Antioxidant Activity of Protein Hydrolyzates from Aquatic Species

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ABSTRACT: Antioxidant activity of protein hydrolyzates from capelin (*Mallotus villosus*) and harp seal (*Phoca groenlandica*) was examined in a β-carotene/linoleate model system. Capelin protein hydrolyzates exhibited a significant ($P < 0.05$) antioxidant effect during the entire or a portion of the 120-min incubation period when added to model systems at 1 to 10-mg levels per 5 mL of lipid emulsion. However, systems containing 10 mg of seal protein hydrolyzate (SPH) displayed a significant ($P < 0.05$) prooxidative effect during the experimentation period, but the effect was less pronounced at 5 mg of SPH addition. This effect was reversed to a weak, but significant ($P < 0.05$), antioxidant effect when 1 or 2 mg of SPH were present after 45, 105–120, and 30–120 min of incubation. Two-dimensional thin-layer chromatographic separation of both hydrolyzates gave spots with antioxidant and prooxidant activities.
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KEY WORDS: Antioxidant and prooxidant activity, capelin, hydrolysates, peptides, seal, TLC.

Protein hydrolyzates and amino acids have frequently been tested as antioxidants in many experimental systems. Autolyzed yeast and hydrolyzed soybean proteins were effective antioxidants in a freeze-dried model system that contained tocopherol-free corn oil (1), and exhibited synergistic effects with butylated hydroxyanisole (BHA), butylated hydroxytoluene, and tocopherols (1,2). Good antioxidant activity was noticed for histidine and tryptophan in both linoleic acid and methyl linoleate (3). A combination of tryptophan and lysine was effective in inhibiting oxidation of butterfat (4). Methionine was a good antioxidant for vegetable oils (5), whereas histidine, threonine, lysine, and methionine showed antioxidant activity in sunflower oil emulsions (6). Antioxidant action, *in vivo,* is suggested for taurine, hypotaurine (7), carnosine, and anserine (8). Antioxidative capacity of proline was equivalent to that of BHA in sardine oil (9), and a nitroxide derivative of proline was reported to have antioxidant activity (10). A polar fraction of krill extract, which was identified as a mixture of numerous amino acids, was reported as having strong antioxidant activity (11). On the other hand, some amino acids, such as cysteine, may act as prooxidants (3,12).

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The present study reports on the antioxidant activity of protein hydrolyzates from two aquatic species, namely capelin and harp seal.

EXPERIMENTAL PROCEDURES

Protein hydrolyzates were prepared, in triplicate runs, from capelin *(Mallotus villosus)* and harp seal *(Phoca groenlandica)* according to Shahidi *et al.* (13,14). Antioxidative properties of these hydrolyzates were investigated in a β-carotene-linoleate model system. A linoleate model emulsion was prepared according to the procedure of Miller (15). Five-milliliter aliquots of the emulsion were transferred to glass tubes, to which 0.2 mL distilled water, containing hydrolyzates (either 1, 2, 5, or 10 mg), were added. All samples were incubated simultaneously for 2 h in a water bath (Lab-Line Instruments, Inc., Melrose Park, IL) at 50°C; absorbance values at 470 nm were recorded every 15 min with an HP 8452A photodiode array spectrophotometer (Hewlett-Packard Corp., Mississauga, Ontario, Canada).

Two-dimensional thin-layer chromatographic (TLC) separation of capelin and seal protein hydrolyzates (CPH and SPH) was carried out on silica gel high-performance TLC plates (Sigma, St. Louis, MO) with a propanol/water (7:3, vol/vol) mixture, followed by an *n*-butanol/acetic acid/water (4:1:2, vol/vol/vol) system (16). Spots from peptides were visualized on plates after spraying with a ninhydrin solution, which affords red- or violet-colored complexes. Plates were also sprayed with a β-carotene/linoleate solution to examine pro- or antioxidative effects of compounds on each. Plates were placed in an oven at 60°C for 10 min to hasten the oxidation of linoleate (17). Faster bleaching of spots, as compared to that of the whole plate, is taken as an indication of prooxidative nature of peptides, while antioxidative spots retain a yellow color even after two hours from the time of spraying.

In all experiments, samples were analyzed in triplicate, and mean values ± standard deviation were recorded. Significant differences between the control and test samples were determined at a 95% level of probability (18).

RESULTS AND DISCUSSION

Addition of CPH to model emulsions resulted in an antioxidative effect by delaying the bleaching of β-carotene (Fig. 1).

This effect was more pronounced during the early phase of the incubation period. Differences in absorbance readings at 470 nm between the control and the system containing 1 mg of CPH were significant ($P < 0.05$), but only during the first 105 min of experimentation. However, systems that contained 2, 5, and 10 mg of CPH were significantly ($P < 0.05$) different from that of the control during the entire incubation period. Furthermore, inhibition of oxidation exerted by 5 and 10 mg of CPH in the systems was similar, and the existing effects were significantly ($P < 0.05$) higher than those imparted by 1 and 2 mg CPH. However, the addition of SPH at 10 mg showed a significant ($P < 0.05$) prooxidative activity, as compared with the control, during the entire incubation period (Fig. 2). The system that contained 5 mg SPH exhibited a significant ($P < 0.05$) prooxidative effect only after 60 and 75 min of incubation. Weak antioxidative effects were noticed when 1 or 2 mg of SPH was used. The effect was noted after 30–120 min for the system that contained 2 mg SPH and after 45, 105, and 120 min for that containing 1 mg SPH. This observation gains support from the findings of Marcuse (3), who reported that most amino acids possess marked antioxidant activity at low concentrations, but with an increasing concentration many became prooxidative.

TLC analysis of both hydrolyzates was characterized with a large number of ninhydrin-positive spots—7 for CPH and 18 for SPH (Figs. 3 and 4, respectively). Three groups of peptides from CPH (Fig. 3) were noticed on the TLC plates—peptides 1–6, 7–12, and 13–17. The less polar peptides of SPH (7–18) had different R_f values than those from capelin. The peptides close to the starting point, i.e., more polar components, possessed antioxidative activity. Prooxidant compounds from both hydrolyzates appeared as elongated spots and did not give a positive reaction with ninhydrin. Two additional spots with antioxidative peptides were also noticed for capelin hydrolyzate on the TLC plate, which further emphasizes the stronger antioxidant properties of this preparation in a β-carotene/linoleate system. Further work is in progress to shed light on the structural characteristics of peptides involved.

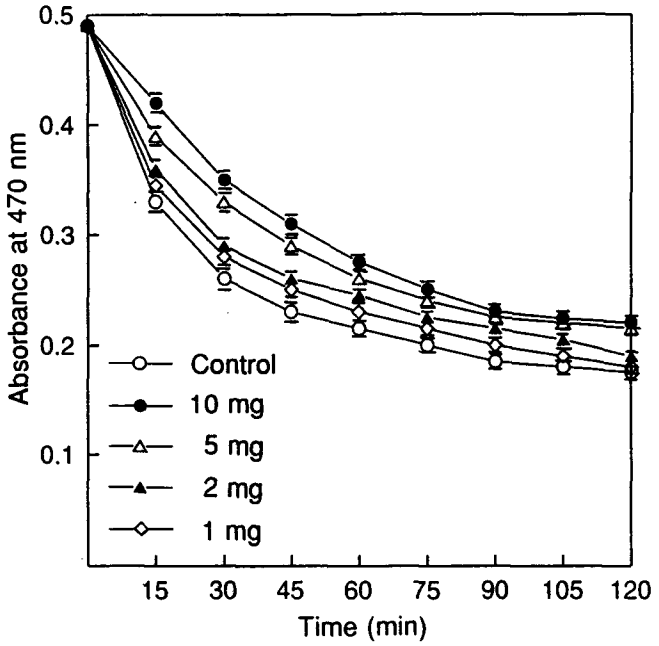

FIG. 1. Antioxidant activity of capelin protein hydrolyzate in a β-carotene/linoleate model system, as measured by changes in absorbance values at 470 nm. Error bars represent standard deviation from means.

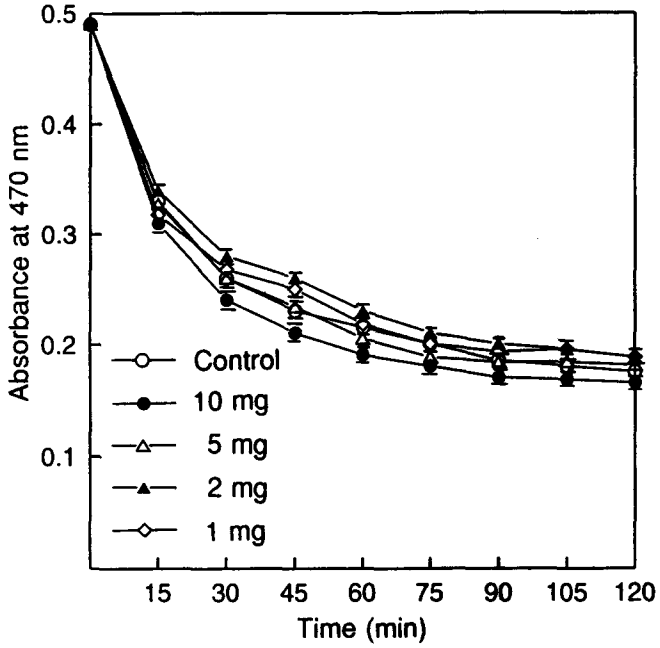

FIG. 2. Antioxidant activity of seal protein hydrolyzate in a β-carotene/linoleate model system, as measured by changes in absorbance values at 470 nm. Error bars represent standard deviation from means.

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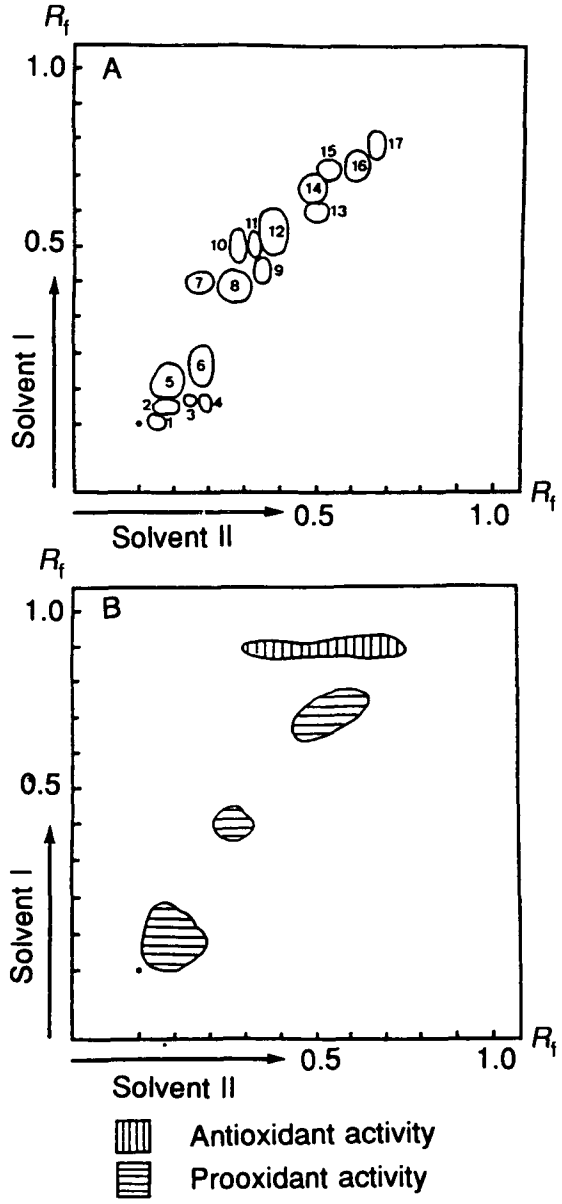

FIG. 3. Two-dimensional thin-layer chromatography of capelin protein hydrolyzate. Plate was sprayed with: A, ninhydrin solution to give colored complexes of peptides; and B, β-carotene/linoleate solution to evaluate antioxidant or prooxidant activity of compounds of each spot.

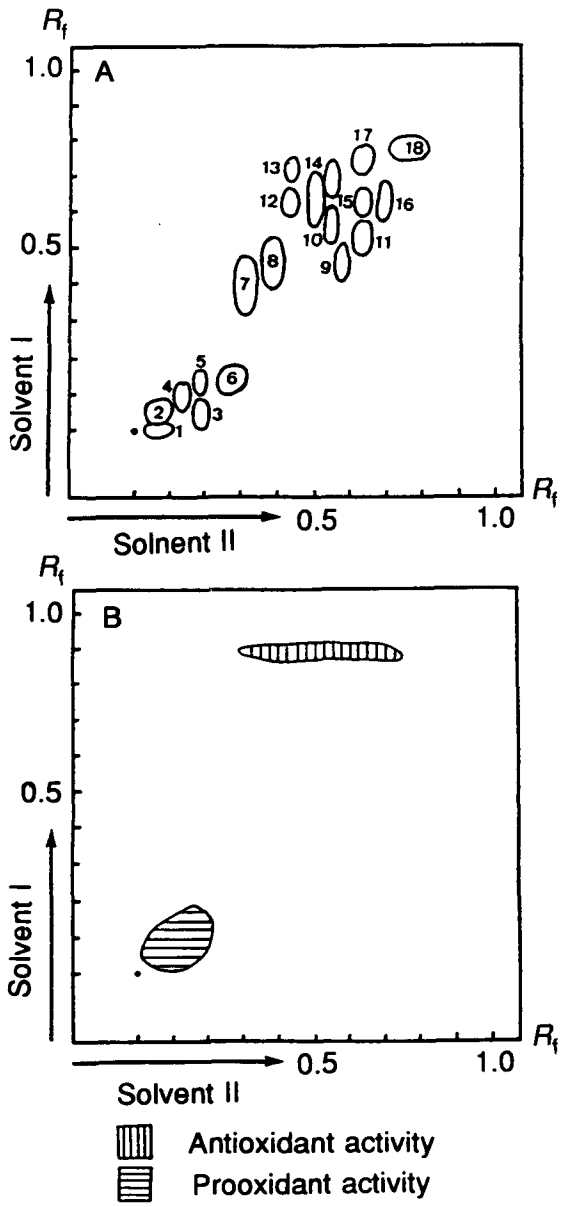

FIG. 4. Two-dimensional thin-layer chromatography of seal protein hydrolyzate. Plate was sprayed with: A, ninhydrin solution to give colored complexes of peptides; and B, β-carotene/linoleate solution to evaluate antioxidant or prooxidant activity of compounds of each spot.

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