短 報

THE RELATIONSHIP BETWEEN
CHEMICAL STRUCTURES OF
CHLOROPHENOLS AND THEIR
BIOLOGICAL ACTIVITIES

クロロフェノールの構造と生物学的活性

To know the relationship between chemical structures and biological activities, the effect of several chlorophenols on the oxidative phosphorylation of rat-liver mitochondria has been investigated.

MATERIALS AND METHODS

All reagents were of special grade obtained from commercial sources and dissolved in buffer solution at use.

The effect of chlorophenols on the oxidative phosphorylation of rat-liver mitochondria was studied by means of the Warburg manometric method. ATPase activity of intact mitochondria was estimated by determining the net release of phosphate from ATP. Other experimental details were the same as previously reported¹⁾.

Dissociation constants for chlorophenols were measured either by comparison of absorption spectra in solutions of different pH values²⁾ or by potentiometric titration³⁾.

RESULTS

Satisfactorily high P/O ratio (2.65 ± 0.15 , $N=13$) was obtained in control experiments. All compounds inhibited inorganic phosphate uptake of mitochondria with little or no effect on oxygen uptake. The effects of a series of chlorophenols on oxidative phosphorylation are represented in Fig. 1. It is apparent from these data that there was an

Fig. 1. Inhibitory effect of chlorophenols on oxidative phosphorylation.

increase in the inhibitory effect with progressive chlorination, although the difference in activity was not a little among the compounds with the same number of substituted chlorine atoms.

Each chlorophenol stimulated the latent ATPase of intact mitochondria, with increase of the chlorine substitution, there was also an increase in activation of ATPase. The effect of each compound was compared with the maximal result obtained simultaneously with $5 \times 10^{-5} M$ of 2,4-dinitrophenol and expressed as a percentage⁴⁾.

The data listed in the table show that with different phenols the value of the dissociation constant ranged from 5.02 to 7.83. As the number of chlorine substituents goes up, there was a progressive fall in pK value. The valid factor which determined pK seemed to be the positions of chlorine substituents in the ring.

Equi-effective concentrations, causing 50% inhibition for oxidative phosphorylation and 20% activation for ATPase activity, were summarized against pK in Fig. 2. Gross inspection suggests that the compounds appear to fall into two classes, with or without substituents at 2,6-positions.

Table. Dissociation constants of chlorophenols.

Compound	pK value
2,4-Dichlorophenol	7.83
2,5-Dichlorophenol	7.45
2,6-Dichlorophenol	6.83
2,4,5-Trichlorophenol	7.01
2,4,6-Trichlorophenol	6.10
2,3,4,6-Tetrachlorophenol	5.30
2,3,4,5,6-Pentachlorophenol	5.02

Fig. 2. Relationship between pK and equi-effective concentration. ●; Oxidative phosphorylation, ○; ATPase activity.

DISCUSSION

It has been known that there is a little difference in toxic effect between higher and lower chlorophenols. The general effect of increasing the chlorination of phenols is a reduction of the convulsant action but an increase in the inhibition of oxidative phosphorylation⁵⁾. Consequently, di-, tri-isomers and tetra-, pentachlorophenol were chosen as materials.

All compounds were proved to be potent uncouplers for oxidative phosphorylation by the observations that they dissociated the respiration of mitochondria from inorganic phosphate uptake and activated the latent ATPase of freshly prepared mitochondria. As the number of chlorine atoms substituted in the ring is increased, the impairment of oxidative phosphorylation progressively augmented. Because the chlorophenols with 2,5-substitutions were more effective than any other isomers, the enhancement of activity may result from the chlorine atoms at 2,5-positions.

Dissociation constants, determined by a spectrophotometric technique and by a potentiometric titration procedure, did not differ greatly from values reported previously^{2,3)}. An inference can be made from these observations that the impurities in each compound should be of no importance, even if any. The addition of a chlorine seemed to decrease the dissociation constant in general, but the definite pK was dependent on the position of substituent.

Chlorophenols were apparently grouped, according to their equi-effective concentrations and pK values, into two classes. The first group is consisted of 2,4-, 2,5-dichlorophenol and 2,4,5-trichlorophenol, which have higher activities with reference to their pK values. The members of the other

group are 2,6-, 2,4,6-, 2,3,4,6- and pentachlorophenol, which are substituted in *di-ortho* position in common. Within each group, there was an inverse linear relationship between equi-effective concentrations and pK values. The chlorophenols with 2,6-substitutions, which Blackman²⁾ found to deviate from the linear relationship, can be plotted on a line separately.

It seems probable, as Farquharson⁶⁾ stated, that the anion produced by ionization of 2,6- positions can not react with some intermediates of phosphorylation mechanisms by virtue of the steric or electronic interference by the *ortho* chlorine atoms.

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