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INHIBITION OF OLIGOPEPTIDE TRANSPORT IN S. CEREVISIAE BY A PEPTIDE-POLY (ETHYLENE GLYCOL) CONJUGATE

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Summary: A soluble macromolecule-peptide conjugate, [$(Met)_3$-OPEG] inhibited the uptake of Met-Met-[^{14}C] Met into S. cerevisiae. Uptake of leucine into this strain was not affected by Met_3-OPEG under identical conditions. Inhibition by the macromolecular inhibitor was competitive (K_I = 5.1 x 10^{-5}M)and followed the structural requirments of the peptide transport systems in S. cerevisiae and C. albicans. These findings give the first example of inhibition of metabolite transpor by a synthetic macromolecular competitor.

Numerous studies have been conducted on the transport of metabolites into cells. With the exception of the periplasmic localization of binding proteins involved in transport of some compounds into gram-negative bacteria (1) little is known about the exact location of transport proteins in the cell envelope. This is especially true with respect to the yeast Saccharomyces cerevisiae which has a very complex envelope composed of β-glucans, mannans, glycoproteins and chitin(2).

We have previously reported on the structural specificity of the peptide transport system in S. cerevisiae using radioactive trimethionine as the transport substrate. We found that S. cerevisiae has a peptide transport system which recognizes a variety of di- and tripeptides but which exhibits a size limit at the tetrapeptide(3,4). In order to probe the basis for this size limit further we have synthesized trimethionine attached to a soluble polymer-poly(ethylene glycol) (MW=5000). Poly(ethylene glycol) of molecular weight greater than 600 cannot pass through the cell wall of S. cerevisiae(5). Thus the ability of the Met_3-OPEG conjugate to affect uptake of radioactive trimethionine in S. cerevisiae should give information on the location of the peptide transport system in the yeast cell envelope. Our report gives the first evidence for the specific interation of a synthetic macromolecular inhibitor with an active transport system in an intact microorganism.

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Methods

Peptide Synthesis: The peptide-poly(ethylene glycol) conjugates were prepared using the liquid phase method of Bayer and Mutter(6). Briefly tert-butoxycarbonyl-L-methionine anhydride $(Boc\text{-}Met\text{-})_2O$ was coupled to poly (ethylene glycol) monomethyl ester (MW = 5,000) in CH_2Cl_2 in the presence of pyridine. The amino acid polymer conjugate was isolated and purified by recrystallization from ether. The tert-butoxycarbonyl (BOC) protecting group was removed using 50% trifluoroacetic acid in CH_2Cl_2 in the presence of 5% (v/v) anisole and the peptide chain extended by reaction with $(Boc\text{-}Met)_2\text{-}O$. All couplings and cleavages were monitored by a ninhydrin assay and judged to be 99.8% complete. The final peptide was cleaved from the poly(ethylene glycol) and found to be homogenous on silica thin layers. Radioactive trimethionine (Met-Met-[^{14}C]Met, 1 mCi/mmole), was synthesized as described previously(3).

Peptide transport: Assay of the uptake of Met-Met-[^{14}C]Met and radioactive amino acids into both S. cerevisiae 139 and C. albicans WD 18-4 was carried out as described previously (3,7). Briefly, cells were grown to mid-log phase, harvested by centrifugation, washed and suspended in uptake buffer containing 0.25% glucose. A solution containing radioactive trimethionine or amino acid and the appropriate competitor was added to the cells, and samples were removed intervals, filtered through membrane filters, and counted.

Results

The ability of $(Met)_3$ -OPEG to affect the transport of two amino acids and a peptide into S. cerevisiae 139 is summarized in Table I. When present in 20-fold molar excess the macromolecule-bound peptide decreased the initial velocity of $(Met)_3$ uptake by 82%. In contrast poly(ethylene glycol) alone had no effect on trimethionine transport, and $(Met)_3$ -OPEG did not inhibit leucine transport. Both

TABLE I

EFFECT OF VARIOUS COMPOUNDS ON TRIPEPTIDE AND AMINO ACID TRANSPORT IN S. CEREVISIAE

	PERCENT INHIBITION OF INITIAL RATE OF TRANSPORT[a]		
COMPETITOR	$(Met)_3$	Methionine	Leucine
$(Met)_3$-OPEG[b]	82	11	0
poly(ethylene glycol)[c]	0	0	0
Boc-$(Met)_3$-OPEG[d]	7	0	0

[a]Transport rates were linear in the interval measured. The percent inhibition was the rate with competitor divided by the rate without competitor times 100.

[b]$(Met)_3$-OPEG is poly(ethylene glycol) covalently attached to trimethionine via the peptide carboxyl terminus.

[c]A poly(ethylene glycol) of 5,000 daltons was used for competition experiments and for synthesis of the $(Met)_3$-OPEG.

[d]Boc-$(Met)_3$-OPEG is poly(ethylene glycol) covalently attached to the carboxyl terminus of amino-terminus blocked (by Boc, tertiarybutoxycarbonyl) trimethionine.

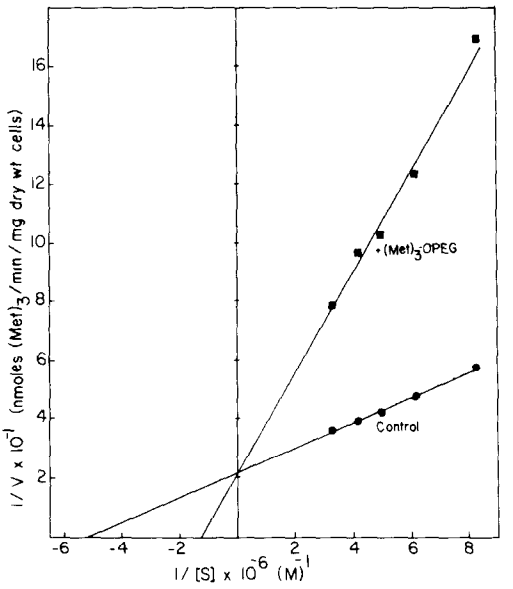

Fig. 1. Competitive inhibition of $(Met)_3$ transport by $(Met)_3$-OPEG, 4.1×10^{-4}M.

of these results demonstrate that the peptide-poly(ethylene glycol) conjugate acts specifically on the peptide transport system rather than having a general effect on the cell envelope. Interestingly, we found that Boc-$(Met)_3$ -OPEG did not compete with Met-Met-[^{14}C]Met uptake into strain 139. This result is reasonable since the peptide transport system in this yeast does not recognize α-N-acylated peptides. Moreover the failure of Boc-$(Met)_3$-OPEG to interact with the peptide transport system eliminates the possibility that the inhibition observed with $(Met)_3$-OPEG is due to the hydrophobicity of the tripeptide moiety. Rather, the inhibition by these compounds reflects the structural specificity expected for the peptide uptake system in strain 139. Finally, in contrast to its effect in S. cerevisiae, (Met_3-)OPEG did not inhibit the uptake of Met-Met-[^{14}C]Met into C. albicans WD 18-4. This finding is consistent with the fact that the peptide transport system in strain WD 18-4 does not recognize peptide esters.

Kinetic analysis of the inhibition of Met-Met-[^{14}C]Met by Met_3-OPEG showed that the polymer-peptide conjugate was a competitive inhibitor (Fig. 1). The K_I of the inhibitor was 5.1×10^{-5}M which is close to the K_M for Met-Met-[^{14}C] Met

uptake. Thus attachment of trimethionine to poly(ethylene glycol) does not significantly reduce its affinity for the peptide transport system in S. cerevisiae.

It was possible that trimethionine was cleaved from the $(Met)_3$-OPEG conjugate and subsequently competed with $(Met)_3$ uptake. We believe that this possibility is unlikely since it would not be expected to lead to a typical competitive inhibition as seen in Figure 1. More than 80% of $(Met)_3$-OPEG would have to be cleaved during the transport assay (1 minute) to account for the large inhibition of uptake, and studies in our laboratory could not detect any free trimethionine after incubation of strain 139 with $(Met)_3$-OPEG for 30 minutes. Furthermore, $(Met)_3$ OPEG at 1.4×10^{-5}M did not serve as a methionine source for a methionine auxotroph of S. cerevisiae, although $(Met)_3$ or methionine at that concentration yielded good growth. These results indicate that neither $(Met)_3$-OPEG nor its peptide or amino acid components are available to the yeast for cellular metabolism.

Discussion

A number of investigators have shown that haptens, substrates, or inhibitors bound to macromolecules maintain their biological activity. Especially noteworthy in this regard is the work of Bayer and coworkers (6), who showed that a biotin-ferritin conjugate inhibited biotin uptake into yeast sphaeroplasts, and Jost and Yaron, who showed that a tripeptide inhibitor of thermolysin was active when conjugated to a soluble dextran but inactive when bound to the crosslinked polysaccharide-agarose (9).

The results reported in this paper show that a tripeptide covalently linked to poly(ethylene glycol) acts as a reversible, competitive inhibitor of the peptide transport system in S. cerevisiae. Inhibition is consistent with the structural specificity of the peptide transport systems in both S. cerevisiae and C. albicans, suggesting that macromolecule bound trimethionine can interact specifically with a component(s) of the peptide transport system in S. cerevisiae. This finding represents the first example of inhibition of a transport system in vivo by a competitor bound to a synthetic polymer and is significant from several points of

view. It suggests that when connected to a macromolecule via chemical groupings not necessary for recognition, transport substrates can still interact strongly with their porters. Such macromolecule-bound inhibitors should therefore be useful in studies designed to isolate components of transport systems. In addition the results with $(Met)_3$-OPEG suggest that some component of the peptide transport system is accessible to an inhibitor which should be restricted by the cell wall from entering the cytoplasm. Thus either the trimethionine transport system is external to the barrier for poly(ethylene glycol) in S. cerevisiae or specific pores exist in the cel envelope through which various substrates can interact with specific receptors. This latter possibility has major implications and is consistent with the observation that the yeast α-mating factor (a dodecapeptide) can interact with its receptor in S. cerevisiae despite the fact that tetrapeptides are not substrates for the peptide transport system in this yeast.

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