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PHOTOAFFINITY INHIBITION OF PEPTIDE TRANSPORT IN YEAST

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SUMMARY : A photoaffinity label, 4-azidobenzoyltrimethionine has been synthesized. It competitively inhibits trimethionine uptake in the yeast C. albicans. Upon UV irradiation it irreversibly and specifically blocks oligopeptide uptake. These results give the first example of photoinhibition of peptide uptake in yeast.

The transport and utilization of oligopeptides by bacteria and yeast have been recently reviewed (1,2). E. coli cells can take up a great variety of peptides through a limited number of dipeptide and oligopeptide permeases. Results from different groups suggest a greater variability of peptide permeases in yeast compared to those of E. coli. "Illicit transport" of toxic drugs linked to a peptide vector through the permeases inside the cell is a well documented phenomenon (2). Recently, the efficiency of a conjugate of peptide and the toxic 5-fluorocytosine has been demonstrated in the pathogenic yeast Candida albicans (3). However, the nature and the structural specificities of the peptide permeases of yeast such as Saccharomyces cerevisiae or Candida albicans seem to vary from one strain to another as pointed out by several workers (2,4,5,6). A better knowledge of the peptide transport systems of yeasts is therefore required if one intends to utilize successfully the concept of illicit transport in the pathogenic species.

Photoaffinity labelling with an oligopeptide derivative should prove useful to learn more about the components of the transport systems. Staros and Knowles recently used an azidopeptide derivative to irreversibly inhibit the dipeptide transport system of E. coli (7). We

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have synthesized 4-azidobenzoyltrimethionine and report here the first example of photoinactivation of the oligopeptide transport system of an eukaryotic cell, namely Candida albicans.

MATERIALS AND METHODS

Chemicals : [^{14}C] labelled methionine was obtained from CEA (France). All other reagents were the best commercial grades available.

Synthesis of radioactive trimethionine : Radioactive trimethionine was synthesized using BOC-L-(methyl-^{14}C)-methionyl hydroxysuccinimide ester coupling on dimethionine following classical methods (8). After cleavage of the BOC-protecting group with trifluoroacetic acid, the product, L-(methyl-^{14}C)-methionyl-L-methionyl-L-methionine (1 Ci.mole^{-1}) was purified by preparative TLC on silica gel (eluent butanol : acetic acid:water, 4:1:1) and was identical to a non radioactive sample of tri-L-methionine. Final yield 65 % (based on radioactive methionine).

Synthesis of 4-azidobenzoyltrimethionine : Trimethionine (58 mg, 0,14 mmole) was treated with the hydroxysuccinimide ester of 4-azido-benzoic acid (36 mg, 0,14 mmole) (9) in dioxane : water, 1:1, containing sodium bicarbonate (0,3 mmole). After 24 hours at room temperature, the solvent was removed in vacuo, the mixture acidified to pH 2-3 with 10 % hydrochloric acid and extracted with ethylacetate. The product was purified by preparative TLC on silica gel (eluent toluene : pyridine : acetic acid, 150:50:3.5). In aqueous solution, the UV spectrum shows a maximum at 271 nm (ε=1,9 10^4, see fig. 1). The infrared spectrum includes a strong azide band at 2120 cm^{-1}. Final yield 30 %.

Organism and culture conditions : Candida albicans ATCC 26278, a methionine requiring auxotroph, was cultivated to the early exponential phase under the conditions described by Logan and al (6). For the transport studies, cells were grown at 37° C to about 1,5 x 10^6 cells/ml and then harvested by centrifugation, washed with cold (4° C) 0.03 M citric acid/potassium phosphate buffer (pH 4.5) and resuspended in the same buffer supplemented with 2 % glucose to a final concentration of 1.5 10^7 cells/ml.

Transport studies : After 15 min incubation at 37° C in glucose/citrate/phosphate buffer with stirring, radioactive trimethionine (together with the photoaffinity label when required for competition experiments) was added to the cell suspension and 0,5 ml sample were removed at 15 seconds intervals over a period of 1 minute, filtered on 0,45 µm pore size Millipore filters and washed with 5 ml of buffer. The filters were dried, place in 10 ml Bray's solution (10) and the radioactivity was measured in a Kontron MR 300 scintillation spectrometer.

Photoinactivation by 4-azidobenzoyltrimethionine : After 15 min incubation at 37° C in glucose/citrate/phosphate buffer with stirring, 4-azidobenzoyltrimethionine was added to the cell suspension (together with oligopeptides when required for protection experiments). The cells were immediately irradiated for 2 min at 37° C under stirring with a Philips SP 500 W UV lamp mounted 8 cm above the surface of the solution. Then 2 ml of the cell suspension were poured in 10 ml citrate/phosphate buffer, centrifuged and the pellet resuspended as above for trimethionine uptake measurement.

RESULTS

Photolysis of 4-azidobenzoyltrimethionine : The time dependent absorption spectra of the photolabile oligopeptide analogue upon exposure to light are shown in Fig. 1. Over 55 % decrease of absorbance at 271 nm was observed on photolysis for 2 min. This period was chosen for photolabelling of C. albicans cells.

Reversible inhibition of trimethionine uptake by 4-azidobenzoyltrimethionine : Trimethionine uptake in C. albicans ATCC 26278 is mediated by more than one transport system and a diffusion controlled process could partially contribute to the overall rate of entry of oligopeptides in this organism (unpublished results). 4-azidobenzoyl-

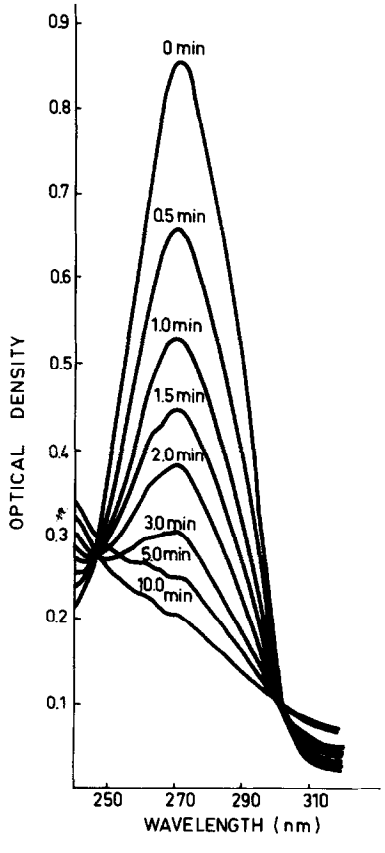

Fig. 1 : Ultraviolet spectral changes of 4-azidobenzoyl trimethionine on exposure to light ; a time study. Concentration = 4.5×10^{-5} M in 0.03 M citric acid, potassium phosphate buffer (pH 4.5). Temperature 37° C. Distance from the lamp (Philips SP 500 W) = 8 cm.

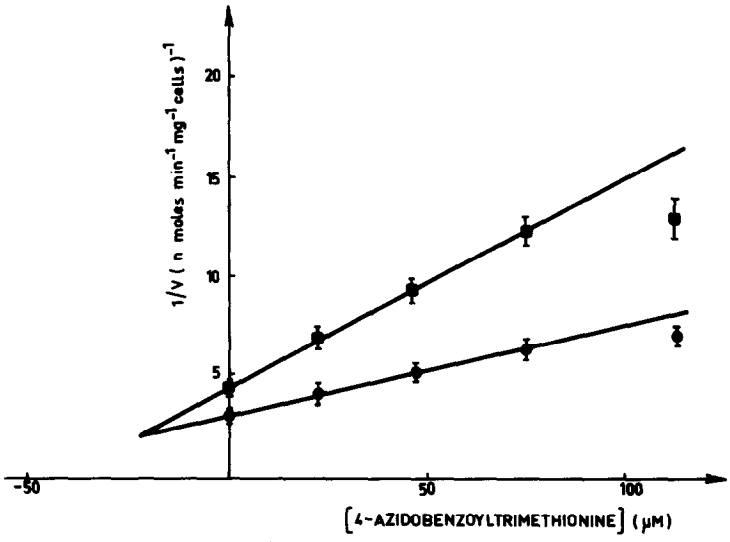

Fig. 2 : Dixon plot of [^{14}C] trimethionine uptake in the presence of 4-azidobenzoyltrimethionine. (●) 1.5 x 10^{-4} M trimethionine, (■) 6.0 x 10^{-5} M trimethionine. For further details, see Materials and Methods.

trimethionine inhibits trimethionine transport in a competitive way as shown in Fig. 2. The apparent Ki for 4-azidobenzoyltrimethionine is 2.1 x 10^{-5} M, a value similar to the apparent Km for trimethionine uptake determined by us (5.5 x 10^{-5} M) and others (6).

Photoinhibition of trimethionine uptake by 4-azidobenzoyltrimethionine : When suspensions of cells are irradiated for 2 min in the presence of 4-azidobenzoyltrimethionine one observes an irreversible inactivation of trimethionine uptake (Table 1). The specificity of 4-azidobenzoyltrimethionine is demonstrated by its inability to inhibit radioactive methionine uptake. This experiment furthermore confirms that trimethionine is not hydrolysed before entering the cell as it was previously shown by Logan et al (6). Trileucine also utilizes trimethionine uptake system in C. albicans with an affinity similar to that of trimethionine (data not shown and ref.6). Table 1 shows that this peptide efficiently protects the transport system against photoinactivation by 4-azidobenzoyltrimethionine. Trimethionine protective effect is poorer than that of trileucine. This result can be accounted for by an unexpected partial photoinactivation observed with trimethionine

TABLE 1

Photoinhibition of trimethionine transport in C. albicans

Additive	UV irradiation	Per cent inhibition of initial rate of transport	
		Trimethionine (5×10^{-5} M)	Methionine (9×10^{-6} M)
none	+	0	0
4-ABTM	-	0	0(a)
4-ABTM	+	90	0(a)
4-ABTM + Leu_3	+	15	N.D.
Leu_3	+	0	N.D.
4-ABTM + Met_3	+	60	N.D.
Met_3	+	35	N.D.

Abbreviations :
4-ABTM = 4-azidobenzoyltrimethionine ca 5×10^{-4} M
Met_3 = trimethionine ca 1.7×10^{-3} M
Leu_3 = trileucine ca 1.7×10^{-3} M
N.D. = not determined
(a) : concentration of 4-ABTM = 1.2×10^{-3} M
For further details, see Materials and Methods.

alone at the concentration used for protection experiments (1.7 mM, see table 1). However, under concentration conditions identical to those used for photoinactivation with 4-azidobenzoyl trimethionine (i.e. 0.5 mM), trimethionine alone only slightly inactivates the transport system (inhibition < 5 %). The photoinactivation of peptide transport with high concentrations of trimethionine is presently under investigation in our laboratory.

DISCUSSION

A number of studies have demonstrated that the peptide transport systems of bacteria and yeast could be used to carry toxic compounds attached to peptides inside the cell (1,2). Little is known about the number and nature of the peptide transport systems in the pathogenic yeast C. albicans. Logan et al. (6) reported that acetyltrimethionine is an effective competitor of trimethionine uptake in this yeast. On the contrary, Davies (5) using a different strain of C. albicans recently demonstrated that acetyltrialanine does not reduce the rate of trialanine transport. Our results with C. albicans ATCC 26278 confirm

that N-acylation of trimethionine leads to a derivative which is recognized by (one of) the oligopeptide permease (s). We have kinetical evidence that trimethionine uptake is mediated by more than one transport system (unpublished results). Affinity labelling should contribute to a better knowledge of the peptide transport phenomena in yeast. This paper shows that 4-azidobenzoyltrimethionine is a suitable photoaffinity label for at least one of the oligopeptide transport systems of C. albicans. The unphotolyzed peptide is a good competitive inhibitor of trimethionine uptake, with a Ki very similar to the apparent Km of trimethionine. After irradiation of a suspension of C. albicans in the presence of the photoaffinity label, trimethionine transport is irreversibly and specifically inhibited. These experiments extend in yeast previous results from Staros and Knowles (7) demonstrating the usefulness of azidopeptide derivatives to study the peptide transport systems of E. coli. Further studies are in progress, using a radioactive label to identify the components of the oligopeptide permease(s) of C. albicans.

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