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The WALMART Peptide

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Abstract

The "name-to-peptide" method was used to design a 7-residue peptide amide with the amino acid sequence: Tryptophan-Alanine-Leucine-Methionine-Alanine-Arginine-Threonine. Abbreviation of this sequence with the International Union of Pure and Applied Chemistry single letter symbols for amino acids yields W-A-L-M-A-R-T, or WALMART. This abbreviation corresponds to the prominent name of an American multinational retail corporation that operates chains of large discount department and warehouse stores. The WALMART amino acid sequence occurs in several natural proteins, such as the acyl CoA dehydrogenase of *Pseudomonas aeruginosa*.

The WALMART peptide was designed to have a carboxyl-terminal amide group in order to eliminate the negative charge that would normally occur at this end of the peptide at neutral pH, thereby enhancing the net positive charge character of the peptide. The peptide is predicted to be relatively hydrophobic (57% hydrophobic amino acid residues), to have a net charge of +2 at pH 7, and to absorb UV light at 180-220 nm (peptide bond) and 280 nm (Tryptophan). Molecular modeling of the peptide as an α-helix indicated that it was capable of forming 1.5 turns of helix, contained 4-5 hydrogen bonds, and had a length [amino-terminal amino group to carboxyl-terminal amide group] of 11 Å. When modeled as a β-strand, the peptide had a length of 21 Å.

Peptide WALMART was synthesized as an HCl salt, in order to avoid potential undesirable effects of the trifluoroacetate anion, and its purity, molecular weight, and amino acid sequence were determined by reverse phase HPLC and mass spectrometry. The peptide was found to be readily soluble in water.

The peptide inhibited proliferation of human female colorectal adenocarcinoma cells (HT-29), and growth of human male and female urinary bladder carcinoma cells (5637 and TCCSUP cells, respectively). Inhibition occurred at concentrations 5-fold lower than the concentrations of dichloroacetate used to obtain the same biological effects. A preliminary test indicated that the peptide may inhibit the growth of *Staphylococcus simulans*.

Introduction

Traditional methods for obtaining novel peptides include their discovery during the study of natural products [1], by synthetic modifications of naturally occurring peptides [2], or by combinatorial peptide chemistry methods [3].

The "name-to-peptide" method for designing novel peptides was described in 2003 [4, 5], and it uses the English language and alphabet, rather than nature, as a source of peptides. The method is a 2-step process consisting of choosing a name (or word or phrase) that is composed of letters of the English alphabet, except for the letters, B, J, O, X, and Z (e.g., WALMART), and then considering the letter sequence of the name to be a sequence of International Union of Pure and Applied Chemistry (IUPAC) single letter symbols for the trivial names of amino acids (AAs) (Table 1) [6, 7] [e.g., Tryptophan (W)-Alanine (A)-Leucine (L)-Methionine (M)-Alanine (A)-Arginine (R)-Threonine (T)]. The method was first tested in 2004 with a peptide designed using the personal name of former US Secretary of State, Colin Powell [8, 9]. Since the letter sequence of the name contained the letter, "O", which had not been assigned to any AA by the IUPAC, it was arbitrarily assigned to the AA, Ornithine, an assignment which had been previously used in the biochemical literature. After synthesis, preliminary testing showed the

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Table 1. International Union of Pure and Applied Chemistry (IUPAC) single letter symbols for the names of amino acids [6], modified to include the IUPAC recommendations for Selenocysteine [7].

Trivial name	Symbols		Systematic name
	3-Letter	1-Letter	
Alanine	Ala	A	2-Aminopropanoic acid
Arginine	Arg	R	2-Amino-5-guanidinopentanoic acid
Asparagine	Asn	N	2-Amino-3-carbamoylpropanoic acid
Aspartic acid	Asp	D	2-Aminobutanedioic acid
Cysteine	Cys	C	2-Amino-3-mercaptopropanoic acid
Glutamine	Gln	Q	2-Amino-4-carbamoylbutanoic acid
Glutamic acid	Glu	E	2-Aminopentanedioic acid
Glycine	Gly	G	Aminoethanoic acid
Histidine	His	H	2-Amino-3-(1*H*-imidazol-4-yl)-propanoic acid
Isoleucine	Ile	I	2-Amino-3-methylpentanoic acid
Leucine	Leu	L	2-Amino-4-methylpentanoic acid
Lysine	Lys	K	2,6-Diaminohexanoic acid
Methionine	Met	M	2-Amino-4-(methylthio)butanoic acid
Phenylalanine	Phe	F	2-Amino-3-phenylpropanoic acid
Proline	Pro	P	Pyrrolidine-2-carboxylic acid
Serine	Ser	S	2-Amino-3-hydroxypropanoic acid
Threonine	Thr	T	2-Amino-3-hydroxybutanoic acid
Selenocysteine	Sec	U	3-Selanyl-2-aminopropanoic acid
Tryptophan	Trp	W	2-Amino-3-(l*H*-indol-3-yl)-propanoic acid
Tyrosine	Tyr	Y	2-Amino-3-(4-hydroxyphenyl)-propanoic acid
Valine	Val	V	2-Amino-3-methylbutanoic acid

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peptide to be inactive against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci bacteria, and herpes simplex virus. However, it was capable of inducing migration of human monocytes and neutrophils, and inhibiting the proliferation of human breast cancer cells, and it had no effect on plasma coagulation.

Several theoretical articles about personal, mythical, and company "name peptides" followed publication of the COLINPOWELL peptide results [10-19]. This article presents preliminary information about the second synthetic "name peptide", which was designed based upon the letter sequence in the company name, Walmart.

Methods and Results

BLAST search

In order to determine if the AA sequence, WALMART, occurs in natural, or other, proteins, a Basic Local Alignment Search Tool (BLAST) [20] search of the National Center for Biotechnology Information's (NCBI) non-redundant protein databases was done. The search yielded 90 exact matches among the 54,027,943 AA sequences in the database as of December 22, 2014 (Tables 2 and 3). None of these exact matches were found to have a Protein Data Bank (PDB) identification code, indicating that the WALMART sequence does not occur among proteins whose three dimensional (3D) structures are known. Most of the exact matches were found to occur in the protein enzyme, acyl-CoA dehydrogenase (ACAD,) of the bacterium, *Pseudomonas aeruginosa*, a medically important Gram-negative, aerobic and facultative anaerobic, opportunistic bacterial pathogen of humans [21, 22]. ACADs are a class of

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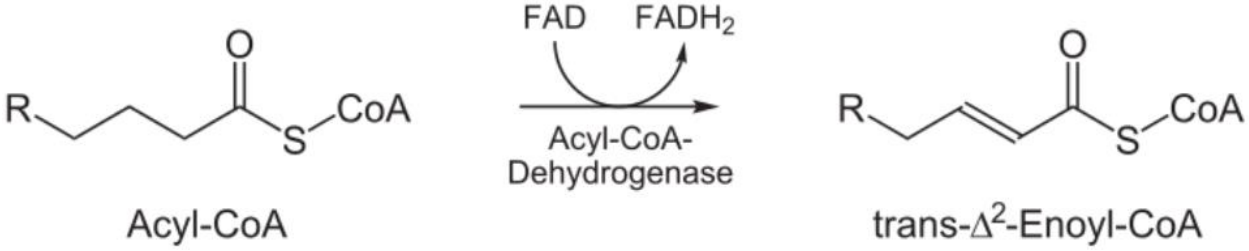
Table 2. A BLAST search of the NCBI non-redundant protein databases (54,027,943 sequences) for the amino acid sequence, WALMART resulted in 90 exact matches. The exact matches occurred predominantly in *Pseudomonas* species (98% of all matches; 86% in *P. aeruginosa*), and 82 (91%) of the exact matches occurred in the protein, acyl-CoA dehydrogenase.

Organism	Number	Percent	Protein (Number of Occurrences)
Pseudomonas:			
" (MULTISPECIES)	2/90	2.2	Acyl-CoA dehydrogenase (2)
" (MULTISPECIES)	1/90	1.1	Hypothetical protein (1)
Pseudomonas aeruginosa:			
"	3/90	3.3	Hypothetical protein, partial (3)
"	25/90	27.8	Acyl-CoA dehydrogenase, partial (25)
"	1/90	1.1	Acyl-CoA dehydrogenase (Fragment) (1)
"	44/90	48.9	Acyl-CoA dehydrogenase (44)
"	1/90	1.1	Acyl-CoA dehydrogenase domain-containing protein, partial (1)
"	1/90	1.1	Putative acyl-CoA dehydrogenase (1)
Pseudomonas aeruginosa DHS29	1/90	1.1	Acyl-CoA dehydrogenase (1)
Pseudomonas aeruginosa PA38182	1/90	1.1	Putative acyl-CoA dehydrogenase (1)
Pseudomonas alkylphenolia	1/90	1.1	Acyl-CoA dehydrogenase (1)
Pseudomonas sp. HYS	1/90	1.1	Acyl-CoA dehydrogenase (1)
Pseudomonas sp. S9	1/90	1.1	Acyl-CoA dehydrogenase (1)
Pseudomonas plecoglossicida	2/90	2.2	Acyl-CoA dehydrogenase (2)
Pseudomonas putida	1/90	1.1	Acyl-CoA dehydrogenase domain-containing protein (1)
Pseudomonas psychrophila	1/90	1.1	Acyl-CoA dehydrogenase (1)
Pseudomonas vranovensis	1/90	1.1	Acyl-CoA dehydrogenase (1)
Roseovarius mucosus DSM 17069	1/90	1.1	Amino acid/amide ABC transporter membrane protein 1, HAAT family(1)
Vibrio splendidus	1/90	1.1	Integrase (1)
TOTAL	90/90	99.8	

Figure 1. The reaction catalyzed by Acyl-CoA dehydrogenase (ACAD).



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enzymes that function to catalyze the initial step in each cycle of fatty acid β-oxidation in the mitochondria of mammalian cells (Figure 1) [23, 24].

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Table 3. Selected examples from the Table 2 BLAST results showing the protein size [number of amino acids (AAs)], the location of the WALMART AA sequence within the protein, and reference information.

				Database	
Organism	Protein source	AAs in protein	Location of WALMART	Sequence identification code	Ref.
Pseudomonas aeruginosa PA38182	Putative acyl-CoA dehydrogenase (d.h.)	334	AAs 277-283	emb|CDI88668.1	25, 26
P. alkylphenolia	Acyl-CoA d.h.	592	535-541	gb|AIL59642.1	27
P. sp. HYS	"	"	"	ref|WP_010221413.1	28
P. plecoglossicida	"	"	"	ref|WP_028625708.1	29
P. psychrophila	"	"	"	ref|WP_019409823.1	30
P. putida	Acyl-CoA d.h. domain-containing protein	"	"	ref|WP_009404199.1	31
P. sp. S9	Acyl-CoA d.h.	"	"	ref|WP_010485976.1	32
P. vranovensis	"	"	"	ref|WP_028942181.1	33
Roseovarius mucosus DSM 17069	Amino acid/amide ABC transporter membrane protein 1, HAAT family	308	176-182	gb|KGM89871.1	34
Vibrio splendidus	Integrase	733	356-362	ref|WP_004734287.1	35

Molecular modeling

1. Modeling of the peptide within a protein:

Of the 90 exact matches obtained in the BLAST results, none occurred within proteins of known 3D structure. Knowledge of the structure of peptide WALMART within a protein of known 3D structure might provide clues to potential biological functions of the peptide. Consequently, a hypothetical 3D structure was generated for one of the ACADs that contained the WALMART AA sequence. This was done using the program SWISS-MODEL [36-40], and the results were transferred to the Microsoft Paint program for the construction of figures (Figure 2). In this model, peptide WALMART is completely α-helical.

2. Modeling of the isolated peptide:

Molecular models were constructed using the AA sequence, WALMART, and the DeepView / Swiss-PdbViewer (v4.1.0) molecular modeling program [43]. The sequence was modeled in both the α-helical and β-strand conformations. The models were then energy minimized using the steepest descent method, and default parameters of the program. The number of hydrogen bonds present after each round of minimization were noted, and after 10,001 steps of minimization, the model was saved and used for display with the RasMol program [44]. RasMol models were then transferred to the Microsoft Paint program for construction of figures (Figure 3).

Custom synthesized peptide

Peptide WALMART was custom synthesized by Peptides International (P.I., Louisville, KY, USA) as a HCl salt. The reason for making peptide WALMART as a HCl, rather than a trifluoroacetate (TFA), salt, is due to potential toxicity problems and other undesirable effects that might arise from the presence of the TFA anion (CF_3-COO^-) [45, 46]. In contrast, chloride (Cl^-) is the most abundant anion in organisms [47], with a normal concentration range in human serum of 100-106 mM [48]. Consequently, no toxicity or other undesirable effects are expected from use of the HCl salt of peptide WALMART. In addition, there is at least a 2-fold size difference between the TFA and Cl^- ions (≥ 3.5 Å [49] vs. 1.7 Å [50]) which might complicate any biological results obtained with a TFA salt of the peptide by introducing steric effects. The purity and molecular weight (MW) of the peptide were determined by P.I.

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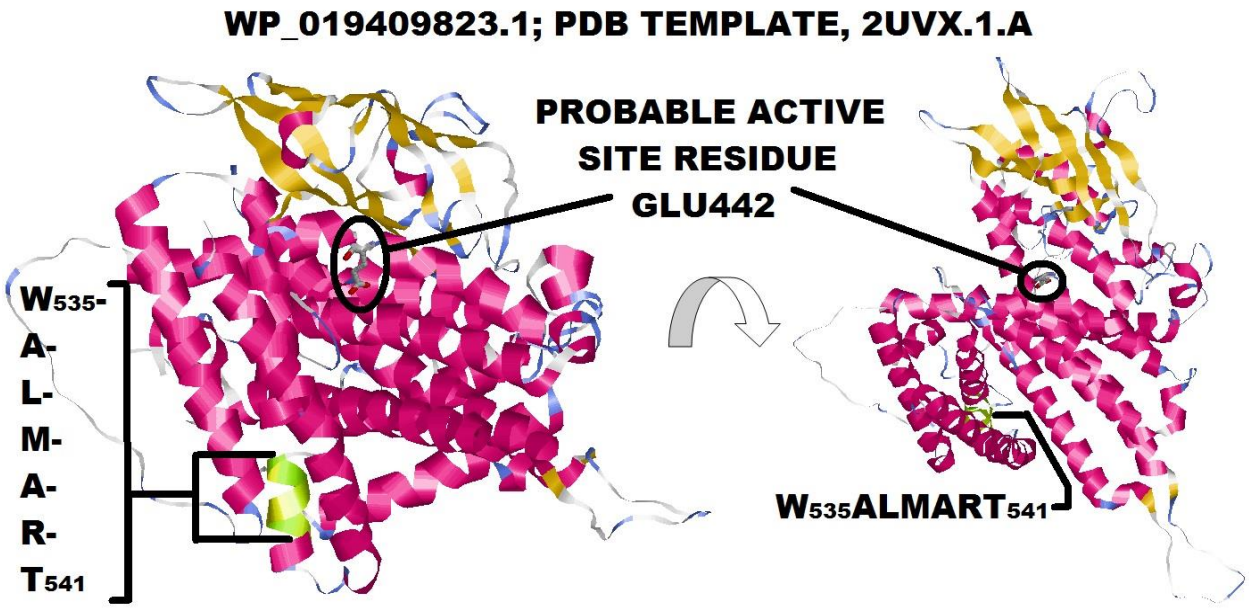
Figure 2. Ribbon diagram of a hypothetical model of *Pseudomonas psychrophila* acyl CoA dehydrogenase (ACAD; sequence identification code WP_019409823.1 [30]) that was generated by the SWISS-MODEL automated protein structure homology-modelling server [36-40]. The template for the program was PDB structure 2uxw.1.A (crystal structure of human very long chain ACAD [41]). The WALMART sequence (colored yellow-green) occurs between AA residues 535-541, and is located within an α-helix. In 2uxw.1.A, the substrate is adjacent to Glu462, which occurs in a loop between two α-helices, and extends into the active site where it probably participates in catalysis. Another ACAD of similar size and known 3D structure is medium-chain acyl-CoA dehydrogenase from pig liver mitochondria (PDB 3MDD and 3MDE) [42], and in this ACAD, Glu376 also occurs in a loop between two α-helices, and extends into the active site where it participates in catalysis. Glu442 in the model shown below retains the features seen for Glu462 of 2uxw.1.A and Glu376 of 3MDD, and, therefore, could serve as an active site residue in this hypothetical model. Trp535, in the WALMART sequence of WP_019409823.1, is located 24 Å from the Glu442, and, therefore, is probably not located near the active site of the *Pseudomonas psychrophila* ACAD.



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using RP-HPLC and mass spectrometry (MS) (Figure 4), and its MW and AA sequence were verified independently (Figure 5). The peptide was found to be readily soluble in water.

Ultraviolet (UV) absorbance:

Although untested, it is expected that the peptide will exhibit UV absorbance at 180-220 nm for the peptide bonds [51, 52], and 280 nm for Tryptophan [53]. Since the peptide was found to be readily soluble in water, the UV absorbance of peptide WALMART may provide a simple means for measuring its concentration in aqueous solutions.

Biological testing

1. Effect of peptide WALMART on the growth of *Staphylococcus simulans*:

Preliminary tests indicated that synthetic peptide WALMART was sterile (data not shown), and that it inhibited the growth of *Staph. simulans* on a Luria/lysogeny broth (LB) agar plate (Figure 6). The peptide had no activity on a mixed bacterial culture from a urine sample (data not shown).

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Figure 3. (Top) Stick figure models of peptide WALMART in the (left) β-strand and (right) α-helical conformations. Single letter symbols for AAs are shown adjacent to the AA side chain, and the location of two positive charges that occur at neutral pH [N-terminus and side chain of Arginine (R)] are shown. (Bottom) Electrostatic potential diagrams of peptide WALMART in the (left) β-strand and (right) α-helical conformations. The peptides are shown as wireframe models, colored black, and in the same orientations as the stick figure models directly above them. Blue color indicates regions of positive electrostatic potential, which are associated with the N-terminal $–NH_3^+$ group, and the side chain guanidino group of Arginine.

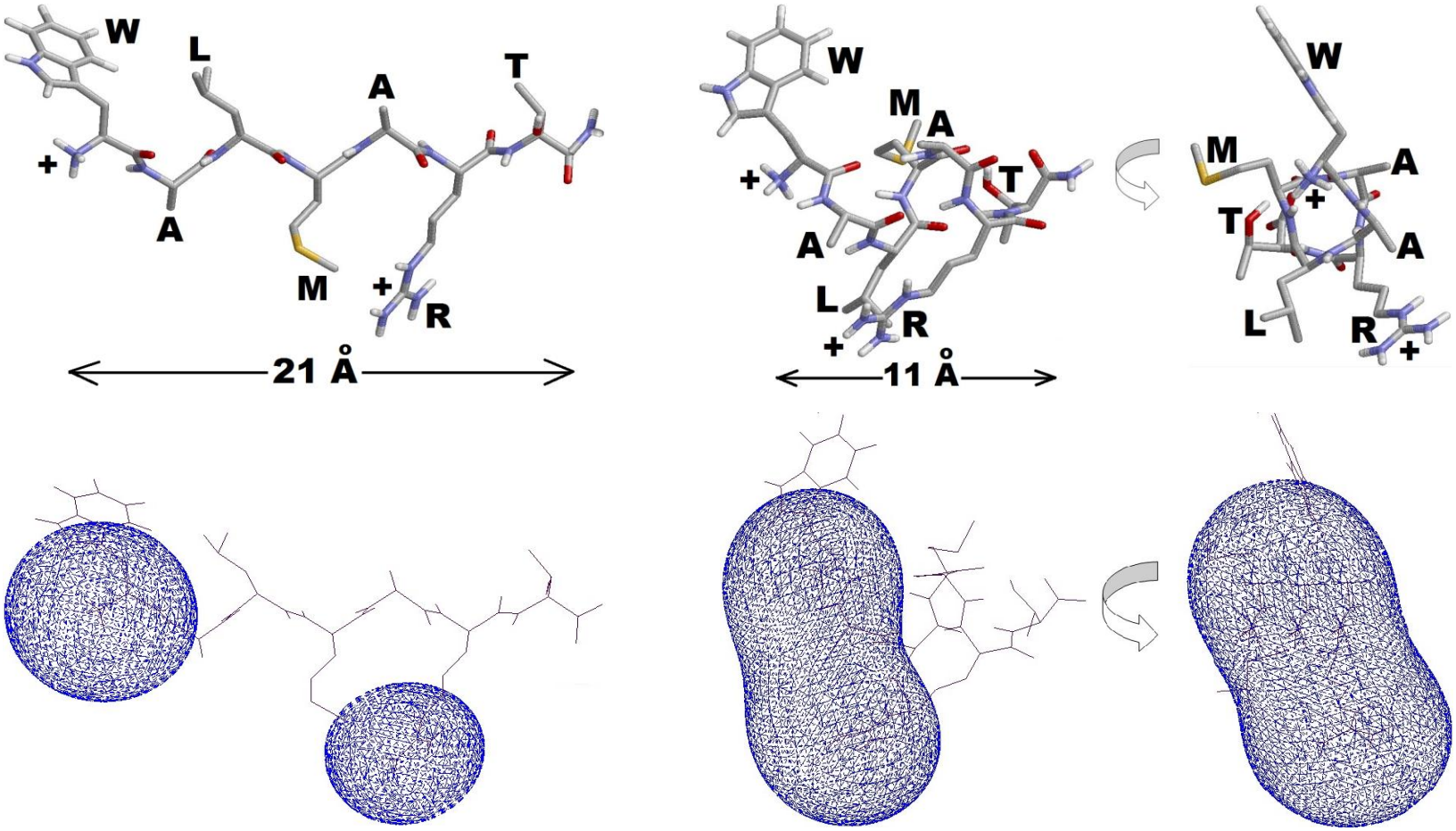

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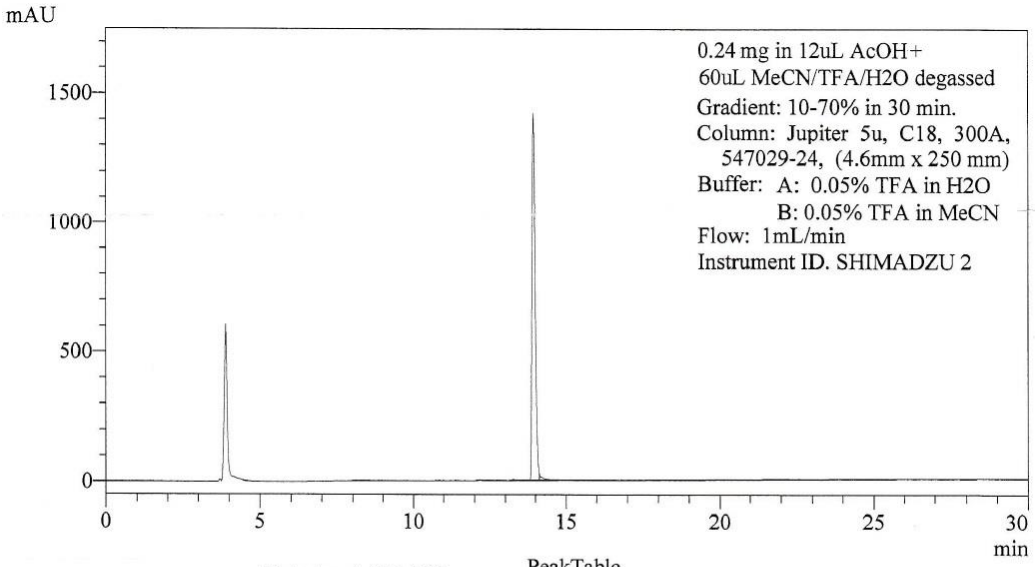
Figure 4. (Below left) HPLC chromatogram, chromatographic conditions, and peak table, and (below right) mass spectral data obtained for synthetic peptide WALMART. The average molecular weight is 847.06. (Courtesy of Peptides International, Louisville, KY, USA.)



Detector A Ch1 220nm PeakTable

Ret. Time	Area	Height	Area %
13.260	20922	3844	0.197
13.654	5655	1157	0.053
13.932	10440813	1419777	98.551
14.142	126942	19318	1.198
			100.000

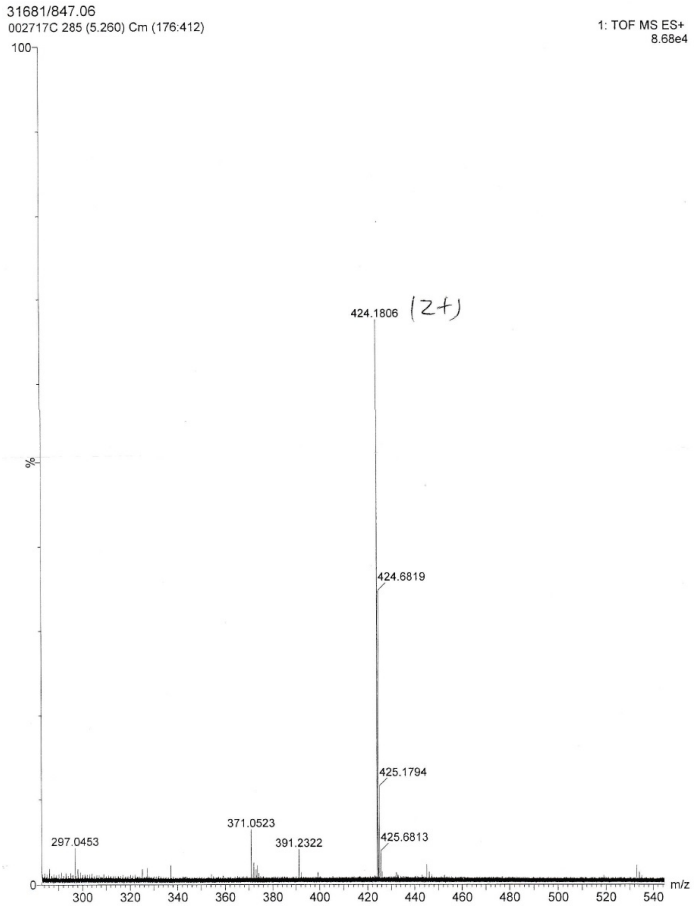


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Figure 5. Amino acid sequence of purified peptide WALMART. The MS/MS data is consistent with the desired peptide sequence. (Courtesy of Dr. Andrew Keightley, Biological Mass Spectrometry and Proteomics Facility, School of Biological Sciences, University of Missouri, Kansas City, MO, USA).

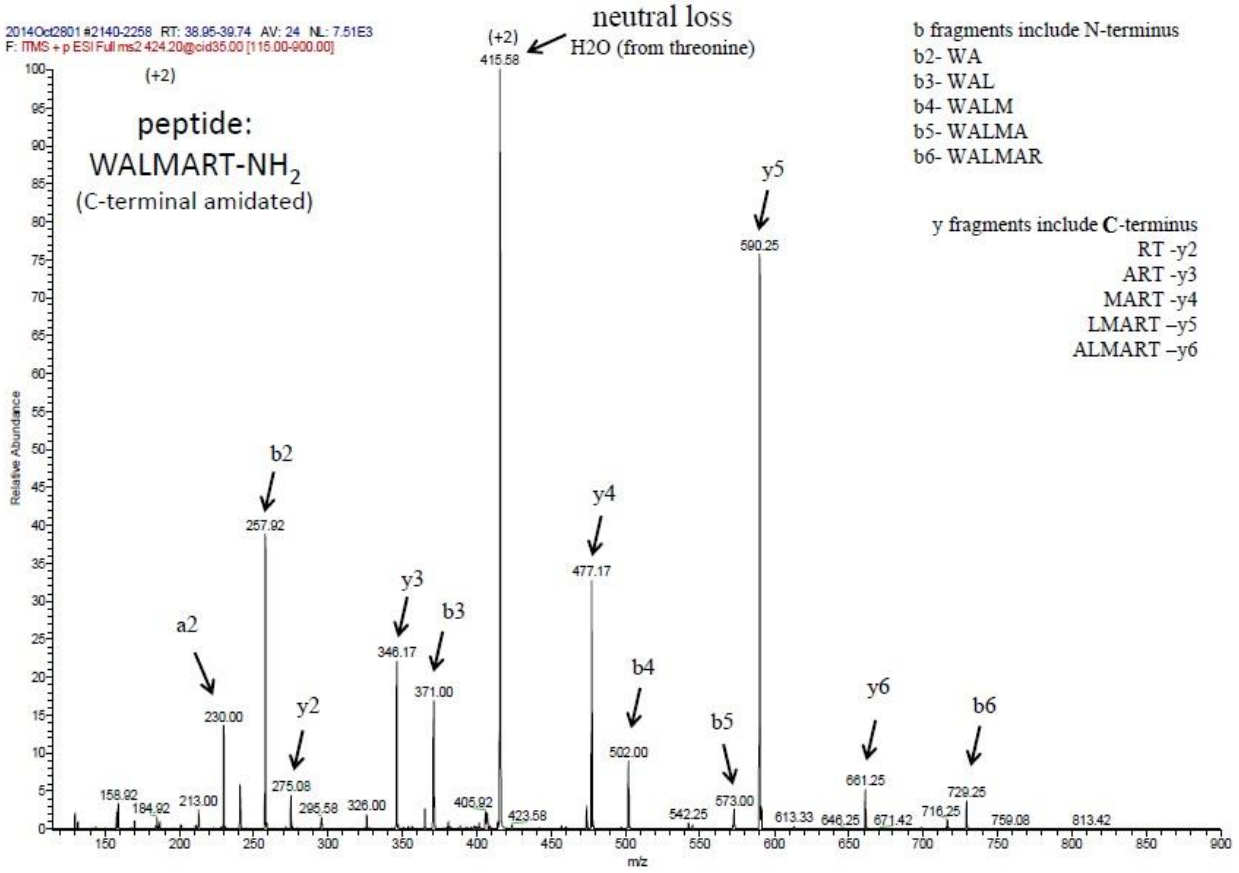

Figure 6. Peptide WALMART inhibits the growth of *Staphylococcus simulans*. A few flakes of peptide WALMART were added to the surface of a Luria/lysogeny broth (LB) agar plate coated with *Staph. simulans*, and also containing discs of ciprofloxacin [CIPRO; 10 mg/disc] and augmentin [AUG; 10 mg/disc]. After overnight incubation at 37 ºC, clear zones were readily apparent around the antibiotic discs. A few, smaller, clear zones were apparent where peptide WALMART had been added. When peptide WALMART was added to a sterile LB plate for 2 weeks, there was no growth, indicating that the peptide preparation was sterile. WALMART had no activity on a mixed bacterial culture from a urine sample. [Courtesy of Dr. George Pieczenik (private laboratory).]

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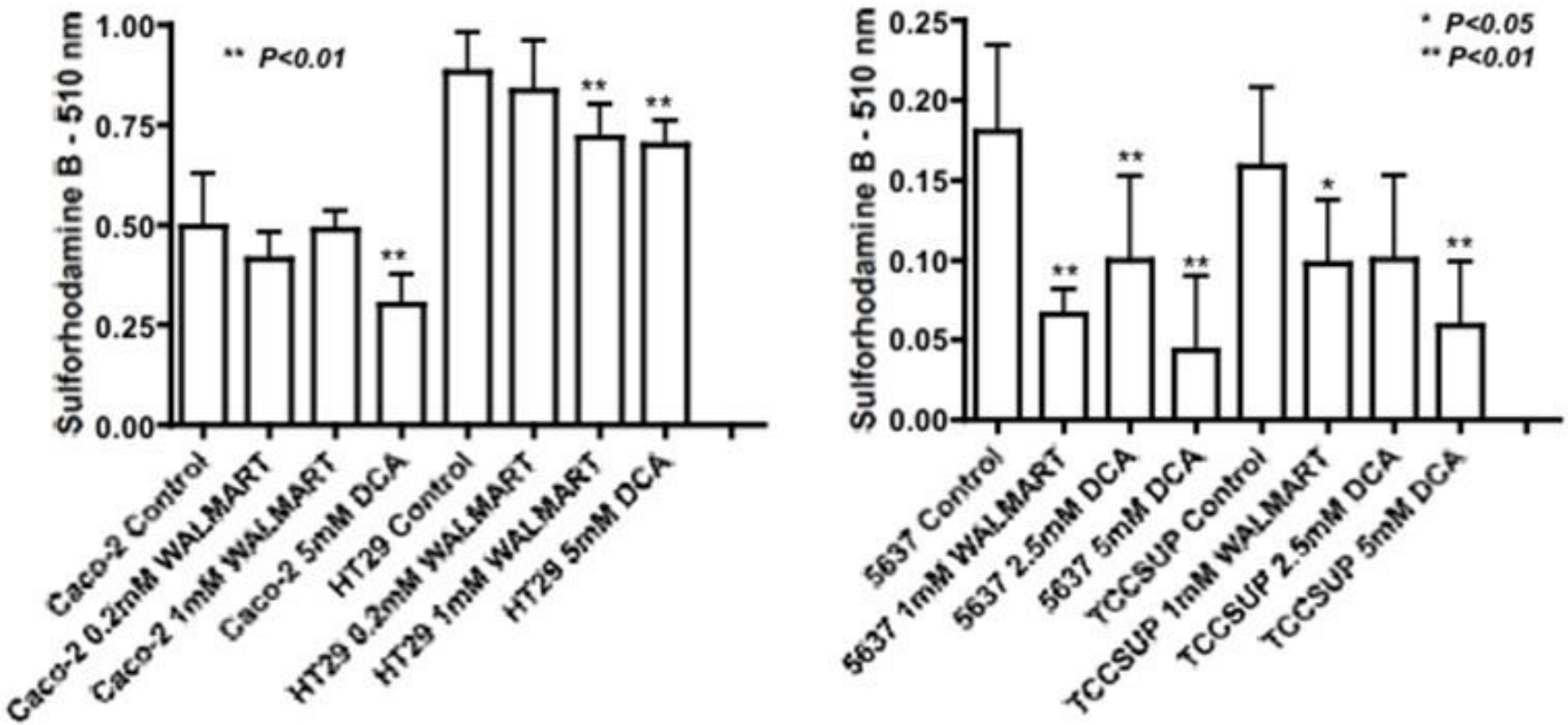
Figure 7. Effect of peptide WALMART and dichloroacetate (DCA) on (below left) the proliferation of human colorectal adenocarcinoma cells (Caco-2, male; HT-29, female), and (below right) the growth of human urinary bladder carcinoma cells (5637, male; TCCSUP, female). Caco-2 and HT29 human colon cancer cells, and 5637 and TCCSUP human bladder cancer cells, were obtained from the American Type Culture Collection, Rockville, MD, United States, and were incubated at 37 °C in RPMI-1640 medium with 5% fetal calf, and with or without DCA or WALMART for 72 hours [54]. Cell proliferation was generally monitored by the increase in protein. In studies with 96-well plates, the procedure involved staining with sulforhodamine B. Cells were routinely allowed to attach to tissue culture dishes or 96-well plates for 24 h before changing the medium. The cells were then incubated for a further 72 h before determining the impact of the compounds under study on cell proliferation as judged by protein mass. Data are presented as means and standard deviations of single experiments with six or more replicates. For each cell line the treated group was compared with its respective control using Dunnett's test for multiple comparisons. DCA (5 mM), but not WALMART (0.2-1.0 mM), inhibited the proliferation of Caco-2 cells. Both DCA (5 mM) and WALMART (1 mM) inhibited proliferation of HT-29 cells. DCA (2.5-5 mM) and WALMART (1 mM) both inhibited the growth of 5637 cells. DCA (5 mM) and WALMART (1 mM) also both inhibited the growth of TCCSUP cells.



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2. Effect of peptide WALMART on cancer cells:

Figure 7 shows the results of tests of peptide WALMART and DCA on Caco-2 (male) and HT29 (female) human colorectal adenocarcinoma cells, and 5637 (male) and TCCSUP (female) human urinary bladder carcinoma cells. DCA is a compound that is currently undergoing clinical trials for use as an anticancer therapeutic agent [55]. DCA (5 mM), but not peptide WALMART (0.2-1.0 mM), inhibited the proliferation of Caco-2 cells. Both DCA (5 mM) and peptide WALMART (1 mM) inhibited proliferation of HT-29 cells. DCA (2.5-5 mM) and peptide WALMART (1 mM) both inhibited the growth of 5637 cells. DCA (5 mM) and peptide WALMART (1 mM) also both inhibited the growth of TCCSUP cells.

Statistical analysis [54]

Data are presented as means and standard deviations. Statistical significance of the results was determined by a Dunnett's test for multiple comparisons using the Instat program from GraphPad Software, Inc., La Jolla, CA, United States. A probability of less than 5% was considered significant and differences compared to the control are shown.

Discussion

Although peptide WALMART has not yet been subjected to the same extent of testing as was peptide COLINPOWELL, the second test of the "name-to-peptide" method for creating novel, bioactive peptides appeared to be as successful as the first. The major difference between the two peptides was that peptide COLINPOWELL is not found in nature, whereas peptide WALMART is found among the amino acid sequences of naturally occurring proteins.

The positive result obtained in preliminary antimicrobial tests provides encouragement for more extensive testing with a variety of microorganisms, including drug resistant strains. The positive results obtained in assays with cancer cells were especially striking, in that peptide WALMART inhibited growth and proliferation of most of the cell types tested, and also at concentrations less than that of DCA, a compound currently undergoing clinical trials for possible use as an anticancer therapeutic agent. It is premature to speculate on possible mechanisms whereby peptide WALMART inhibits the growth of microbial and cancer cells, but it is hoped that further testing will provide clues to these mechanisms.

Acknowledgements

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