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Screening of a synthetic pentapeptide library composed of D-amino acids against fructose-1,6-biphosphate aldolase

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SUMMARY

A synthetic peptide library, theoretically composed of 537 824 D-amino acid pentapeptides anchored on polystyrene beads, was prepared with each bead bearing a single pentapeptide sequence. This library was screened for interaction with fructose-1,6-biphosphate aldolase of *T. brucei* labelled with biotin. Affinity beads that bound the enzyme were selected with streptavidin-coated magnetic beads. A total of 19 beads were isolated and individually subjected to Edman microsequence analysis. The corresponding peptide sequences were synthesized and evaluated for enzyme activity inhibition.

INTRODUCTION

Trypanosoma brucei, the cause of sleeping sickness, is very susceptible to compounds interfering with glycolysis, because of its high glucose consumption, which is 50 times faster than the glucose consumption of mammalian cells, and the lack of storage forms for metabolic energy. By blocking its energy supply in a specific manner, we should be able to stop the evolution of the disease.

In this paper, we describe the selection procedure of peptide ligands found by screening a peptide library for binding to fructose-1,6-biphosphate aldolase (Aldo) of *T. brucei*, their identification and their evaluation as enzyme inhibitors.

METHODS

The peptide library was prepared using the split-synthesis approach [1] and assembled on a Tentagel-S-NH_2 support ($\phi \cong 130$ μm) using Fmoc chemistry [2].

The enzyme Aldo was labelled overnight with Biotin-XX-NHS [3]. Labelled enzyme was incubated with the peptide library overnight. Positive beads were selected with streptavidin-coated magnetic beads using a Magnetic Particle Concen-

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TABLE 1
PEPTIDE SEQUENCES IDENTIFIED AFTER HYBRIDISATION OF THE LIBRARY WITH ALDOLASE[a]

No.	Sequence	No.	Sequence
1	R-K-Thi-Y-K	8	Y-Y-Y-K-E
2	P-E-Y-W-Thi	9	K-Thi-Y-Y-V
3	M-V-Thi-K-S	10	F-Y-E-K-P
4	K-A-F-R-V	11	A-K-S-P-M
5	P-S-Cha-A-R	12	K-F-Cha-R-V
6	R-F-P-R-V	13	W-K-K-M-M
7	K-P-M-N-Thi	14	K-S-K-P-R

[a] Cha = cyclohexylalanine; Thi = thienylalanine.

trator [5]. The peptide sequences were identified by Edman degradation. All identified pentapeptides were synthesized as soluble peptide amides on a Rink amide MBHA resin (347 mg, 0.49 mmol/g; Novabiochem) using standard Fmoc chemistry on a peptide synthesizer (ABI 431A). The peptides were purified by HPLC on a reversed phase column (Biogel®). The identity of the lyophilized peptides was verified by mass spectrometry [4]. Inhibition of the aldolase enzyme activity by the peptides was assayed as described [6].

RESULTS

Screening of the peptide library with the biotin-labelled enzyme resulted in the identification of 14 pentapeptides. The enzyme inhibitory activity of the thus prepared pentapeptide amides was investigated at the 1 mM level. However, none of these peptides showed any significant inhibition.

DISCUSSION AND CONCLUSIONS

While a large number of different molecules was present in the primary hybridization assay, the experiments described here demonstrate that the use of a peptide library for the identification of an inhibitor for an enzyme without any prior knowledge is not as straightforward as originally expected. This is perhaps due to the fact that many interactions are possible with large enzymes like aldolase (MW = 160 000), without interference with the enzymatic activity. This possibility will be evaluated by microcalorimetry, a sensitive method for the determination of the affinity of ligands for their ligate. On the other hand, the labelling procedure itself causes random enzyme modifications, which is unattractive for the reproducibility of the method.

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