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Study of the Metabolism of Flucytosine in *Aspergillus* Species by ^{19}F Nuclear Magnetic Resonance Spectroscopy

N. CHOUINI-LALANNE,[1] M. C. MALET-MARTINO,[1] R. MARTINO,[1]* AND G. MICHEL[2]

Biomedical NMR Group, Laboratory of IMRCP,[1] and Laboratory of Industrial Microbiology,[2] Université Paul Sabatier, 118, route de Narbonne, 31062 Toulouse Cédex, France

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The metabolism of flucytosine (5FC) in two *Aspergillus* species (*Aspergillus fumigatus* and *A. niger*) was investigated by ^{19}F nuclear magnetic resonance spectroscopy. In intact mycelia, 5FC was found to be deaminated to 5-fluorouracil and then transformed into fluoronucleotides; the catabolite α-fluoro-β-alanine was also detected in *A. fumigatus*. Neither 5-fluoroorotic acid nor 5-fluoro-2′-deoxyuridine-5′-monophosphate was detected in perchloric acid extracts after any incubation with 5FC. 5FC, 5-fluorouracil, and the classical fluoronucleotides 5-fluorouridine-5′-mono-, di-, and triphosphates were identified in the acid-soluble pool. Two hydrolysis products of 5-fluorouracil incorporated into RNA, 5-fluorouridine-2′-monophosphate and 5-fluorouridine-3′-monophosphate, were found in the acid-insoluble pool. No significant differences in the metabolic transformation of 5FC were noted in the two species of *Aspergillus*. The main pathway of 5FC metabolism in the two species of *Aspergillus* studied is thus the biotransformation into ribofluoronucleotides and the subsequent incorporation of 5-fluorouridine-5′-triphosphate into RNA.

Flucytosine (5FC) is an antimycotic agent used in the treatment of many fungal infections (11). Although the metabolic fate of 5FC has been extensively investigated in yeasts (15 and references cited therein), much less is known of it in filamentous fungi of the *Aspergillus* type (2, 8, 10, 13, 14). Among the studies done, one in particular has held our attention as it proposed an unexpected pathway for 5FC metabolism, with 5-fluoroorotic acid (5FO) being the principal metabolite (14). We thought that fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectroscopy, already used to study the metabolic transformation of 5FC in *Candida* yeasts (3, 12), could lead to better knowledge of the metabolism of this antifungal agent and confirm or invalidate the results obtained by the authors cited above. Indeed, this technique allows the simultaneous detection of all fluorinated metabolites in a sample and does not require a labeled drug, as the observed probe, the ^{19}F nucleus, is an inherent part of the parent drug and its metabolites. We therefore carried out an investigation of 5FC metabolism in two species of *Aspergillus* (*Aspergillus fumigatus* and *A. niger*) by ^{19}F NMR.

MATERIALS AND METHODS

Chemicals. 5FO, 5FC, 5-fluorouracil (5FU), 5-fluorouridine, 5-fluoro-2′-deoxyuridine, OMP, UMP, dUMP, OMP decarboxylase (from bakers' yeast), and alkaline phosphatase (from calf intestine) were supplied by Sigma Chemical Co., St. Louis, Mo. 5-Fluorouridine-5′-mono-, di-, and triphosphates (FUMP, FUDP, and FUTP) and 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) and 5-fluoro-2′-deoxyuridine-5′-diphosphate were purchased from Sierra Bioresearch, Tucson, Ariz. α-Fluoro-β-alanine was from Koch-Light Laboratories, Colnbrook, United Kingdom.

Organisms, culture conditions, and PCA extract preparation. *A. fumigatus* 437 (5FC MIC, 3 μg ml^{-1}) was kindly provided by A. Polak from Hoffmann-La Roche Laboratories, Basel, Switzerland. The *A. niger* strain (5FC MIC, 1 μg ml^{-1}) was a recent clinical isolate. The MICs of 5FC were determined by the agar dilution method after 3 days of incubation at 37°C (9). The strains were maintained on Sabouraud agar medium (Bio-Mérieux, Craponne, France) at 22 to 25°C. For the present study, spores were suspended in 20 ml of Czapek-Dox medium (Difco Laboratories, Detroit, Mich.) and incubated for 48 h at 30 ± 1°C under moderate shaking. The mycelium was then macerated to obtain hyphal fragments. For identical absorbance between experiments, the suspended hyphal fragments were standardized with a spectrophotometer at 640 nm. The hyphal suspension was then added to 150 ml of Czapek-Dox medium and incubated for 48 h under the same conditions. The medium was then centrifuged (20 min, 1,500 × *g*, 20°C), and the pellet was suspended in 150 ml of Czapek-Dox medium and incubated for a further 1 h. 5FC (50 μg ml^{-1}) was added to the culture, which was incubated under shaking for 1.5 or 3.5 h for the intact mycelia studies and 1.5, 3.5, or 14 h for studies on the perchloric acid (PCA) extracts. All of these experiments were done in triplicate. After centrifugation, the cells were washed three times with a 0.9% solution of NaCl to eliminate 5FC and extracellular metabolites. The pellet was then either placed in an NMR tube for immediate analysis or extracted with PCA. The pellet was extracted in an equal volume of ice-cold 7% PCA and homogenized (Polytron homogenizer; Bioblock, Illkirch, France) for 30 s on ice. After standing for 30 s, the mixture was homogenized once more and then was left standing for 30 min at 0°C. After centrifugation (15 min, 5,000 × *g*, 4°C), the supernatant was collected. The pellet was washed twice with PCA, the supernatants were pooled, and the pH was adjusted to the required value with 5 M KOH. After centrifugation to remove $KClO_4$, the acid-soluble fraction was lyophilized and suspended in 3 ml of distilled water for the NMR study. The acid-insoluble fraction was obtained by heating the residual pellet for 20 min at 70°C in an equal volume of 7% PCA. After centrifugation, the supernatant was collected and the

* Corresponding author.

pH was adjusted. After lyophilization, the acid-insoluble fraction was suspended in 3 ml of distilled water.

Incubation with OMP decarboxylase. The acid-soluble fraction of a PCA extract of *A. fumigatus*, incubated for 1.5 h with 5FC, was brought to pH 8. After addition of 1 U of enzyme diluted in 10 mM phosphate buffer (pH 8) containing 5 mM 2-mercaptoethanol, the mixture was incubated for 1 h at 37°C. The pH was adjusted to 1.5 for NMR analysis. A control sample with no enzyme was prepared under identical conditions.

It has been verified preliminarily that the enzyme OMP decarboxylase transformed OMP into UMP in a blank acid-soluble fraction of a PCA extract of *A. fumigatus*, under the conditions described above, by measuring the decrease in A_{286} (16).

Treatment with $NaIO_4$ and methylamine. A 100-μl portion of 0.5 M $NaIO_4$ prepared daily, followed a few minutes later by 125 μl of a 4 M solution of methylamine which had been slowly brought to pH 7.5 with H_3PO_4, was added to a neutralized PCA-soluble or -insoluble extract. After being mixed, the sample was incubated for 40 min at 37°C. A 25-μl amount of a 1 M solution of rhamnose was then added to destroy the remaining IO_4^-. The sample was then acidified, and the ^{19}F NMR spectrum was recorded.

Incubation with alkaline phosphatase. A PCA-insoluble extract was adjusted to pH 9 by addition of Tris buffer (150 mM; pH 9.2) containing 0.2 mM magnesium acetate. After addition of 2 U of alkaline phosphatase, the sample was incubated at 30°C for 3 h and then acidified. A control sample with no enzyme was treated under identical conditions. After recording of its ^{19}F NMR spectrum, the enzymatic sample was treated with $NaIO_4$ and methylamine as described above.

^{19}F NMR analysis. Proton-decoupled ^{19}F NMR spectra were recorded at 282.4 MHz on a Bruker WB-AM 300 spectrometer, using 10-mm-diameter NMR tubes. The magnetic field was shimmed by using the ^1H NMR resonance of water. The chemical shifts (δ) were relative to the resonance peak of the external reference CF_3COOH (5%, wt/vol, aqueous solution). Spectra were recorded under the following conditions: probe temperature, 4°C for intact mycelia, 25°C for PCA extracts; sweep width, 29,411 Hz; 32,768 data points zero-filled to 65,536; pulse width, 7 μs (i.e., flip angle ≈ 40°); repetition time, 0.5 s for intact mycelia, 3 s for PCA extracts; number of scans, 8,000 for intact mycelia, 15,000 for PCA extracts; line broadening caused by exponential multiplication, 15 Hz for intact mycelia, 0 to 3 Hz for PCA extracts.

Peaks were assigned by adding standard fluorinated compounds to the PCA extracts.

TLC. Acid-soluble fractions of PCA extracts from *A. niger* and *A. fumigatus* after, respectively, 1.5 and 3.5 h of incubation with 5FC were obtained as described above. After neutralization and lyophilization, they were suspended in 0.3 ml of distilled water and chromatographed on thin-layer chromatography (TLC) plates (aluminium-backed silica gel 60 F_{254}, 20 by 20 cm; E. Merck AG, Darmstadt, Federal Republic of Germany). Two solvent systems were used: system 1, methanol; system 2, ethyl acetate-methanol-concentrated ammonium hydroxide, 75:25:1. Standard 5FC, 5FU, 5FO, FUMP, FdUMP, UMP, dUMP, FUDP, 5-fluoro-2′-deoxyuridine-5′-diphosphate, and FUTP were cochromatographed with the acid-soluble fractions analyzed. After development, the chromatograms were examined at 254 nm.

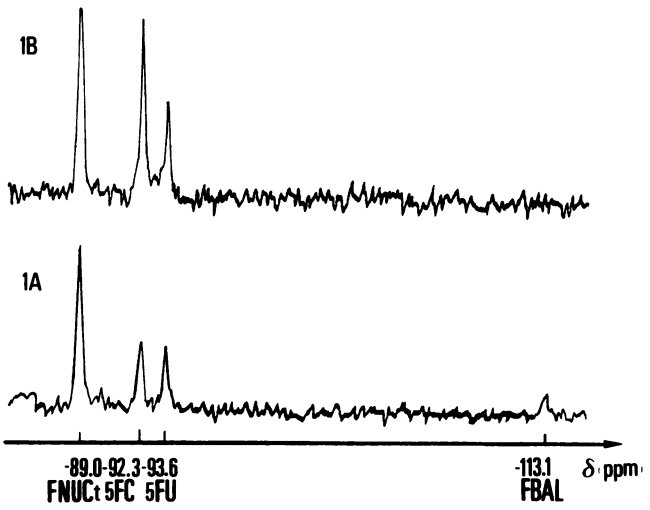

FIG. 1. Typical ^{19}F NMR spectra of intact mycelia of *A. fumigatus* (A) and *A. niger* (B) incubated for 3.5 h with 5FC. Spectra were recorded for 2 h at 4°C. FBAL, α-Fluoro-β-alanine.

RESULTS

Intact mycelium study. The mycelia from the two strains of *Aspergillus* after incubation with 5FC were analyzed directly, i.e., intact, at 4°C to avoid possible degradation of metabolites. Signals corresponding to the following intracellular fluorinated compounds were detected in both strains: unmetabolized 5FC (−92.3 ppm) and 5FU (−93.6 ppm) coming from deamination of 5FC, as well as fluoronucleotides (FNUCt; −89.0 ppm) resulting from the anabolic conversion of 5FU. The signal from α-fluoro-β-alanine (−113.1 ppm), a catabolite of 5FU, was only found in *A. fumigatus* (Fig. 1). However, due to the similarity of their structures, the various FNUCt have very close ^{19}F NMR chemical shifts. It was therefore possible that the signals of several FNUCt might be present in the broad signal observed in intact mycelia at −89.0 ppm. In addition, at intracellular pH, the chemical shift of 5FO is very close to those of FNUCt, and so the signal corresponding to this compound, if present in *Aspergillus* spp., might have been included in the broad signal attributed to FNUCt. We could not decide from these results, therefore, whether 5FO was present in *Aspergillus*. Since the mycelium is a heterogeneous medium leading to broad NMR signals, we thus examined PCA extracts in an attempt to discriminate the ^{19}F peaks easily, especially with respect to signals corresponding to FNUCt and 5FO.

PCA extract study. (i) Demonstration of the absence of 5FO. Figure 2A shows a typical ^{19}F NMR spectrum of the acid-soluble fraction of a PCA extract of *A. fumigatus*. The spectrum was recorded at a strongly acidic (≈1.5) pH since the chemical shift of 5FO at this pH is deshielded and cannot be confused with the peaks caused by FNUCt. We observed signals due to 5FC (−93.58 ppm), 5FU (−93.41 ppm), and three FNUCt (−89.00, −89.08, and −89.43 ppm; the attribution of these signals to FNUCt is discussed below) and two signals at −80.67 and −84.49 ppm. Addition of 5FO to this extract led to the appearance of a signal at −86.37 ppm (Fig. 2B). The addition of 5FO to an acid-insoluble fraction of *A. fumigatus* showing the two signals at −80.7 and −84.5 ppm also indicated that this metabolite was not present in the acid-insoluble pool.

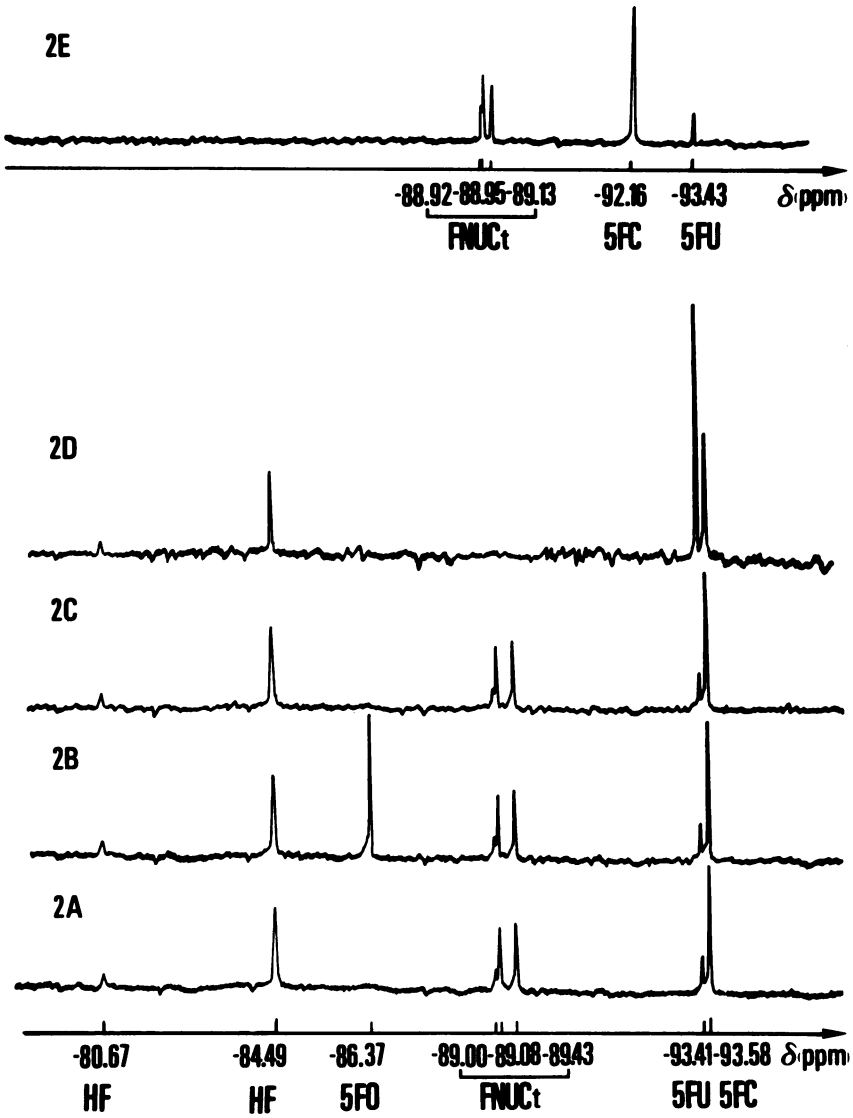

FIG. 2. Typical ^{19}F NMR spectra of the acid-soluble fraction of a PCA extract of *A. fumigatus* incubated for 1.5 h with 5FC. (2A) pH 1.5; (2B) 2A plus 5FO, pH 1.6; (2C) 2A after action of OMP decarboxylase, pH 1.5; (2D) 2A after treatment with periodate and methylamine, pH 1.6; (2E) 2A brought to pH 6.3.

Since the signals at −80.7 and −84.5 ppm did not correspond to 5FO, we thought that one of them might have been due to 5-fluoroorotidine-5′-monophosphate. In the absence of a reference sample of this compound, we examined the action of the enzyme OMP decarboxylase, which transforms OMP to UMP and CO_2 (16) and is also active on 5-fluoroorotidine-5′-monophosphate (1). No change in the signals was observed after incubation with this enzyme (Fig. 2A and C). Thus, the signals at −80.7 and −84.5 ppm were not due to 5FO or a nucleotide derived from 5FO.

In view of the drastic treatment of samples for preparation of the PCA extracts and the fact that the −80.7- and −84.5-ppm signals were observed in both acid-soluble and acid-insoluble fractions, we surmised that these signals were not due to metabolites but to degradation products of these metabolites, namely, fluoride ion (or more precisely, fluorhydric acid [HF] at the low pH used). This was confirmed by bringing the pH of the acid-soluble fraction of the PCA extract shown in Fig. 2 to 6.3. At this pH, the fluoride ion resonates at −43.5 ppm. Increasing the pH led to the disappearance of the −80.7- and −84.5-ppm signals (Fig. 2E) and gave rise to a signal at −43.5 ppm (zone of δ not shown in Fig. 2E). A further confirmation was obtained by the addition of HF to an acid-insoluble fraction of *A. fumigatus* prepared after 3.5 h of incubation with 5FC. There was a marked increase in the two peaks at −80.7 and −84.5 ppm. We verified that these two signals were also present in an aqueous solution and a blank PCA extract of *Aspergillus* spiked with NaF (10^{-3} M) and recorded at ~pH 1.3. As shown by the study of HF δ variation as a function of its concentration, the signal at −84.5 ppm corresponds to the HF monomer, while the signal at −80.7 ppm probably corresponds to an oligomeric form of HF. Indeed, the intensity of the −84.5-ppm signal increased and that of the −80.7 ppm decreased as the samples were diluted.

Since 5FO and FdUMP have been found in the acetic acid-soluble pool of *Aspergillus* extracts (14), we thought that these compounds could have been degraded during

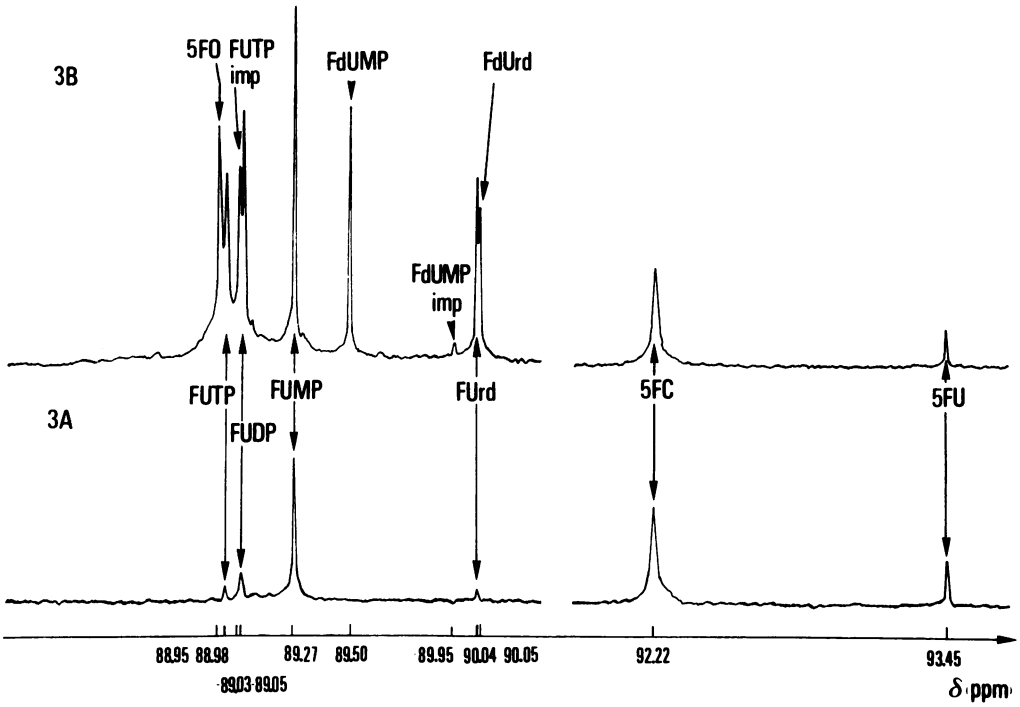

FIG. 3. Typical ^{19}F NMR spectra of the acid-soluble fraction of a PCA extract of *A. fumigatus* incubated for 1.5 h with 5FC. (A) Before addition of standards, pH 5.5; (B) after addition of 5FO and various FNUCt and fluoronucleosides. FUrd, 5-Fluorouridine; FdUrd, 5-fluoro-2'-deoxyuridine; imp, impurity (present in commercial standards).

treatment of the cell pellet with PCA. We thus verified with standard 5FO and FdUMP that these compounds were not, in fact, affected by the procedure used for preparation of the PCA-soluble extracts.

(ii) Identification of FNUCt. In general, three signals of FNUCt were observed in the acid-soluble pool of PCA extracts of *A. fumigatus* or *A. niger*. They were first identified as ribofluoronucleotides. Indeed, it is known that a treatment with periodate and methylamine leads to a complete destruction of ribonucleosides and ribonucleotides into corresponding base but is ineffective on the corresponding deoxy derivatives (4, 5). Treatment of a PCA-soluble extract with periodate and methylamine led to a loss of the FNUCt signals and an increase in the 5FU signal (Fig. 2D).

The various ribofluoronucleotides were then identified by addition of standards to the acid-soluble fraction of a PCA extract of *A. fumigatus* incubated for 1.5 h with 5FC (Fig. 3). A further confirmation of the absence of 5FO should be noted. The main FNUCt was FUMP (−89.27 ppm). The difference in its chemical shift between Fig. 2 and 3 is due to differences in pH. At low pH, FUMP and FdUMP are more shielded than at pH 5.5 to 6. FUDP (−89.05 ppm) and FUTP (−88.98 ppm) were also present, but in small proportions. That FUTP was not the principal FNUCt was almost certainly due to its instability during preparation (7) or conservation of the PCA extracts. FdUMP is more shielded (−89.50 ppm) than the above-mentioned ribofluoronucleotides. It was not present in the acid-soluble fraction, which was also demonstrated by treatment with periodate and methylamine. The fluoronucleoside signal at −90.04 ppm corresponded to 5-fluorouridine. This signal was not observed in all PCA extracts, and it was not detected in the intact mycelia (Fig. 1). It almost certainly stemmed from degradation of FNUCt during preparation of the PCA extracts.

Two signals (−89.47 and −89.73 ppm at ~pH 1.5) were observed in the spectra of the PCA-insoluble fractions. These signals were not the same as those observed in the acid-soluble fractions, and they did not correspond to FdUMP or other deoxyribofluoronucleotides that are more deshielded than FdUMP. The incubation of a PCA-insoluble fraction with alkaline phosphatase, an enzyme that dephosphorylates nucleotides to nucleosides, led to the complete disappearance of these two signals and the occurrence of a signal of fluoronucleosides. This fluoronucleoside signal was attributed to 5-fluorouridine since treatment of the last sample with periodate and methylamine led to 100% 5FU. The two signals therefore correspond to ribofluoronucleotides. However, they remained unchanged after a direct treatment of a PCA-insoluble fraction with periodate and methylamine. This means that the 2' or 3' position (or both) of the sugar moiety is esterified by phosphate. These results suggest that the two signals observed in the PCA-insoluble fractions correspond almost certainly to 5-fluorouridine-2'-monophosphate and 5-fluorouridine-3'-monophosphate, resulting from the acid hydrolysis of 5FU incorporated into RNA. In the two strains of *Aspergillus*, the proportions of the fluorinated compounds found in the PCA-insoluble fraction increased with increasing duration of incubation with 5FC.

Culture medium study. The ^{19}F NMR spectrum of the incubation media of the two *Aspergillus* strains showed only the signals corresponding to 5FC and 5FU. The proportion of 5FU released into the extracellular medium was very low at 1.5 h of incubation with 5FC and increased with the duration of incubation.

TLC. Since in a previous study TLC was used to identify 5FC metabolites in aspergilli (14), we also used this technique to compare the results we obtained. Adsorbent and solvent systems were identical to those used by Wagner and

TABLE 1. R_f values of compounds detected by TLC in the acid-soluble fraction of PCA extracts of *A. niger* and *A. fumigatus* incubated with 5FC for 1.5 or 3.5 h, respectively, and comparison with standards

Solvent system[a]	R_f values of standards[b]										Acid-soluble fraction of PCA extracts[b]			Acid-soluble fraction of TCA extracts (14)	
	5FC	5FU	5FO	FUMP	FdUMP	FUDP	FdUDP	FUTP	UMP	dUMP	*A. niger* (R_f)[c]	*A. fumigatus* 437 (R_f)[c]	Identification	*A. niger* 4.55 (R_f)[c]	Identification
1															
Spot 1		0.79	0.75	0.76						0.79	0.74	0.71	5FU, 5FO, FUMP, dUMP	0.81	5FU, FdUMP
Spot 2	0.65				0.64				0.68		0.62	0.61	5FC, FdUMP, UMP	0.60	5FC, 5FO
Spot 3						0.06	0.08	0.03			0	0	FUDP, FdUDP, FUTP		
2														0.68	FdUMP
Spot 1		0.52									0.52	0.54	5FU	0.41	5FU
Spot 2	0.31										0.33	0.33	5FC	0.30	5FC
Spot 3			0	0	0	0	0	0	0	0	0	0	5FO, FUMP, FdUMP, FUDP, FdUDP, FUTP, UMP, dUMP	0	5FO

[a] Solvent systems are given in Materials and Methods.
[b] R_f values are means of seven to nine experiments.
[c] *A. niger*: MIC = 1 μg ml^{-1}, 1.5-h incubation with 5FC; *A. fumigatus* 437: MIC = 3 μg ml^{-1}, 3.5-h incubation with 5FC; *A. niger* 4.55: MIC = 0.05 μg ml^{-1}, 1-h incubation with 5FC.

Shadomy. The R_f values of spots detected in the acid-soluble fractions of PCA extracts of *A. niger* and *A. fumigatus* incubated with 5FC for, respectively, 1.5 or 3.5 h are listed in Table 1 with those of authentic standards. In system 1, three major spots were detected with $R_f = 0$, $R_f \approx 0.6$, and $R_f \approx 0.7$. In solvent system 2, three spots were detected, the major one with $R_f = 0$ and the two other spots with $R_f \approx 0.3$ and $R_f \approx 0.55$. In the two solvent systems, minor spots were also observed. System 1 showed that the spot with $R_f \approx 0.6$ could correspond to 5FC or FdUMP or endogenous nonfluorinated nucleotides such as UMP or to all three; the spot with $R_f \approx 0.7$, to 5FU, 5FO, FUMP, or dUMP; and the spot with $R_f = 0$, to di- and triphosphate FNUCt and probably also to nonfluorinated endogenous di- and triphosphate nucleotides. In system 2, it was possible to distinguish 5FC ($R_f \approx 0.3$) and 5FU ($R_f \approx 0.5$) from 5FO and fluorinated or nonfluorinated nucleotides that do not migrate. Thus, with unlabeled 5FC, TLC allowed us to identify 5FC and 5FU in the acid-soluble fraction of PCA extracts of *A. niger* and *A. fumigatus*, but did not allow us to determine definitively the presence of other fluorinated metabolites.

DISCUSSION

The generally accepted metabolic scheme accounting for the antifungal activity of 5FC is shown in Fig. 4. 5FC is actively taken up into the fungal cell by means of a cytosine permease. Inside the cell, 5FC is rapidly deaminated to 5FU by means of a cytosine deaminase. After intracellular formation of 5FU, two pathways are possible. One leads to FUTP, which is incorporated into RNA, and the other leads to FdUMP, a potent inhibitor of thymidylate synthetase (11).

In aspergilli, 5FC is only fungistatic, whereas in yeasts it also shows fungicidal activity (10). This difference in action may be partly related to differences in the metabolic fate of 5FC. Moreover, less is known about the metabolic pathways

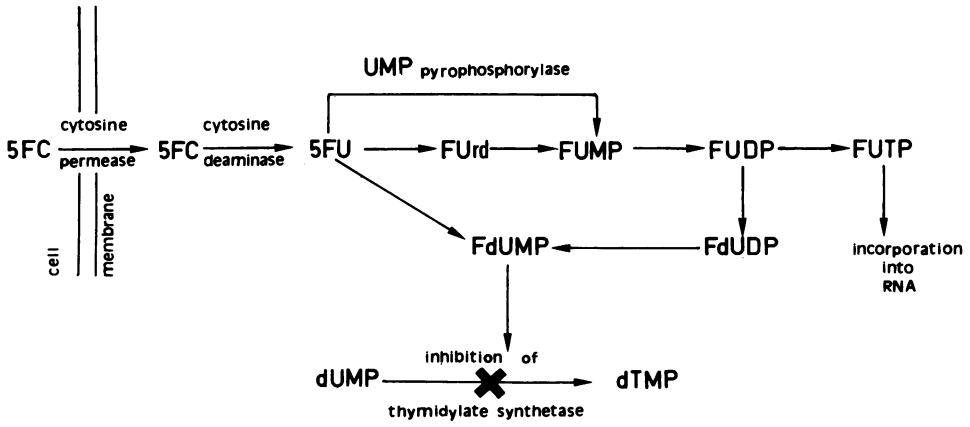

FIG. 4. Intrafungal pathway and mode of action of 5FC. FUrd (5-fluorouridine) is a fluoronucleoside; FUMP, FUDP, FUTP, FdUMP, and FdUDP (5-fluoro-2'-deoxyuridine-5'-diphosphate) are FNUCt.

of 5FC in aspergilli than in yeasts, and an intriguing pathway leading to the formation of 5FO has been described in *Aspergillus* sp. (14). The present study was therefore designed to investigate the metabolism of 5FC in two species of *Aspergillus* (*A. fumigatus* and *A. niger*) by ^{19}F NMR.

From the similarities of the spectra (Fig. 1), 5FC appears to be metabolized similarly in both strains, although α-fluoro-β-alanine was only detected in *A. fumigatus*. However, this compound was not detected in PCA extracts prepared immediately at the end of the culture period. The catabolic pathway may thus only operate under the unfavorable survival conditions existing during the NMR recording since the mycelia are packed in the NMR tube. The same phenomenon was observed when *Escherichia coli* cells were incubated with 5FU. Indeed, when the cell pellet was recorded immediately after the cells had been harvested by centrifugation, 5FU catabolites could not be detected, whereas 5,6-dihydro-5-fluorouracil and α-fluoro-β-ureidopropionic acid, two 5FU catabolites preceding α-fluoro-β-alanine in the 5FU catabolic pathway, were observed when the cell pellet was recorded after standing for several hours in the NMR tube (unpublished results).

With TLC, 5FO has been identified as the major 5FC metabolite in the acetic acid-soluble intracellular pool of aspergilli after incubation for 1 h with 5FC. 5FC, 5FU, and FdUMP have also been detected, but in much smaller amounts (14). We were unable to detect the presence of 5FO in the intracellular pool of either strain of *Aspergillus* after any incubation with 5FC. This would tend to discount a mechanism of action based on 5FO. In the PCA-soluble fraction, both 5FC and 5FU were detected, but among the FNUCt detected, FdUMP was not observed, and we only detected the ribofluoronucleotides FUTP, FUDP, and FUMP.

Since our ^{19}F NMR results differed strikingly from those of Wagner and Shadomy (14), a TLC study was carried out and the results obtained were compared with those of these authors (Table 1). Concerning 5FC and 5FU, our results are in agreement. In solvent system 2, the R_f value of 5FO was identical to that given by these authors. However, 5FO migrated as 5FU and not as 5FC in solvent 1. This discrepancy may be explained by the fact that Wagner and Shadomy did not dispose of standard 5FO and used published R_f values for this compound. In the two solvent systems, our R_f values for FdUMP did not agree with those obtained by Wagner and Shadomy. In solvent 1, FdUMP migrated like 5FC and not 5FU, while FUMP migrated like 5FU. In solvent 2, FdUMP, like all other fluorinated or nonfluorinated nucleotides, did not migrate. Thus, the important radioactivity detected by Wagner and Shadomy for an R_f value of 0 in solvent 2 and attributed to 5FO may also be due to FUMP, FdUMP, or other FNUCt. It corresponds almost certainly to FUMP, that is, the major FNUCt detected by NMR. The identification of 5FC metabolites by Wagner and Shadomy with TLC therefore is not relevant.

By using radiolabeled 5FC, incorporation of 5FU into RNA has been demonstrated in *Aspergillus* spp. (10, 13, 14). Our results show that fluorinated metabolites found in the PCA-insoluble fractions are ribofluoronucleotides and correspond to 5-fluorouridine-2′-monophosphate and 5-fluorouridine-3′-monophosphate. They are therefore hydrolysis products of 5FU incorporated into RNA. If the low intrinsic sensitivity of the ^{19}F NMR method does not allow us to deny a 5FC mechanism of action via the inhibition of thymidylate synthetase by FdUMP, our results clearly show that the main pathway of 5FC metabolism in the two strains of *Aspergillus* studied is the biotransformation into ribofluoronucleotides and the subsequent incorporation of FUTP into RNA.

In conclusion, ^{19}F NMR showed that, in *Aspergillus* spp., 5FC is deaminated into 5FU. 5FU is further transformed into FNUCt via the classical anabolic route. 5FO and FdUMP were not detected in any of our experiments, which conflicts with previous data (14). Our results suggest that the metabolism of 5FC in *Aspergillus* spp. closely resembles that in yeasts such as *Candida* (3, 12) or that of 5FU in other filamentous fungi such as *Nectria* (6).

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