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Antioxidant activity of peptide fractions of capelin protein hydrolysates

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Four peptide fractions were separated from protein hydrolysates of capelin (*Mallotus villosus*) using Sephadex G-10 gel filtration column chromatography. Antioxidant activity of each fraction was determined in a β-carotene-linoleate model system. One isolated fraction possessed a notable antioxidant activity, another two had a weak efficacy while the fourth exerted a prooxidant effect. Two-dimensional thin layer chromatography (TLC) of isolated fractions gave spots with both antioxidant and prooxidant activities. Two prooxidant compounds were separated from the hydrolysate by preparative TLC using silica gel plates. One compound exhibited a maximum absorption at 254 nm while the other absorbed at 260 nm. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Hydrolysed proteins from many animal and plant sources, individual peptides and amino acids have been found to possess antioxidant activity. Large quantities of yeast and soybean protein hydrolysates were used to inhibit the oxidation of corn oil (Bishov & Henick, 1972; Bishov & Henick, 1975). Some amino acids were reported as having strong antioxidant activity in linoleic acid and methyl linoleate model systems (Marcuse, 1962). A polar fraction from krill extract, which was identified as containing a mixture of numerous amino acids, possessed strong antioxidant activity (Seher & Löschner, 1985). A combination of tryptophan and lysine was effective in butter fat (Merzametov & Gadzhieva, 1976). Furthermore, the antioxidative action of proline in sardine oil (Revankar, 1974), methionine in vegetable oils (Sims & Fioriti, 1977), and histidine, threonine, lysine and methionine in a sunflower oil emulsion (Riison *et al.*, 1980) has been reported. An antioxidative effect, *in vivo*, is suggested for taurine, hypotaurine (Aruoma *et al.*, 1988), carnosine and anserine (Aruoma *et al.*, 1989). S-Nitrosocysteine (RSNO), a compound which has been shown to be generated during the curing process of meat, was found to act as an antioxidant (Kanner, 1979).

Capelin (*Mallotus villosus*) is a small silvery fish found in the coastal waters of Newfoundland. While female capelin is a popular food item in Japan, male capelin remains underutilized. Recently, we reported the production of capelin protein hydrolysate (Shahidi *et al.*, 1995); it may be used in a variety of applications such as athletic drinks, protein extenders in emulsified meat products and cereal based foods and for patients with gastrointestinal tract complications.

The present study reports on the antioxidant activity of peptide fractions isolated from protein hydrolysates of capelin.

MATERIALS AND METHODS

Protein hydrolysates were prepared from capelin (*Mallotus villosus*) according to Shahidi *et al.* (1995). Peptides were separated on a column (1 × 100 cm) packed with Sephadex G-10 using water and 0.1 M acetic acid for elution (Rochat *et al.*, 1970). The antioxidative properties of isolated fractions were investigated in a β-carotene-linoleate model system. To 5 mL of the model emulsion (Miller, 1971) were added 0.2 mL of water containing 5 mg of dissolved peptide fractions. Samples were incubated for 2 h at 50°C and absorbance readings were monitored at 470 nm every 15 min. Two-dimensional TLC of the peptide fractions was carried out on silica gel HPTLC plates (Sigma, St. Louis, MO) using propanol-water (7:3, v/v) followed by a *n*-butanol-acetic acid-water (4:1:2, v/v/v) system (Matsumoto *et*

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al., 1976). Spots from peptides were visualized on plates after spraying with a ninhydrin solution. A second set of plates was sprayed with the β-carotene-linoleate solution (Philip, 1974) in order to evaluate the effect of separated compounds on the oxidative state of the sprayed solution. While spots of prooxidative peptides were quickly bleached, those of antioxidative compounds remained yellow even after several hours from the time of spraying.

For isolation of prooxidant compounds, 1 g of capelin hydrolysate was suspended in 50 mL of methanol and then sonicated for 10 min. After filtration, the methanolic extract was concentrated to 2 mL and then applied on a PK5F silica gel preparative plate (thickness 1 mm; Whatman). The plate was developed using chloroform-methanol-water (65:35:10, v/v/v, lower phase) (Amarowicz *et al.*, 1995*a*). Among compounds separated, two were ninhydrin-negative but prooxidative. These compounds were further examined using analytical silica gel TLC plates (Sigma-Aldrich) and the same developing system. Plates were sprayed with solutions of ninhydrin, ferric chloride and ferric chloride-potassium ferricyanide (Krebs *et al.*, 1969). UV spectra of two compounds in methanol were recorded using a Hewlett Packard 8452A diode array spectrophotometer.

RESULTS AND DISCUSSION

Four peptide fractions (I-IV) were separated from capelin protein hydrolysate using Sephadex G-10 gel filtration column chromatography based on absorbance readings at 220 and 280 nm (Fig. 1). Fractions I-III were eluted from the column by water while fraction IV was eluted using a 0.1 M acetic acid solution. For all fractions, the optical density (OD) at 220 nm (peptides bonds) was greater than that at 280 nm (aromatic ring). Relatively high OD values at 280 nm were noted for fraction III, which might indicate the presence of a high content of tyrosine and tryptophan in the peptides of this fraction or the existence of other aromatic compounds.

Application of peptides of fractions I, II and IV to model emulsion systems resulted in a delay in the bleaching of β-carotene (Fig. 2), thus reflecting their antioxidant action. However, the effect for fractions II and IV was noticeable only during the first part of the incubation period. Fraction I exhibited a much higher antioxidant efficacy than fractions II and IV. Addition of fraction III to the system hastened the bleaching of β-carotene during the first 45 min of heating, thus reflecting a prooxidative nature. Shahidi & Amarowicz (1996) have previously reported the prooxidant activity of seal protein hydrolysates. The antioxidant activity of fraction I was similar to that of the lesser active phenolic fractions separated by column chromatography from flax (Amarowicz *et al.*, 1994) and mustard seeds (Amarowicz *et al.*, 1996*a*). A majority of phenolic fractions separated by column chromatography from plant extracts and examined in a β-carotene-linoleate model system, exhibited a much stronger antioxidant activity than fraction I (Amarowicz *et al.*, 1993, 1995*b*, 1996*b*; Amarowicz & Shahidi, 1995).

The weak antioxidant activity of fractions II and IV may be due to the presence of prooxidative compounds. While some amino acids such as cysteine may act as prooxidants (Pratt & Hudson, 1990), histidine has been shown to revert from being an antioxidant to a prooxidant at higher concentrations; in the case of tryptophan this tendency was less. Copper bound to amino acids or peptides has a strong catalysing effect on the oxidation

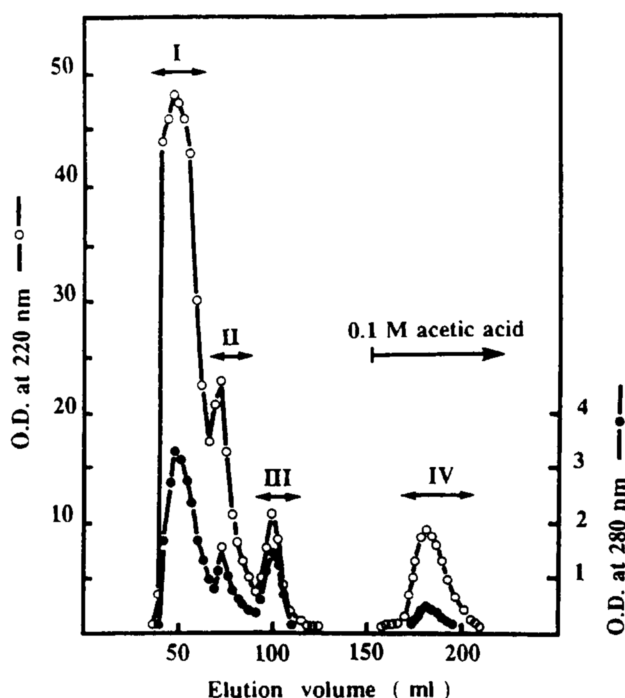

Fig. 1. Separation of peptide fractions from capelin protein hydrolysate by Sephadex G-10 gel filtration.

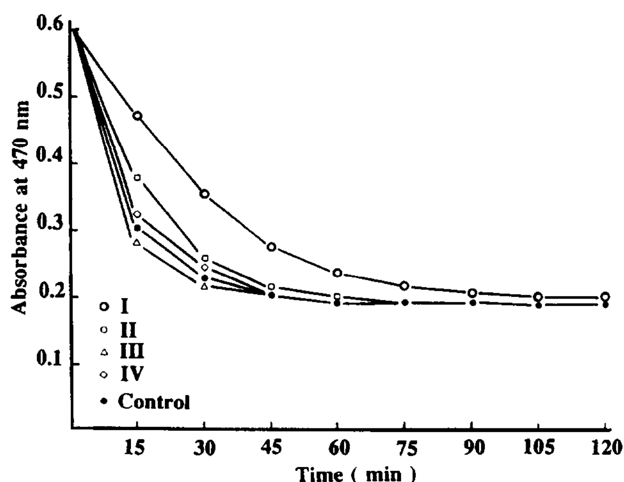

Fig. 2. Antioxidant activity of peptide fractions of capelin protein hydrolysates in a β-carotene-linoleate model system, as measured by changes in absorbance values at 470 nm.

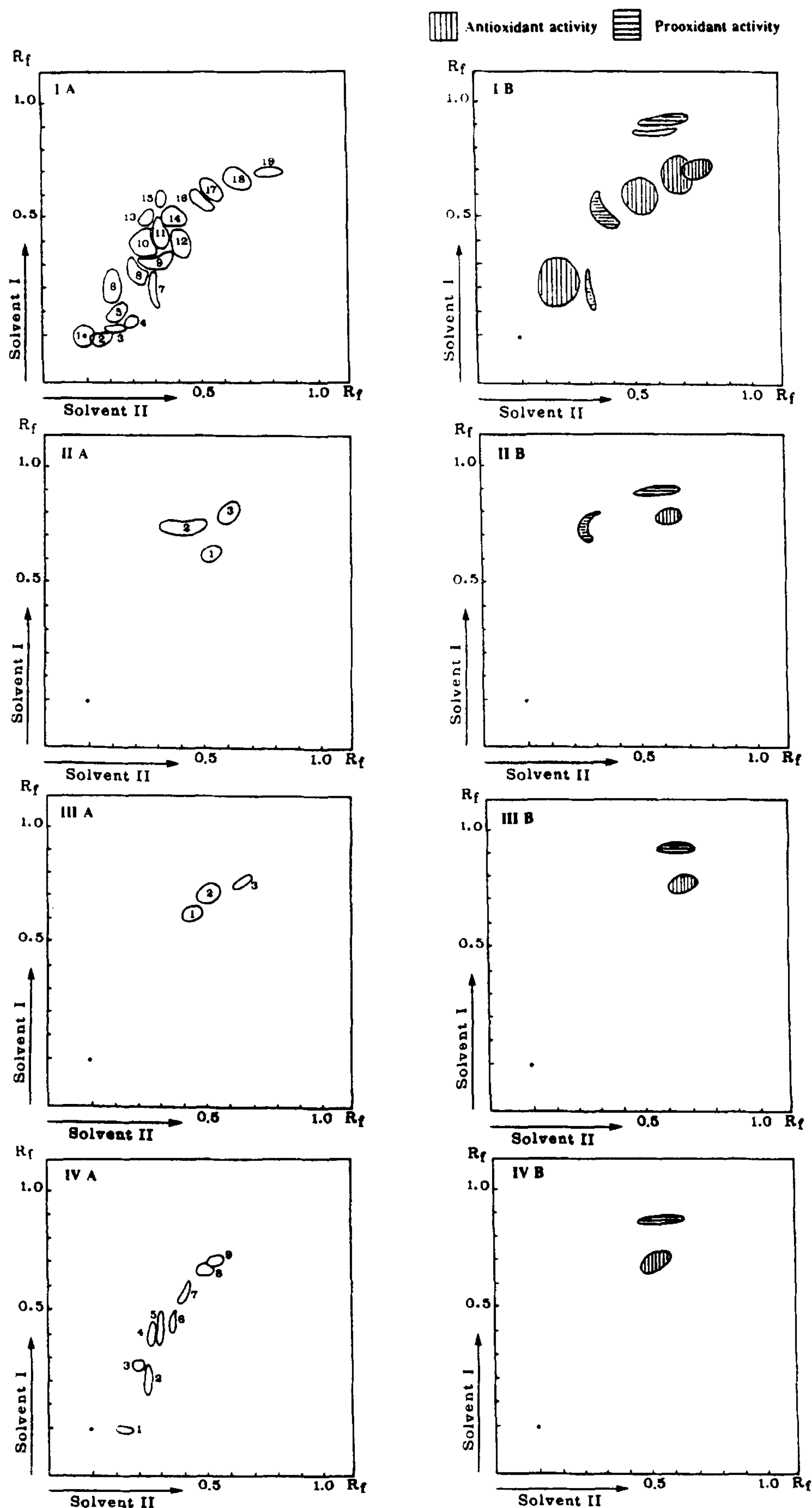

Fig. 3. Two-dimensional thin layer chromatography of peptide fractions of capelin protein hydrolysate; plates were sprayed with: (A), a ninhydrin solution to give spots of peptides and (B), a β-carotene-linoleate solution in order to evaluate antioxidant or prooxidant activity of compounds of each spot; I–IV – fraction numbers.

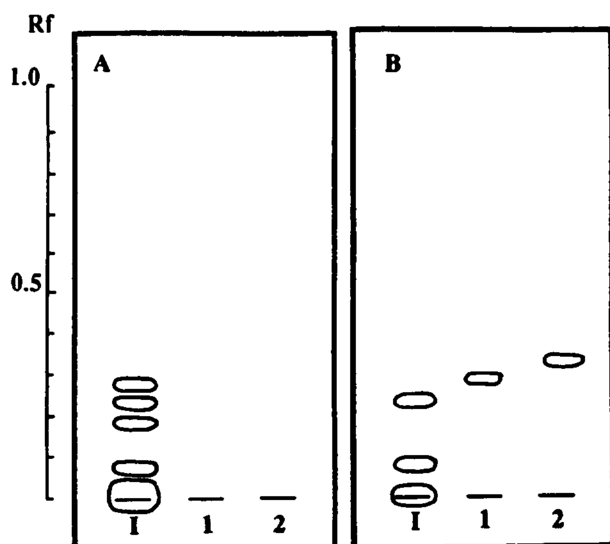

Fig. 4. Silica gel TLC chromatogram of methanolic extract of capelin protein hydrolysate (I) and two prooxidant compounds (1 and 2); plates were sprayed with: (A), a ninhydrin solution to identify peptides spots and (B), a ferric chloride-potassium ferricyanide solution in order to identify reducing compounds.

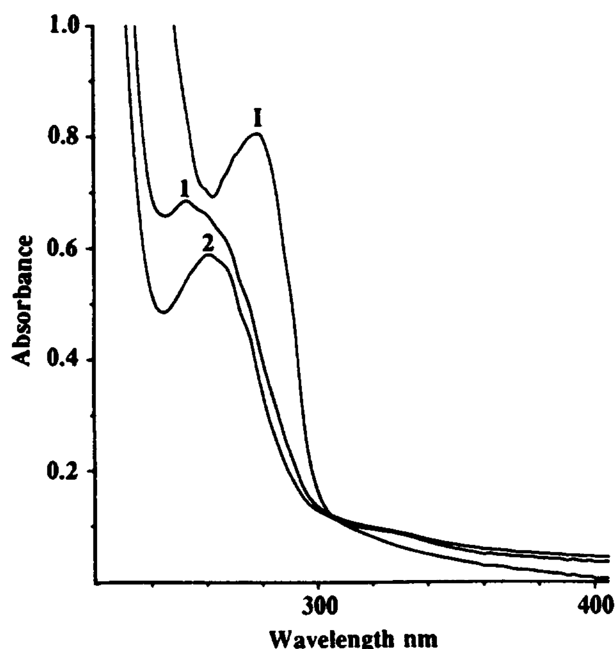

Fig. 5. UV spectra of methanolic extract of capelin protein hydrolysate (1) and two prooxidant compounds (1 and 2).

of linoleic acid (Marcuse, 1962). Differences in the antioxidant properties of peptide fractions might originate from their synergistic action with the emulsifier (Tween 40) used in the analysis or with peptides in the system. Emulsifiers are known to decrease the particle size of peptides and increase the contact surface of the phases (Marcuse 1962).

Thin layer chromatographic analysis of the hydrolysate from capelin resulted in a large number of spots - 19 in fraction I, 3 in each of II and III, and 9 in IV (Fig. 3). Using the same chromatographic method for capelin and seal protein hydrolysates (without gel filtration), 17 and 18 peptide spots, respectively, were detected on TLC plates (Shahidi & Amarowicz, 1996). In comparison with capelin protein hydrolysate, seal protein hydrolysate possessed a smaller number of lesser polar peptides. Furthermore, hydrolysates from seal muscle protein, analyzed by paper and thin layer chromatography, were shown to contain more than 22 peptide compounds (Matsumoto *et al.*, 1976).

Spraying of plates with the β-carotene-linoleate solution indicated the presence, in each fraction, of both antioxidant and prooxidant compounds. The majority of antioxidant compounds detected were found in fraction I; compounds from spots 5, 6, 8, 16, 17, 18 and 19 showed antioxidant activity. Such antioxidant efficacy was also demonstrated for spot 3 in fractions II and III, and for spots 8 and 9 in fraction IV. The presence of many antioxidant compounds in fraction I is in line with results of the β-carotene-linoleate experiments (Fig. 2). Prooxidant compounds appeared on TLC plates from all fractions as elongated spots and they did not give a positive reaction with ninhydrin. In addition, spots 7, 14 and 15 in fraction I and a ninhydrin-negative spot in fraction II (Fig. 1, plate IIB) exhibited prooxidant activity.

Two prooxidant compounds isolated from methanolic extract of capelin hydrolysates were characterized on TLC plates; their R_f values were 0.29 (1) and 0.32 (2) (Fig. 4). Both compounds did not give a positive reaction with ninhydrin and ferric chloride. However, spraying of the plate with a solution of ferric chloride-potassium ferricyanide gave blue spots which indicated the reducing properties of compounds involved.

The UV absorption maxima of compounds 1 and 2 were 254 and 260 nm, respectively (Fig. 5). For methanolic extract of capelin hydrolysates (Fig. 5 — spectra 1), the absorption maximum was at a longer wavelength of 278 nm. The negative reaction with ferric chloride and UV absorption characteristics of the compounds (a shorter wavelength) indicated that these prooxidative compounds were not phenolic in nature. Therefore, these substances with reducing properties may belong to the amine or thiol group of compounds (Krebs *et al.*, 1969).

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