Inactivation of Some Coliphages with Copper-Thiol Complexes

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When copper salts are mixed with substances containing thiol groups, complexes are formed whose composition is determined by the concentration of the two components. After mixing copper and thiol compounds in certain concentrations the mixtures became strongly virucidal for T3, T5 and φX174 phages, while T2 and T4 were affected only at higher concentrations of copper. Unless otherwise stated, coliphage T5 was used as test organism in these experiments.

Together with a final concentration of 5 × 10^{-5} M-CuCl₂ the thiol compounds cysteine and dithio-pentaerythritol at certain concentrations had a strong virucidal activity, while no such activity was demonstrated for the corresponding disulphide compounds (Fig. 1). Dithio-pentaerythritol but not cysteine was active with a CuCl₂ concentration of 5 × 10^{-6} M. At this copper concentration thiglycollic acid, 2,3-dimercaptopropanol and dihydrothioctic acid were also virucidal, while glutathione was active only with 5 × 10^{-5} M-CuCl₂, and the disulphide compound thiotic acid (α-lipoic acid) was inactive even at the higher concentration of copper. No activity was demonstrated after mixing with this copper concentration for any of a great number of non-thiol amino acids tested at concentrations 5 × 10^{-5} to 5 × 10^{-4} M. The thiol compounds alone were not toxic at the concentrations tested. As shown for cysteine and dithio-pentaerythritol (Fig. 1), the effect of all copper + thiol mixtures tried was reduced at low and high concentrations of the thiol compound. Higher concentrations of thiol compounds were required in the presence of tris buffer than in distilled water. Low concentrations of organic substances (Difco nutrient broth, 1 g./l.) extinguished the effect.

When CuCl was mixed with cysteine, a reaction similar to that of CuCl₂ occurred. Mg²⁺, Ca²⁺, Zn²⁺, Cd²⁺, Mn²⁺, Co²⁺ and Ni²⁺ had no effect, either alone nor when mixed with 5 × 10^{-5} M-cysteine. The toxic effect of Ag⁺ and Hg²⁺ alone was reduced in the presence of cysteine. The virucidal effects of Pb²⁺, Al³⁺ and Cr²⁺ alone were weak, moderate and very strong, respectively, but were slightly enhanced in the presence of cysteine. The effect of Fe²⁺ was increased or decreased depending on the concentration.

Dialysis against distilled water for 3 days did not reverse the inactivation of T5 by the copper-thiol complexes, although it abolished the inactivating effect of copper + thiol mixtures. Some morphological changes were observed after the inactivation: the bunch of fibres at the tip of the tail (Pl. 1, fig. 1) was replaced by a more cone-shaped structure (Pl. 1, fig. 2). The length of the tail to the tip of the ‘cone’ of this inactivated phage seemed to be the same as the length of the tail to the tips of the tail fibres of a virulent phage. This implies that tail fibres sticking to each other could account for the formation of the ‘cone’ in Plate, fig. 2. Other inactivated phages had a blunter tip to the tail. The length of the tail to the tip was shorter in these phages. Aggregates were observed in which the distal parts of the tails adhered to each other (Pl. 1, fig. 3), similar in arrangement to but less regular than the rosettes described for the T-even phages (1). This phenomenon could be a consequence of adhesion of tail.
fibres from different phages. All phages observed in preparations from suspensions with more than 99% inactivation appeared either in aggregates with their tails inwards or as single phages with the tips of the tails cone-shaped. The adsorption of the inactivated phages was impaired.

![Graph](image)

**Fig. 1.** Inactivation of T 5. One ml. of the sulphur compound (final conc. given in fig.) was mixed with one ml. of $2 \times 10^{-4}$M (filled symbols) or $2 \times 10^{-5}$M (open symbols) CuCl$_2$. After 1 hr at room temperature 2 ml. of T 5 in 0.005 M-tris buffer (pH = 7.0) was added, and assayed after a further hr. ■, □ = cysteine; ▲ = cystine; ●, ○ = dithio-pentaerythrite; ♦ = 4,4-bis-hydroxymethyl-dithiolane.

In some respects this system seems similar to the inactivating cyanide complexes of Zn$^{2+}$ described for the T-even phages (2, 3). Zinc in triosephosphate dehydrogenase is present in the bacterial cell membrane (4). Similarly copper is a constituent of the cytochrome c-oxidase system which is located in the same cell structure (5). At least part of this copper is supposed to be bound to sulphur (6). The cytochrome c-oxidase system functions in the electron transport chain as the final link to oxygen. This indicates a superficial location, which would be convenient if its copper complexes were essential in processes which lead to injection of bacteriophage nucleic acid into bacteria.
Short communications

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REFERENCES


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EXPLANATION OF PLATE

T5 phages negatively stained with uranyl acetate.

Fig. 1. The tails of two phages from the untreated suspension.

Fig. 2. A phage from a suspension > 99% inactivated with a mixture of $5 \times 10^{-4} \text{M-CuCl}_2$ and an equal concentration of dithio-pentaerythritol.

Fig. 3. Four phages from the same preparation as fig. 2 aggregated at the tips of their tails.