Peptide Contamination from Cellulose Dialysis Membrane

Cellulosic dialysis membranes were shown to release peptides into the dialysate and retentate during equilibrium dialysis, pervaporation, and electrodialysis procedures. Two-dimensional peptide mapping revealed the presence of eight peptide zones in the water extract of the cellulosic membranes. Gel filtration chromatography on Sephadex G-10 and G-25 columns indicated that free amino acids were not responsible for the ninhydrin positive zones and established a molecular weight range of 700 to 5000 for the peptides. Thin-layer high-voltage electrophoresis at pH 4.5 showed the presence of both positive and negative charged peptides in the isolated mixture. Amino acid compositions of the peptides were consistent with the observed electrophoretic mobilities. Protein binding of the peptides was not confirmed. However, peptides released during protein isolation, desalting, or concentration procedures could introduce artifactual evidence in studies involving the processing of proteins at low levels of concentration.

Isolation of peptides following electrodialysis of various pure protein fractions from bovine milk has been reported by numerous researchers [Kim et al., 1965; Gizis et al., 1965; Dorris, 1968; and Kirk et al., 1972]. Kirk et al. (1972) reported that approximately 30 to 40 mg of peptide material was obtained after concentrating and lyophilizing 4000 ml of electrodialysate from 3 to 5 g of 4 × β-lactoglobulin (β-Lg) suspended in 125 ml of deionized water. Results from the study reported here show the origin of these peptides to be the cellulosic dialysis membrane used in the isolation and electrodialysis techniques.

Crevasse and Pearson (1969) reported the presence of nitrogenous contaminants from dialysis tubing in the diffusate of acid-soluble calf-skin collagen after the following pretreatment of the dialysis membrane: boiling in 10% sodium carbonate for 2 h, thoroughly rinsing with distilled water and 0.05% acetic acid. The present study characterized the peptides derived from the cellulosic membranes after a thorough pretreatment procedure as described by McPhie (1971).

EXPERIMENTAL SECTION

Treatment of Membranes. Cellulosic membranes were obtained from Union Carbide Corporation (Films-Packaging Division, Chicago, Ill.). Two pretreatment procedures were used to eliminate impurities from the membranes. Initially, membranes were treated by boiling in a diluted EDTA solution and thoroughly rinsing with deionized water. A more extensive pretreatment of the membranes (McPhie, 1971) was also used. This procedure consisted of simmering the membranes in 50% ethanol for 1 h, followed by a 1-h soaking of the membranes in approximately 0.01 N sodium bicarbonate and EDTA solutions, respectively. Prior to use, the membranes were rinsed with deionized water.

Electrodialysis–Isolation of Membrane Peptides. Electrodialysis was carried out according to the procedure of Kirk et al. (1972). Cellulose membranes used in the electrodialysis chamber were subjected to a potential of 200 V for 2 to 48 h in deionized water. The dialysate was collected, pervaporated, and lyophilized.

Thin-Layer High-Voltage Electrophoresis. Single-dimension thin-layer high-voltage electrophoresis was performed according to the procedure of Kirk et al. (1972). Two buffer systems of pyridine–acetic acid–water were used: 1:10:189 at pH 3.5 and 5:9:986 at pH 4.5. Visualization of peptide zones was accomplished by spraying the plates with ninhydrin and drying at 90 °C (von Arx and Neher, 1963).

Peptide Mapping. Two-dimensional peptide mapping was performed on thin-layer chromatography plates coated with MN300 cellulose (500 μm). Samples were made to 0.1% in 0.1 N ammonium acetate solution applied as a small spot (5 μl) and thoroughly dried. High-voltage thin-layer electrophoresis was performed in the first dimension using the pyridine–acetic acid–water (5:9:986) buffer (pH 4.5) at a field strength of 40 V/cm for 20 min. The plates were dried in an oven at 90 °C and cooled to room temperature. Development in a second dimension was carried out by ascending thin-layer chromatography with a solvent system consisting of 2-propanol–formic acid–water (40:20:10). After developing the chromatogram the solvent front was marked and the plates dried at 90 °C. Peptides were located as previously described.

Glycopeptides. Peptides isolated from peptide maps were spotted on TLC plates (silica gel G) and developed with butanol–acetic acid–water (80:20:20). After development, the plates were sprayed with ninhydrin and held at 90 °C until the peptide spots were visible. The plates were then sprayed with orcinol–sulfuric acid reagent (0.1% orcinol in 2 N H_2SO_4) and held at 100 °C for 10 to 15 min (Moczar, 1973). Brown colored spots indicated the presence of carbohydrates. Color development for glycoprotein zones was not influenced by previous treatment with ninhydrin.

Amino Acid Analysis. Amino acid analyses were carried out on 22-h hydrolysates of the isolated peptides employing a Beckman Spinco amino acid analyzer (Model 120 C) according to the method of Moore et al. (1958).

Gel Filtration Chromatography. Gel filtration chromatography using Sephadex G-25 (2.5 cm × 37.5 cm) and G-10 (1.6 cm × 31 cm) was used for molecular weight estimations of the peptides. The eluent was 0.1 N ammonium acetate. Elution patterns were observed using an Isco Model UA-2 Column Monitor at 254 nm.

RESULTS AND DISCUSSION

Characteristics of Peptides. The lyophilized peptide preparation was light brown and had a sticky consistency. Separation of the peptide mixture by single dimension high-voltage thin-layer electrophoresis on cellulosic thin-layer plates at pH 4.5 is shown in Figure 1. Four peptide zones, classified according to the nomenclature described by Kirk et al. (1972), were observed: (1) negative 1 peptide (*neg 1*) migrating slightly toward the anode; (2) negative 3 peptide (*neg 3*) migrating the farthest toward the anode; (3) positive 2 peptide (*pos 2*) migrating slightly toward the cathode; (4) positive 3 peptide (*pos 3*), the remaining peptide migrating toward the cathode.

Two-dimensional peptide mapping showed eight peptide zones (Figure 2). The original *pos 2* peptide was shown to contain five peptide components designated as: *pos 2-1*, *pos 2-2*, *pos 2-3*, *pos 2-4*, and *pos 2-5* peptides, with R_f values of 0.36, 0.44, 0.56, 0.71, and 0.86, respectively. Peptides *pos 2-1* and *pos 2-2* gave positive color reactions with both ninhydrin and orcinol–sulfuric acid reagent,

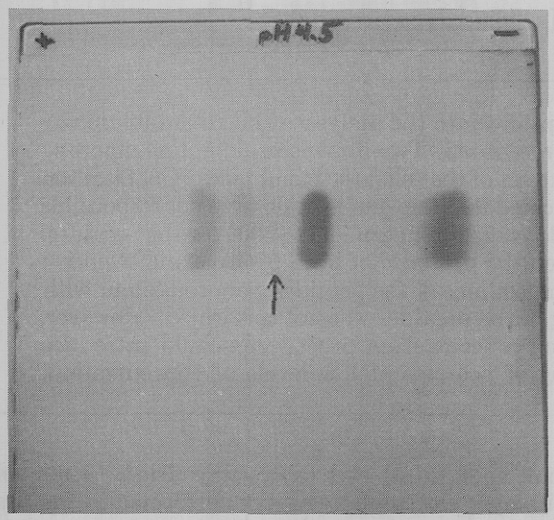

Figure 1. Thin-layer high-voltage electropherogram of the peptide mixture electrodialyzed from the cellulosic membranes: support, MN 300 cellulose; buffer, pyridine–acetic acid–water (pH 4.5). (Sample applied at location designated by arrow.)

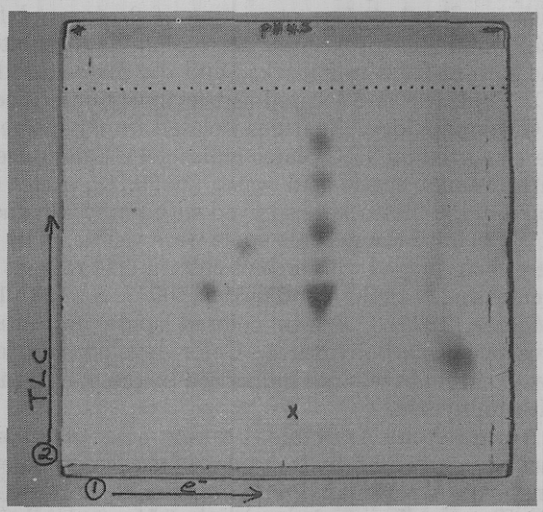

Figure 2. Two-dimensional peptide map of the peptide mixture electrodialyzed from the cellulosic membranes: support, MN 300 cellulose; electrophoresis, pyridine–acetic acid–water (pH 4.5); chromatography, 2-propanol–formic acid–water (40:2:10). (Sample applied at location designated by X.)

indicating the presence of carbohydrate.

Gel filtration chromatograms, developed by mapping the eluate, of the isolated peptides over a Sephadex G-10 column (exclusion limit, 700; V_0 = 21 ml) confirmed that all peptide components were present in the void volume. A second ninhydrin negative peak with an elution volume (V_e) of 44.5 ml was eluted after the peptides. A calibration mixture of three amino acids (tyrosine, phenylalanine, and tryptophan) was passed over the column, yielding elution volumes of 40, 47, and 88 ml, respectively. These data indicate that free amino acids were not responsible for the peptide zones.

Gel filtration chromatography of the peptides over Sephadex G-25 (exclusion limit, 5000; V_0 = 62 ml) resulted in three peaks at V_e of 127, 145, and 185 ml. The first two peaks contained all eight peptides, the third peak being ninhydrin negative. Phenylalanine exhibited a V_e of 182 ml on this column.

The molecular weight range of 700 to 5000 observed for the peptides was consistent with the findings of Kirk et al. (1972) who employed Bio-Gel P-2 and P-10 to obtain values of between 1500 and 3600 daltons for peptides recovered in the electrodialysate of β-Lg.

Amino Acid Analysis. Amino acid analyses of the peptide mixture indicated the presence of high concentrations of glycine, glutamic acid, lysine, and serine which was consistent with high-voltage thin-layer electrophoresis patterns. This observation was consistent with that of Kirk (1971) who reported high concentrations of these amino acids in peptide fractions neg 3, pos 2, and pos 3. Amino acid analyses were not performed on the individual peptides because of the difficulty of obtaining a sufficient sample of each purified peptide fraction.

Equilibrium Dialysis and Pervaporation. Additional studies with treated membranes showed the presence of all eight peptides in the dialysate and retentate following equilibrium dialysis and to a lesser extent in the retentate after fresh deionized water was pervaporated in the cellulose membranes. These results support the findings of Crevasse and Pearson (1969), who reported the contamination of the dialysate of acid-soluble collagen with nitrogenous material from dialysis tubing following equilibrium dialysis.

Steven and Tristram (1962) reported nitrogenous contaminants in distilled deionized water originating from the desalting resins. However, our studies revealed an absence of peptides in fresh distilled deionized water.

CONCLUSION

Peptides are released from regenerated cellulosic membranes during equilibrium dialysis, pervaporation, and electrodialysis procedures. Because these peptides are present in small quantities, they should not affect the physical and chemical properties of major protein fractions. However, in studies involving the preparation of minor protein fractions, particularly if long periods of dialysis or pervaporation are required, these peptides could lead to significant interpretational errors.

LITERATURE CITED

Crevasse, G. A., Pearson, A. M., *J. Food Sci.* **34**, 52 (1969).
Dorris, J. C., Ph.D. Dissertation, Michigan State University, East Lansing, Mich., 1968.
Gizis, E., Brunner, J. R., Schweigert, B. S., *J. Dairy Sci.* **48**, 1704 (1965).
Kim, Y. P., Gizis, E., Brunner, J. R., Schweigert, B. S., *J. Nutr.* **86**, 394 (1965).
Kirk, J. R., Ph.D. Dissertation, Michigan State University, East Lansing, Mich., 1971.
Kirk, J. R., Brunner, J. R., Stine, C. M., Schweigert, B. S., *J. Nutr.* **102**, 699 (1972).
McPhie, P., *Methods Enzymol.* **22**, 23 (1971).
Melachouris, N., *J. Dairy Sci.* **52**, 456 (1969).
Moczar, E., *J. Chromatogr.* **76**, 417 (1973).
Moore, S., Speckman, D. H., Stein, W. H., *Anal. Chem.* **30**, 1185 (1958).
Stevens, F. S., Tristram, G. R., *Biochem. J.* **83**, 245 (1962).
von Arx, E., Neher, R., *J. Chromatogr.* **12**, 329 (1963).

R. McDonald
J. Kirk*
J. Brunner
Department of Food Science and Human Nutrition
Michigan State University
East Lansing, Michigan 48824

Received for review August 27, 1975. Accepted November 20, 1975. Michigan Agricultural Experiment Station Journal Article No. 7238.