Published in final edited form as:

J Microbiol Methods. 2007 October ; 71(1): 87–89.

Commercial ampholytes used for isoelectric focusing may interfere with bioactivity based purification of antimicrobial peptides

Shadi Riazi¹, Sara Dover^{1,2}, Yevgeniy Turovskiy¹, and Michael L. Chikindas^{1,*}

¹Department of Food Science, Rutgers, the State University of New Jersey, New Brunswick, New Jersey 08901, USA

²The Royal Institute of Technology, Stockholm, Sweden

Abstract

BioRad's Rotofor[®] system has been frequently used for the purification of proteins and smaller peptides such as bacteriocins. In this study, we report that some commercially available ampholytes used with the Rotofor[®] isoelectric focusing system possess antimicrobial activity, which may interfere with the purification of bacteriocins and bacteriocin-like substances.

Keywords

Ampholytes; Rotofor; isoelectric focusing; bacteriocins

Rotofor[®] is an isoelectric focusing apparatus which has been used for more than a decade for the purpose of protein purification (Ayala et al., 1998). Preservation of the biological activity may be crucial when biologically active proteins and peptides are studied using this purification method. Little attention is given, however, to the fact that the ampholytes used for the isoelectric focusing may actually interfere with some proteins' biological activity or with the activity detection assays.

Bacteriocins are small antimicrobial proteins that are synthesized ribosomally by virtually all bacteria (Cleveland et al., 2001). Bacteriocins of lactic acid bacteria have attracted much attention due to their food-related, personal care and medical applications. Purification of these antimicrobials using isoelectric focusing has been reported by several research groups (i.e., Green et al., 1997, Venema et al., 1997).

In Rotofor[®], the separation of proteins is caused by formation of a gradually increasing pH gradient. This pH gradient is formed by ampholytes, zwitterionic molecules with low molecular weight, as the electric field is applied. The ampholyte systems used for the isoelectric focusing commonly contain a mixture of compounds with different isoelectric point (pI) values. When the electric field is applied, ampholyte molecules tend to migrate according to their net charge and then align themselves in a specific position between anode and cathode based on their

* Author for correspondence. Tel: +1-732-932-9611 Ext. 218; Fax: +1-732-932-6776 E-mail: tchikindas@aesop.rutgers.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

isoelectric point (Stoyanov et al., 2005). In this study, we investigated the antimicrobial activity of the commercially available ampholytes commonly used for purification of the small antimicrobial peptides.

The ampholytes used in the study were Bio-Lyte® pH 3-10 (BioRad, Hercules, CA), Pharmalyte pH 3-10 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and Ampholine pH 3.5-9.5 (Amersham Biosciences AB, Uppsala, Sweden). The activity of ampholytes was tested against *Micrococcus luteus* ATCC 10420, *Escherichia coli* O157:H7 ATCC 43859, *Listeria monocytogenes* Scott A, *Salmonella enterica* serovar Typhimurium UMR1, *Lactobacillus rhamnosus* 160 (L 160), *Lactobacillus* 710 (L 710), *Lactobacillus lactis* NCK 401 (NCK 401), *Pediococcus pentosaceus* ATCC 43200, and *Lactobacillus casei* strain Shirota (LcS) in well diffusion assay as described by Lyon and Glatz (Lyon and Glatz, 1992). All assays were performed in duplicate. Nisin (10 mg/ml) and nisin diluent (hydrochloric acid solution, pH 1.7) were used as positive and negative controls for this experiment. Bio-Lyte®, Ampholine™ and Pharmalyte™ total samples were active against *L. monocytogenes*, *P. pentosaceus*, *M. luteus*, *L. casei* Shirota, *L. rhamnosus* 160, *L. lactis* NCK 401, *L. casei* Shirota, and *Lactobacillus* 710. All tested ampholytes were inhibitory to the Gram-positive bacteria used in this experiment and non-inhibitory to Gram-negative strains (Table 1). The inhibitory effects of ampholytes on Gram-positive and Gram-negative bacteria are similar to those of bacteriocins (Green et al. 1997). In Gram-negative bacteria, the outer membrane, which consists of phospholipids, proteins and lipopolysaccharides (LPS), protects the cell from antimicrobial action of bacteriocins and other small antimicrobial peptides (Abee et al 1995).

The sample for Rotofor® was prepared by adding 2% (w/v) of ampholyte, 10% (v/v) glycerol (FisherChemicals, Fairlawn, NJ) and glycine (Merck, Darmstadt, Germany) at a final concentration of 5% (w/v) to 50 ml deionized water. The sample was loaded into the Rotofor® cell. After the procedure was completed the fractions were harvested and the pH of each fraction was measured and adjusted to approximately 5.0. The activity of each fraction was tested in well-diffusion assay using *M. luteus* ATCC 10420 as an indicator microorganism (Venema et al., 1997). The investigated electrolyte samples seemed to display a common trend in antimicrobial activity against *M. luteus*; the most noticeable inhibitory effect was exhibited by the fractions with higher isoelectric points. For the Bio-Lyte® brand, progressively increasing antimicrobial activity was observed in the fractions 15 through 20 (Fig. 1A). Similarly, the Pharmalyte™ displayed increasing antimicrobial activity in fractions 18 through 20. Interestingly, for this brand, some antimicrobial activity was also noticeable in fraction 1 (Fig. 1C). In the Ampholine™ sample, only fractions 18, 19, and 20 were inhibitory to the indicator microorganism, and though fractions 18 and 19 are the most inhibitory to the indicator microorganism (Fig. 1B), the zone of inhibition in these fractions was not sharp. Fraction 14 to 20 of the nisin sample (10mg/ml) subjected to isoelectric focusing caused very sharp inhibition zones when used with the Bio-Lyte® ampholyte compared to the Bio-Lyte® sample alone (Fig. 1A). Interestingly, the total sample of Ampholine™, when tested against *M. luteus*, showed a much larger zone of inhibition (12 mm) than the fraction 20 (pI 9-10) that was separated through the Rotofor®.

The commercially available ampholyte systems used in this study contain mixtures of short polypeptide molecules. Each of these molecules has a distinct pI point. There are only two amino acid residues (lysine and tyrosine) with the pK_a of their side chains in the range between 9 and 10. The polypeptide molecules with the pI point in the range 9-10 are likely to be rich in lysine and/or tyrosine. The antimicrobial effect of the ampholyte mixture can be due to various poly-lysines that are well known for their antimicrobial properties (Liang and Kim 1999, Yoshida and Nagasawa 2003). The presence of poly-lysine in the ampholyte mixture is purely speculative, since the specific composition of the commercial ampholytes from Amersham and Bio-Rad is not disclosed by the manufacturers (Stoyanov et al., 2005).

Ultimately, however, these commercial ampholyte systems will have to be reformulated if they are to be used for purification of antimicrobial proteins based on the function. Finally, our observations suggest that of all ampholytes tested, Ampholine™ could be the most favorable choice for use in the Rotofor® system to purify antimicrobial peptides.

Acknowledgements

This research was sponsored by NIH grant “Natural antimicrobials against bacterial vaginosis” NCCAM NIH R21AT002897-01. The authors thank Linda Rosenberg for editorial work.

References

- Abee T, Krockel L, Hill C. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *Int. J. Food Microbiol* 1995;28:169–185. [PubMed: 8750665]
- Ayala A, Parrado J, Machado A. Use of Rotofor preparative isoelectric focusing cell in protein purification procedure. *Appl. Biochem Biotechnol* 1998;69(1):11–116. [PubMed: 9457755]
- Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. *Int. J. Food Microbiol* 2001;71:1–20. [PubMed: 11764886]
- Green G, Dicks LMT, Bruggeman G, Vandamme EJ, Chikindas ML. Pediocin PD-1, a bactericidal antimicrobial peptide from *Pediococcus damnosus* NCFB 1832. *J. Appl. Microbiol* 1997;83:127–132. [PubMed: 9246779]
- Liang JF, Kim SC. Not only the nature of peptide but also the characteristics of cell membrane determine the antimicrobial mechanism of a peptide. *J. Peptide Res* 1999;53:518–522. [PubMed: 10424346]
- Lyon WJ, Glatz BA. Isolation and purification of propionicin PLG-1, a bacteriocin produced by a strain of *Propionibacterium thoenii*. *Appl. Environ. Microbiol* 1993;59(1):83–88. [PubMed: 8439170]
- Stoyanov AV, Das C, Fredrickson CK, Fan ZH. Conductivity properties of carrier ampholyte pH gradient in isoelectric focusing. *Electrophoresis* 2005;26:473–479. [PubMed: 15657903]
- Venema K, Chikindas ML, Seegers FML, Handrikman AJ, Leenhouts KJ, Venema G, Kok I. Rapid and efficient purification method for small, hydrophobic, cationic bacteriocins: purification of Lactococcin B and Pediocin PA-1. *Appl. Environ. Microbiol* 1997;63(1):305–309. [PubMed: 16535495]
- Yoshida T, Nagasawa T. epsilon-Poly-L-lysine: microbial production, biodegradation and application potential. *Appl. Microbiol. Biotechnol* 2003;62:21–26. [PubMed: 12728342]

B

C

Fig. 1.

Antimicrobial activity distribution of Bio-Lyte® (A), Ampholine™ (B), and Pharmalyte™ (C) ampholytes after preparative isoelectric focusing. The inhibitory activity was determined by well diffusion assay using *M. luteus* ATCC 10420 as the indicator strain. Figure inserts iA and iiA show inhibition zones caused by Bio-Lyte® and nisin (10 mg/ml), respectively, in well diffusion assay against *M. luteus*. Similarly figure inserts iB and iC are visual representation of zones of inhibition caused by Ampholine™ and Pharmalyte™, respectively, after being fractioned through Rotofor®.

Zones of inhibition (mm) caused by Bio-Lyte[®], Ampholyte[™] and Pharmalyte[™] against different bacterial strains. The inhibitory activity of ampholytes was determined by well diffusion assay.

Table. 1

Bacteria	Bio-Lyte [®]	Ampholyte [™] Ampholyne [™]	Pharmalyte [™]	Nisin (10mg/ml)	Controls	Nisin diluent
<i>E. coli</i> O157: H7	0	0	0	0		0
<i>S. Typhimurium</i>	0	0	0	0		0
<i>L. monocytogenes</i>	1	1	2	7		0
<i>P. pentosaceus</i>	6	2	4	15		0
<i>M. luteus</i>	18	12	17.5	15		0
<i>L. lactis</i> NCK 401	2	0	2	2		0
<i>L. casei</i> Shirota	3	2	2	10		0
<i>L. rhamnosus</i> 160	1	1	1	16		0
<i>Lactobacillus</i> 710	3	2	4	15		0