Decomposition of Chloro-organic Acids by Fungi

During a study of bacterial decomposition of various chloro-substituted organic acids[1] it was observed that certain strains of *Trichoderma viride* produced small amounts of chloride in synthetic medium with sodium monochloroacetate as sole organic constituent. Extra addition of yeast or soil extract had little effect; but later experiments showed that the fungi became very active when glucose was supplied as an additional carbon source. Ten strains of *T. viride* were tested in liquid medium containing 0·04 *M* sodium monochloroacetate, 0·2 per cent glucose, 0·05 per cent ammonium sulphate and dipotassium phosphate, 0·02 per cent magnesium sulphate and calcium sulphate, and 0·005 per cent ferrous sulphate. Cultures were incubated at 25° C., and chloride was determined by titration with 0·01 *N* silver nitrate, in the presence of potassium chromate as an indicator. The strains proved to comprise two groups, each of five strains. One of these groups (I) was strongly and the other (II) weakly active towards monochloroacetate. In addition, dichloroacetate was decomposed with moderate vigour, but α-monochloropropionate only feebly (Table 1).

Table 1

Substrate and concentration	Incubation (days)	Per cent ionized Cl: Group I	Group II
Monochloroacetate, 0·04 *M*	7	8–53	0–3
	14	76–100	4–7
Dichloroacetate, 0·02 *M*	7	1–17	0–2
	14	7–28	5–7
Monochloropropionate, 0·04 *M*	7	0–9	0–2
	14	2–17	4–5

The fungi of Group I displayed a similar activity when glucose was replaced by cellulose or xylan.

Sixty-two strains of other fungi, partly collection cultures and partly random soil isolates, were tested against monochloroacetate. The majority (46 strains) had no effect, while ten strains produced chloride in amounts comparable to *T. viride*, Group II. Four strains of *Penicillium roqueforti* (collection cultures) and two of *Clonostachys* sp. (soil isolates) showed an activity approaching that of *T. viride*, Group I. *Clonostachys* sp. also attacked dichloroacetate and α-monochloropropionate, while *P. roqueforti* had very little effect upon these compounds (Table 2).

Table 2

Substrate and concentration	Incubation (days)	Per cent ionized Cl: *P. roqueforti*	*Clonostachys* sp.
Monochloroacetate, 0·04 *M*	7	0–33	5–7
	14	45–76	30–37
Dichloroacetate, 0·02 *M*	7	(0)	3–5
	14	< 1–4	16–18
Monochloropropionate, 0·04 *M*	7	(0)	3–4
	14	0–2	13–14

None of the fungi produced chloride from trichloroacetate, α-dichloropropionate, or 2,4-dichlorophenoxyacetate.

Tests with mycelium of *T. viride* grown in submerged culture indicated that formation of a chloride-producing enzyme was induced when mono- or di-chloroacetate was added to the medium. The same enzyme appeared to be responsible for the decomposition of both compounds.

Experiments on the properties of the enzyme and the behaviour of the fungi in soil are being continued, and the results will be published in detail elsewhere.

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[1] Jensen, H. L., *Canad. J. Microbiol.*, **3**, 151 (1957).

Photosensitivity of Hæm Compounds

Haldane and Lorrain Smith[1] discovered that carboxyhæmoglobin is sensitive to light, and several workers have shown that this property is shared by other ferrohæm–carbon monoxide compounds. Until Keilin and Hartree[2] found that the cyanide compounds of ferroperoxidase and myoglobin were light-sensitive, it was generally believed that carbon monoxide compounds were unique in this respect. We have now found that oxyhæmoglobin, nitric oxide hæmoglobin and ethyl isocyanide hæmoglobin are light-sensitive, thus showing that all the known ligands able to combine with ferrohæmoglobin are qualitatively similar.

The compounds were examined by the flash photolysis method[3] the layout of the apparatus following the description of Gibson[4] except that 300*J* were dissipated in each of two borosilicate glass flash tubes and that a Hilger monochromator type *D* 246 was used instead of interference filters. Taking the quantum yield for carboxymyoglobin as 1, the following approximate yields were obtained: oxymyoglobin 0·03, oxyhæmoglobin 0·008, nitric oxide hæmoglobin and nitric oxide myoglobin 0·001, ethyl isocyanide hæmoglobin 0·05. These observations were made at 430 mμ for the hæmoglobin compounds and at 435 mμ for the myoglobin compounds, using a 3-mm. cuvette with 3×10^{-5} *M* hæm pigment, phosphate buffer 0·1 *M*, *p*H 7·1, 1° C.

It should perhaps be pointed out that the photosensitivity of these compounds cannot easily be demonstrated using ordinary light sources. The discharge at present used lasts about 20 μsec., corresponding to an instantaneous rate of work of 3×10^5 kW. In the most favourable circumstances, with a half-time for recombination of oxymyoglobin of 5 msec., a continuous source of about $1{\cdot}2 \times 10^3$ kW. would be needed to maintain half-dissociation if applied in the present apparatus.

For the case of whale oxymyoglobin the absorption spectrum of the product of the photochemical reaction has been determined from the absorption spectrum of the initial solution by measuring the change in optical density produced by the flash at a series of wave-lengths, and subtracting these changes from the initial values. The spectrum so obtained (Fig. 1) corresponded in form to that of reduced hæmoglobin. The recombination reaction has been found to be accurately second-order with rate constant 5×10^6 M^{-1} sec.$^{-1}$ at 1° C. and 12×10^6 at 22° C.

So far we have not been able to cause any ferrihæm compound to dissociate measurably, which, under our conditions, means that the quantum yield must be less than 0·0001. We suggest, therefore, that photosensitivity be regarded, for the present, as a general property of ferrohæm compounds. If further work shows our findings to apply to the hæm enzymes,