Feil, V. J., Hedde, R. D., Zaylskie, R. G., Zackrison, *J. Agr. Food Chem.* **18**, 120 (1970).
Keith, L. H., *Tetrahedron Lett.*, 3 (1971).
Keith, L. H., Alford, A. L., *J. Ass. Offic. Anal. Chem.* **53**, 1018 (1970).
Keith, L. H., Alford, A. L., McKinney, J. D., *Tetrahedron Lett.*, 2489 (1970).
Marchand, A. P., Rose, J. E., *J. Amer. Chem. Soc.* **90**, 3724 (1968).
McCullock, R., Rye, A. R., Wege, D., *Tetrahedron Lett.*, 5163 (1969).
McKinney, J. D., Matthews, H. B., Fishbein, L., *J. Agr. Food Chem.* **20**, 597 (1972).
Parsons, A. M., Moore, D. J., *J. Chem. Soc. C*, 2026 (1966).
Ramsey, N. F., *Phys. Rev.* **78**, 699 (1950).
Rondeau, R. E., Sievers, R. E., *J. Amer. Chem. Soc.* **93**, 1520 (1971).
Smith, W. B., Davenport, D. L., *J. Magn. Resonance* **6**, 256 (1972).

Richard L. Roberts
Gary L. Blackmer*

Department of Chemistry
Texas Tech University
Lubbock, Texas 79409

Received for review March 12, 1973. Accepted February 22, 1974. This work was supported by the Graduate School of Texas Tech University (Summer Research Assistantship Grant No. 191-5983) and also by Robert A. Welch Foundation Grant No. D-531.

Thermal Gelation of Pig-Skin Tropocollagen

Comparisons of thermal gelation curves for untreated pig-skin tropocollagen with identical samples treated with either pepsin, trypsin, chymotrypsin, or elastase were made. In general, the enzyme treatments altered the shape of the thermal gelation curves by increasing the lag phase and the time required for maximum gelation. However, each of the enzymes affected gelation differently, indicating that cleavage of the tropocollagen molecule occurred at different sites.

Tristram *et al.* (1965) have studied the effects of thermal denaturation upon soluble calf-skin collagen. Rubin *et al.* (1965), Drake *et al.* (1966), and Kühn *et al.* (1966) have shown that proteolytic enzyme treatments alter the aggregation properties and the α- and β-component ratios of calf-skin tropocollagen. Crevasse *et al.* (1969) working in our laboratory verified the effects of proteolytic enzymes upon thermal gelation of calf-skin collagen. They demonstrated that prevention of thermal gelation was greater in the case of elastase followed in order by chymotrypsin, trypsin, and pepsin treatments. Since no information is available on thermal gelation of pig-skin tropocollagen, the present study was undertaken to investigate its thermal gelation properties as altered by treatment with trypsin, pepsin, chymotrypsin, and elastase.

MATERIALS AND METHODS

Acid-soluble pig skin was extracted and purified according to a modification of the procedure utilized in preparation of calf-skin collagen by Rubin *et al.* (1965) as modified by Crevasse *et al.* (1969) with the following exceptions: (1) the precipitate of acid-soluble collagen (fraction 2-A) was redissolved and reprecipitated seven times; (3) the KCl precipitate (fraction 2-B) from the supernatant of the acid-soluble collagen preparation was redissolved and centrifuged for long periods of time and then reprecipitated against 2% NaCl. The precipitate was redissolved in 0.05% acetic acid and centrifuged at 25,000*g* for 24 hr. This was repeated several times, but the supernatant still remained slightly cloudy. After dialysis against 2% NaCl, the sample was frozen and stored.

Enzymatic reactions with pepsin, trypsin, chymotrypsin, and elastase were carried out as outlined by Crevasse *et al.* (1969), except that the substrate concentration was 5.8 mg/ml. Furthermore, the protein was not lyophilized after precipitation, but instead was dialyzed free of salts against distilled water and stored at 4°. Before analysis, the samples were dialyzed against 0.05% acetic acid. Nitrogen determinations were made by the micro-Kjeldahl procedure (Crevasse *et al.*, 1969) and hydroxyproline analysis by the method of Woessner (1961).

Short- and long-term thermal gelation was monitored at 230 nm in a Beckman DU-2 monochronometer using a Gilford automatic cuvette positioner and an absorbance converter connected to a recorder as described in more detail by Crevasse (1967).

RESULTS AND DISCUSSION

Figure 1 shows thermal gelation curves for untreated acid-soluble pig-skin collagen and samples after treatment with pepsin, trypsin, chymotrypsin, and elastase. The untreated sample and the pepsin-treated sample showed similar maximum gelation values. However, untreated collagen had a very short lag phase and maximum gelation had occurred within 10 min. Although pepsin-treated

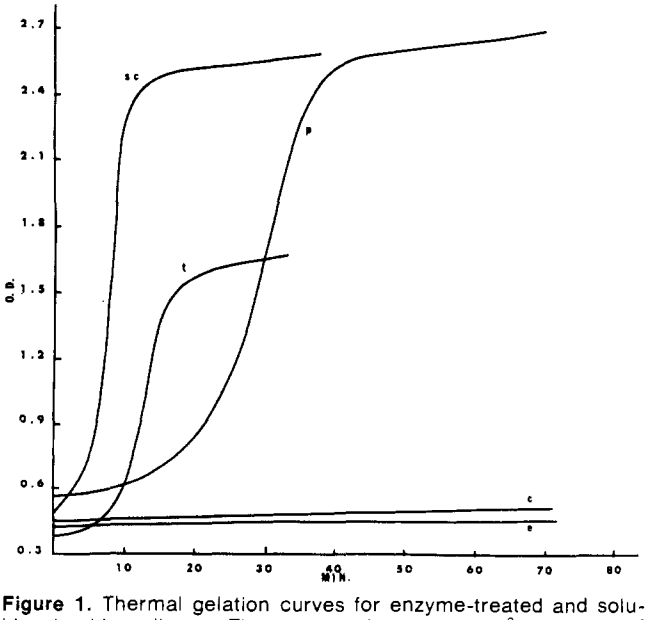

Figure 1. Thermal gelation curves for enzyme-treated and soluble pig-skin collagen. Thermal gelation was at 33° using 0.1 *M* phosphate buffer at pH 7.4: (sc) untreated pig-skin collagen; (p) pepsin treated; (t) trypsin treated; (e) elastase treated; (c) chymotrypsin treated.

collagen reached similar maximum gelation values, appreciable gelation did not occur until 20 min and the maximum was not reached until about 30 min. The trypsin-treated sample had about an 8-min lag phase but then proceeded to gel rapidly, reaching a maximum at about 12 min. However, the maximum amount of gelation in the trypsin-treated sample was decidedly less.

Elastase and chymotrypsin had the greatest affect upon polymerization of pig-skin collagen, essentially preventing gelation. After long periods in 0.6 *M* phosphate buffer, there was some slight fiber formation in chymotrypsin-treated pig-skin collagen, but essentially none in the elastase-treated samples. The results of this study indicate that aggregation or gelation of acid-soluble pig-skin collagen is prevented by enzyme treatment with the following order of effectiveness: elastase > chymotrypsin > trypsin > pepsin. This order is identical with that reported earlier for the gelation of acid-soluble calf-skin collagen (Crevasse *et al.*, 1969), suggesting that the tropocollagen molecules of both species are altered in a similar manner by the enzyme treatments. The differences in the gelation patterns of pig-skin collagen treated by the different enzymes suggest that they cleaved the molecules at different sites and/or a different number of times.

LITERATURE CITED

Crevasse, G. A., Ph.D. Thesis, Michigan State University, East Lansing, Mich., 1967.
Crevasse, G. A., Pearson, A. M., Brunner, J. R., McClain, P. E., *J. Agr. Food Chem.* **17,** 356 (1969).
Drake, M. P., Davison, P. F., Bump, S., Schmitt, F. O., *Biochemistry* **5,** 301 (1966).
Kühn, K., Fietzek, P., Kühn, J., *Biochem. Z.* **344,** 418 (1966).
Rubin, A. L., Drake, M. P., Davison, P. F., Pfahl, D., Speakman, P. T., Schmitt, F. O., *Biochemistry* **4,** 181 (1965).
Tristram, G. R., Worrall, J., Steer, D. C., *Biochem. J.* **95,** 350 (1965).
Woessner, J. F., *Arch. Biochem. Biophys.* **93,** 440 (1961).

Gary A. Crevasse[1]
Albert M. Pearson*
Phillip E. McClain[2]

Department of Food Science and Human Nutrition
Michigan State University
East Lansing, Michigan 48824
[1] The Breechteen Company
Mt. Clemons, Michigan 48043
[2] Protein Nutrition Laboratory
Nutrition Institute, Agricultural Research Service
U. S. Department of Agriculture
Agricultural Research Center
Beltsville, Maryland 20705

Received for review November 5, 1973. Accepted January 14, 1974. Journal Article No. 5627, Michigan Agricultural Experiment Station. This investigation was supported in part by Public Health Service Research Grant No. FD-00097 from the Food and Drug Administration.