,6-Dimethylbenzoquinone (*m*-xyloquinone)	+
2,5-Dimethylbenzoquinone (*p*-xyloquinone)	+
2-Methylbenzoquinone (toluquinone)	+
2,5-Dimethoxybenzoquinone	—
2-Methoxy-6-methylbenzoquinone	+
2-Methoxy-6-propylbenzoquinone	+
2-Methoxy-5-acetylbenzoquinone	+
2-Methoxy-5-acetyl-6-ethylbenzoquinone	—

hydrogen, methyl, propyl or acetyl groups permitted inhibition, whereas a methoxyl group destroyed the inhibitory effect. This effect is illustrated clearly with the isomers of dimethoxy-methyl-*p*-benzoquinone. Of the three isomers, only 2,3-dimethoxy-5-methylbenzoquinone is inhibitory. This is because the unsubstituted position is adjacent to a methyl group rather than a methoxyl as in the other two isomers.

Relation between structure and degree of inhibition

The methyl substituted *p*-benzoquinones were taken and the lowest concentration required to give complete inhibition was found by extrapolation of the activity-inhibitor concentration curve (Table II). It will be seen that the potency of the inhibitor can be correlated with the number of free positions in the nucleus.

The type of inhibition

In view of the functional participation of ubiquinone in the respiratory chain it is of considerable interest to determine whether the inhibitory quinones are acting competitively or non-competitively. This was done using the methylene blue reductase system mediated by an added ubiquinone homologue which is assumed to react similarly to the endogenous ubiquinone[5]. All the quinones which inhibited succinic oxidase also inhibited this system. The ubiquinone homologue or other quinone mediator was used at two different concentrations and the activity of the enzyme system

TABLE II

MINIMAL CONCENTRATIONS OF METHYL-SUBSTITUTED *p*-BENZOQUINONES FOR COMPLETE INHIBITION OF SUCCINIC OXIDASE ACTIVITY

Succinic oxidase activity was determined polarographically at 20° with the following reaction mixture: KH_2PO_4–Na_2HPO_4 buffer, pH 7.4, $7.7 \cdot 10^{-2}$ *M*; KCl, $7.7 \cdot 10^{-3}$ *M*; cytochrome *c*, $3.8 \cdot 10^{-5}$ *M*; sodium succinate, 10^{-2} *M*; enzyme protein, 7.3 mg. The total reaction volume was 3.25 ml. The quinones were added in a small volume (0.01–0.02 ml) of ethanol and allowed to incubate for 2 min at 20° before adding the succinate to start the reaction.

p-benzoquinone derivative	*Least concentration for complete inhibition of succinic oxidase activity*
2-Methyl-	$0.6 \cdot 10^{-4}$ *M*
2,5-Dimethyl-	$3.2 \cdot 10^{-4}$ *M*
2,3,5-Trimethyl-	$7.1 \cdot 10^{-4}$ *M*

measured at a series of concentrations of the quinone inhibitor. In the first experiment aurantiogliocladin was used as the quinone carrier and 2-methoxy-6-propyl-*p*-benzoquinone as the inhibitor. In the second experiment ubiquinone-(5) was the carrier with the same inhibitor, while in the third, 2-methyl-1,4-naphthoquinone (menadione) was the carrier with 2-methyl-*p*-benzoquinone (toluquinone) as the inhibitor. The results when plotted as the reciprocal of the reaction velocity against the concentration of the inhibitor gave graphs characteristic of non-competitive inhibition[6]. The results of the first experiment are shown in Fig. 1.

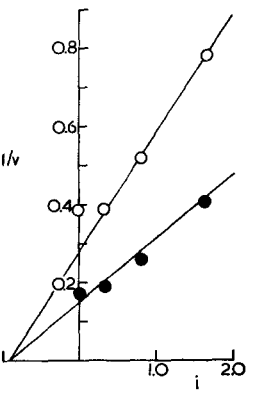

Fig. 1. Inhibition of aurantiogliocladin-mediated methylene blue reductase by 2-methyl-6-propyl-*p*-benzoquinone. *v*, reaction velocity (μl O_2/min/mg protein); *i*, final concentration of 2-methyl-6-propyl-*p*-benzoquinone (10^{-4} *M*). ●—●, $7.5 \cdot 10^{-4}$ *M* aurantiogliocladin; O—O, $3 \cdot 10^{-5}$ *M* aurantiogliocladin (final concentrations).

Reaction of inhibitory quinones with cysteine and 2,3-dimercaptopropanol

SMITH AND LESTER[2] using a spectrophotometric technique showed that 2,3-dimethoxy-5-methyl-6-bromo-*p*-benzoquinone and 2,3-dimethoxy-5-methyl-*p*-benzoquinone reacted with cysteine while aurantiogliocladin and ubiquinone-(50) did not. The same method was used in the present investigation to study the effect of the substituent adjacent to an unsubstituted position on the reactivity with sulphydryl compounds. The results of experiments using cysteine are summarized in Table III.

Similar results were obtained with 2,3-dimercaptopropanol. It will be seen that a methoxyl group adjacent to an unsubstituted position appears to render that position unreactive towards sulphydryl compounds.

TABLE III

REACTION OF BENZOQUINONES WITH CYSTEINE

Quinones were dissolved in 0.1 M KH_2PO_4–Na_2HPO_4 buffer, pH 7.4, to give a final concentration of about $6 \cdot 10^{-5}$ M. Spectra were determined in a recording spectrophotometer (Unicam S.P. 700) An aqueous solution of cysteine (0.03 ml) was added to the quinone solution (2.8 ml) to give a final concentration of $2 \cdot 10^{-4}$ M. The data given are those obtained 15 min after the addition.

p-benzoquinone derivative	*Inhibitory action*	*Main absorption maximum (mμ)*		*Extinction of new absorption maximum as percentage of initial value*
		Before addition of cysteine	*After addition of cysteine*	
2,5-Dimethoxy-	—	285	285	100
2-Methoxy-5-methyl-	+	270	323	45
2,5-Dimethyl-	+	258	307	19
3,5-Dimethoxy-6-methyl-	—	284	284	73
2,3-Dimethoxy-5-methyl-	+	270	310	28

DISCUSSION

It was shown by BERGSTERMANN AND STEIN[7] that *p*-benzoquinone inhibited succinic dehydrogenase and that the probable mechanism was a combination of the quinone with essential sulphydryl groups of the enzyme system by addition reactions similar to those described in organic chemistry[8,9]. Recently it has been shown that certain quinones related to ubiquinone inhibit the enzyme systems of the respiratory chain and that the inhibition is probably exerted by addition reactions with sulphydryl groups[1,2]. The results of the present paper have confirmed and extended these findings.

It has been shown that one criterion for inhibition is that the quinone should have at least one unsubstituted position which enables it to react with a sulphydryl group and that the degree of inhibition can be correlated with the number of unsubstituted

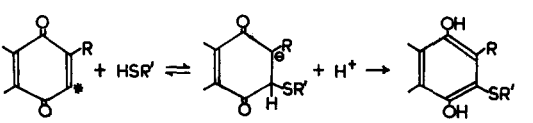

Fig. 2. Reaction of a trisubstituted benzoquinone with a sulphydryl compound.

positions. Furthermore, the nature of the substituent adjacent to an unsubstituted position is important. The reaction of a quinone containing one unsubstituted position with a sulphydryl compound is depicted in Fig. 2. The important determining feature is the electron-deficient site (marked with an asterisk). When the adjacent substituent, R, is a strong electron-attracting group such as acetyl or a weak electron-supplying group such as methyl or propyl, the reaction with a sulphydryl group is able to proceed as shown. However, when R is a strong electron-supplying group such as methoxyl, the reaction is prevented.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. F. M. DEAN for helpful discussions and to Miss J. D. KERR for technical assistance.

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Biochim. Biophys. Acta, 56 (1962) 440–444

THE PURIFICATION AND PROPERTIES OF ALDEHYDE OXIDASE

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(Received April 29th, 1961)

SUMMARY

1. Highly purified aldehyde oxidase has been prepared from pig liver by reverse ammonium sulphate fractionation, heat treatment and chromatography on DEAE-cellulose and hydroxyl-apatite.

2. Although pure by electrophoresis the enzyme shows several minor components in the ultracentrifuge. The flavin content is 5 mμmoles/mg, and the molybdenum content 1–2 mμmoles/mg. It was not possible to remove the metal from the enzyme by dialysis under several conditions.

3. The presence of large amounts of haem in the preparation can be accounted for by the catalase activity of the preparation.

4. The influence of pH and of chain length of substrate on the activity of the enzyme has been investigated and the protection of the enzyme by ammonium ion noted.

INTRODUCTION

Aldehyde oxidase was first isolated from pig liver in 1940 when it was tentatively identified as a flavoprotein[1]. Since that time the enzyme has also been obtained from

Abbreviations: BSA, bovine serum albumin; DCIP, dichlorophenol-indophenol; TCA, trichloroacetic acid; PMS, phenazine methosulfate.

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