In order to prepare pure enzyme before gene cloning the proteins were separated by fast protein liquid chromatography and used to prepare monoclonal antibodies to papaya proteinase A and B.

Crude spray-dried latex from Powell and Scholefield was dialysed against 1,3-diaminopropane and the enzyme isolated by the method of Goodenough & Owen (1986) using 6 M urea/1,3-diaminopropane as buffer and a gradient of 4 M mm/min of NaCl. The use of an anion-exchange column gave a good separation of the proteins, and fractions containing papaya proteinase A and B were freeze-dried and redissolved in phosphate-buffered saline (0.1 M NaCl/0.06 M phosphate, pH 7.3).

Balb/c mice were injected subcutaneously with 50 µg of a mixture of the redissolved protein in 200 µl of Freund's complete adjuvant. Six weeks later, after an intravenous booster injection, fusion of the spleen cells and mouse myeloma cells, P3 × 63-Ag 8-653 was obtained. Eight hybridomas were shown to have immunological activity when reacted against mixed papaya proteinase A and B. Western immunoblotting and enzyme-linked immunosorbent assays were as described by Goodenough et al. (1986).

When lysates from uninfected baby hamster kidney (BHK) cells in S-phase are centrifuged in the sucrose gradient only DNA polymerase and thymidine kinase co-sediment at the rates observed with the lysates from the HSV-I-infected cell. The situation in the HSV-I-infected cell for the virus-induced DNA polymerase is not that of the uninfected cell. There is a slowly sedimenting component of the thymidine kinase. The co-sedimentation of enzymes is destroyed if the lysates are exposed to EDTA, instead of EGTA, as seen with the virus-induced enzymes in its genome which are synthesized after infection of the host cell. These include a DNA polymerase (Keir et al., 1979), deoxycytidine deaminase (Jamieson et al., 1979; Mathews et al., 1979). It seemed to us that the herpes virus-infected mammalian cell represented an analogous biological system. Like bacteriophage T4, herpes simplex virus (HSV-I) encodes a number of enzymes in uninfected and HSV-I-infected mammalian cells. These include a DNA polymerase (Keir et al., 1979), deoxycytidine deaminase (Jamieson et al., 1979; Mathews et al., 1979). It seemed to us that the herpes virus-infected mammalian cell represented an analogous biological system. Like bacteriophage T4, herpes simplex virus (HSV-I) encodes a number of enzymes in its genome which are synthesized after infection of the host cell. These include a DNA polymerase (Keir & Gold, 1963), deoxypyrimidine kinase (Jamieson et al., 1979), deoxycytidine deaminase (Chan, 1977), and a ribonucleotide reductase (Cohen, 1972).

We have completed investigations on a number of such enzymes in uninfected and HSV-1-infected mammalian cells and also in yeast, a lower eukaryote. A summary of this work forms the basis of this communication.

We have adopted two previously used approaches to obtain physical evidence for enzyme complexes, including (a) sucrose density gradient centrifugation (Mathews et al., 1979) and (b) gel-filtration chromatography (Wickremasinghe et al., 1982, 1983). Secondly, we attempted to obtain kinetic evidence for a functional enzyme complex, by studying the possible channeling of ribonucleotide precursors into DNA.

When lysates from uninfected baby hamster kidney (BHK) cells in S-phase are centrifuged in the sucrose gradients we observe clear co-sedimentation of a number of DNA precursor pathway enzymes (see Fig. 1). A variety of experimental observations argues against this being simply a non-specific aggregate of enzymes, including: (1) the bulk of the protein sediments near the top and bottom of the gradient, not in the position of the putative complex; (2) co-sedimentation of enzymes is destroyed if the lysates are exposed to 2 M NaCl, followed by dialysis, before centrifugation; (3) radiolabelled RNA or DNA does not sediment with the enzymes, so that a non-specific ionic complex with these molecules can be excluded; (4) when EDTA is used instead of EGTA, to inhibit further enzyme digestion in the preparation of lysates, the previously co-sedimenting enzymes now sediment independently within the first third of the gradient; this suggests the importance of divalent cation, perhaps Mg²⁺ or Mn²⁺, in the maintenance of the observed complex; (5) when lysates from G₂-phase cells are centrifuged in the gradients only DNA polymerase and thymidine kinase co-sediment at the rates observed with the lysates from S-phase cells.

The situation in the HSV-1-infected cell for the virus-induced enzymes is somewhat different. The obvious departure from the situation in the uninfected cell is that the virus-induced DNA polymerase sediments independently near the middle of the gradient from the more rapidly, co-sedimenting virus-induced enzymes ribonucleotide reductase and thymidine kinase. There is also a slowly sedimenting component of the thymidine kinase. The co-sedimentation of these two enzymes is not observed when lysates are exposed to EDTA, instead of EGTA, as seen with the uninfected cell lysates.

When lysates from exponentially growing Saccharomyces cerevisiae were analysed either by sucrose gradient centrifugation or gel-filtration chromatography on Sepharose 6B,
S-phase BHK-21/C13 cells were suspended (about 2 x 10^7 cells/ml) in hypotonic Tris/HCl buffer containing 100 units/ml and phospholipase C (60 units/ml) and incubated for 1 h at 15°C. The reaction was stopped with 8 mM-EGTA (final concentration) and centrifuged (5 min, 15,000 g). The supernatant (400 μl portion) was centrifuged in the sucrose gradients (5 ml of 5%-20%, w/v, sucrose, 35,000 rev./min for 8 h at 4°C, SW50.1 rotor). Sedimentation is from right to left. Protein; α, thymidine kinase; ω, nuclease diphosphate kinase (NDP kinase); β, DNA polymerase; γ, ribonucleotide reductase; α, dihydrofolate reductase.

**Fig. 1. Sucrose gradient centrifugation**

S-phase BHK-21/C13 cells were suspended in hypotonic Tris/HCl buffer containing 100 units/ml and phospholipase C (60 units/ml) and incubated for 1 h at 15°C. The reaction was stopped with 8 mM-EGTA (final concentration) and centrifuged (5 min, 15,000 g). The supernatant (400 μl portion) was centrifuged in the sucrose gradients (5 ml of 5%-20%, w/v, sucrose, 35,000 rev./min for 8 h at 4°C, SW50.1 rotor). Sedimentation is from right to left. Protein; α, thymidine kinase; ω, nuclease diphosphate kinase (NDP kinase); β, DNA polymerase; γ, ribonucleotide reductase; α, dihydrofolate reductase.

**Contribution of endogenous muscle proteinases to tenderization and flavour in cooked muscle**

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Degradation of muscle proteins by endogenous muscle proteinases contributes to (1) metabolic turnover in live animals; (2) conditioning of muscle as meat.

Studies on proteolytic activities in muscle have been mainly focused on degradation of myofibrillar proteins and its role in meat tenderization (e.g. Penny, 1980). Sarcoplasmic proteinase which constitute up to 30% of muscle proteins have so far received little attention (Etherington, 1981).

This study was designed to investigate the proteolytic degradation of muscle proteins during the cooking process. Bovine sternomandibularis muscle was removed 20 min after slaughter. Pieces of muscle 30 g each were vacuum packed and they were kept up to 24 h at room temperature and up to 72 h thereafter at 0-4°C.

Samples were collected as follows:

1. control samples, 3 h and 96 h post-mortem;
2. heated samples,
   (a) fresh (3 h post-mortem, i.e. pre-rigor) and
   (b) aged (96 h post-mortem, i.e. post-rigor)
were heated at 80°C for 7.5, 15, 30 and 60 min;
3. 'drip' formed during cooking;
4. aliquots of sarcoplasmic protein extracts were heated at 80°C for 7.5, 15, 30 and 60 min.

Muscle proteins were extracted from:

(a) minced muscle homogenized in 12 vol. of 4% SDS/4% NH₄OH for 1 min at room temperature and centrifuged at 12,500 g for 20 min extracted total muscle proteins;
(b) diced muscle was extracted sequentially in 4 vol. of 0.0153 M- and in 4 vol. of 0.230 M-Na₂HPO₄/,NaH₂PO₄ at pH 7.4 with mechanical stirring for 3 h at 0-4°C and centrifuged at 1700 g for 15 min to extract sarcoplasmic and myofibrillar muscle fractions respectively. The residual pellet was dissolved in 8 vol. of 4% SDS/4% NH₄OH.

Protein concentration was determined by the Biuret method (Gornall et al., 1949). Denaturation and electrophoresis were carried out as described previously (Patestos & Harrington, 1984) and staining according to the procedure of Neuhoff et al. (1985).

Protein profiles showed that the degradation of sarcoplasmic proteins in terms of amount and spectrum of proteins extracted appeared to be extensive during the early stages of cooking process. Some myofibrillar proteins appeared to be degraded but to a lesser degree than were sarcoplasmic proteins. Drip proteins appeared to degrade also. Sarcoplasmic protein extracts heated at 80°C for 7.5,