Glucose permeability of liposome vesicles prepared with sterol extracts from fenpropimorph-grown fungi

CHRISTOPHER C. STEEL and E. IAN MERCER

Department of Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD, U.K.

Fenpropimorph [N-[3-[(p-tert-butylphenyl)-2-methylpropyl]-cin, 2,6-dimethylhydropyridine] is a fungicide whose primary fungitoxic action appears to be the blocking of fungal sterol biosynthesis; it inhibits both the sterol A\textsubscript{4}-reductase and the sterol A\textsubscript{21}→A\textsubscript{22}-isomerase (Baloch \textit{et al.}, 1984; Baloch & Mercer, 1987). It has been generally assumed, though never proved, that the abnormal sterols that accumulate in fenpropimorph-treated fungi are incorporated into the fungal membranes causing them to behave abnormally. In this paper we provide evidence in favour of this hypothesis by showing (i) that the abnormal sterols are incorporated into the microsomal membranes of \textit{Saccharomyces cerevisiae} and (ii) that liposomal membranes containing the abnormal sterols are more permeable than those containing normal fungal sterols.

\textit{Saccharomyces cerevisiae} (NCYC 739) was grown "semi-aerobically" in tryptone yeast extract at 30°C for 72 h and then harvested, washed and aerobically adapted in a medium (0.1 m-phosphate buffer, pH 6.2 containing 10%, w/v, glucose) containing no fenpropimorph (control) or 125 µg fenpropimorph (treated) at 30°C for 18 h. The resulting cells were homogenized and the microsomal and cytosolic frac-
tions were isolated by differential centrifugation and analysed for their sterol content. No sterols were detected in either cytosolic fraction. Normal sterols were found in the control microsomal fraction while abnormal sterols, principally 24-methylene ignosterol, were found in the treated microsomal fraction (24-methylene ignosterol/ergosterol = 1.42:1).

Liposome vesicles containing entrapped glucose were prepared by the method of Demel et al. (1972), using sterols extracted from (i) the treated and control S. cerevisiae microsomal fractions described above and (ii) Botrytis cinereae, Pyrenophora teres and Pseudocercosporella herpotrichoides cultured normally or in the presence of sufficient fenpropimorph to inhibit their growth by 50%, along with phosphatidylcholine (Sigma type XI-E) and phosphatidic acid in the ratio 5:20:1 (by wt.). After removal of the untrapped glucose by dialysis against two successive volumes of a solution containing 0.075 M-KCl and 0.075 M-NaCl, the permeability of the liposome membranes was assessed at intervals over a period of 4 h by measuring the leakage of glucose from them using the method of Demel et al. (1972). Leakage was expressed as the percentage of the glucose present in the liposomes at time zero that is lost during time t, which was calculated from the expression 100 − 100(%G₀/₁₀₀%Gₜ) where %G₀ and %Gₜ are respectively the percentages of the total glucose in the preparation that is released by Triton from the liposomes at the start of the 4 h of incubation and after a given period of time T. The results shown in Fig. 1 indicate that liposomes prepared with sterol extracts from fenpropimorph-treated fungi are significantly more permeable to glucose than those prepared with sterol extracts from the corresponding untreated fungi.

Thus it appears clear that the abnormal sterols that accumulate in fungi when they are treated with fenpropimorph are incorporated into their plasma membranes and endoplasmic reticulum, which are isolated together as the microsomal fraction. This agrees with Benveniste et al. (1984) and Hartmann et al. (1985), who showed that the abnormal 9β,19-cyclopropyl sterols accumulated by maize seedlings after treatment with morpholine fungicides were incorporated into the microsomal membranes of the root cells. Moreover, the fact that liposomes prepared with the abnormal fungal sterols are abnormally permeable to glucose suggests that these same sterols will also cause the natural fungal membranes to be abnormally permeable to glucose and by implication to other small polar molecules and ions. It is likely that these alterations in membrane permeability lead to a loss of fungal viability and are thus at least part of the reason why fenpropimorph is fungitoxic.

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