Degradation of myofibrillar proteins by trypsin-like serine proteinases

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1. The effects of the Ca^{2+} -activated cysteine proteinase, the rat trypsin-like serine proteinase and bovine trypsin on myofibrillar proteins from rabbit skeletal muscle are compared. 2. Myofibrils that had been treated at neutral pH with the Ca^{2+} -dependent proteinase and with the rat enzyme were (a) analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and (b) examined in the electron microscope. Treatment with each proteinase resulted in loss of the Z-discs, but the rat enzyme caused much more extensive disruption of the ultrastructure and degraded more of the myofibrillar proteins. 3. Purified F-actin was almost totally resistant to the proteinases, whereas G-actin was degraded by the rat trypsin-like proteinase at a rate approx. 15 times faster than was obtained with bovine trypsin. 4. Similar results were obtained with α -actinin, whereas tropomyosin was degraded more readily by bovine trypsin than by the rat trypsin-like proteinase. 5. The implications of these findings for the non-lysosomal breakdown of myofibrillar proteins *in vivo* are considered.

Studies with lysosomotropic agents and specific proteinase inhibitors have shown that, although lysosomes are undoubtedly involved in the catabolism of intracellular proteins (Dean, 1979; Shaw & Dean, 1980; Grinde & Seglen, 1980), only a fraction of the total endogenous protein degraded can be accounted for by this lysosomal pathway (Knowles & Ballard, 1976; Poole *et al.*, 1977). It has been suggested that most cells have a second, non-lysosomal, route for catabolizing their intracellular proteins (for a review, see Kay, 1978). The quantitative importance of this route in tissues such as muscle with a relatively low lysosomal content remains to be determined, but its significance has been underlined by subsequent evidence showing that, although 'general' protein turnover in muscle can be inhibited by chloroquine and leupeptin, the breakdown of myosin is not affected by these inhibitors of lysosomal proteolysis (Wildenthal *et al.*, 1980; Riebow & Young, 1980).

Thus it seems that at least the initial stages in the degradation of myosin (and presumably other myofibrillar proteins) takes place outside the lysosomes in muscle.

A number of neutral proteinases have been identified in muscle extracts (see, e.g., Katunuma &

Kominami, 1977; Pennington, 1977), but most of these enzymes have now been shown to be located in non-muscle cells, e.g., mast cells. Two still remain as perhaps the most likely neutral proteinases that have been characterized *in vitro*, and which might be involved in the non-lysosomal breakdown of myofibrillar proteins *in vivo*.

The Ca^{2+} -activated cysteine proteinase was first isolated from pig skeletal muscle (Dayton *et al.*, 1976a), but it has now been detected in a variety of tissues, including platelets, brain, chicken oviduct and cardiac and smooth muscles (Szpacenko *et al.*, 1980; Guroff, 1964; Vedeckis *et al.*, 1980; Mellgren, 1980; Puca *et al.*, 1977). Elevated Ca^{2+} -activated proteolytic activity has been observed in atrophying muscle (Dayton *et al.*, 1977) and in dystrophy (Kar & Pearson, 1976). The enzyme has been shown (Szpacenko *et al.*, 1981; Dayton *et al.*, 1981; Kubota *et al.*, 1981) to exist in a form which is activated at the micromolar concentrations of Ca^{2+} that can be attained intracellularly in muscle (Jobsis & O'Connor, 1966; Marban *et al.*, 1980). However, the Ca^{2+} -activated proteinase has little effect on most of the contractile proteins of muscle (Dayton *et al.*, 1976b). Its major influence appears to be the disruption of the ultrastructure of the myofibril by solubilization of α -actinin, with consequent disappearance of the Z-discs.

The trypsin-like serine proteinase that was first identified in rat smooth muscle (Beynon & Kay,

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1978) was shown to have a similar activity to other neutral proteinases when acting on peptide substrates or denatured proteins, but it was approx. 100 times more active than, for example, bovine trypsin towards soluble proteins in their native conformation at pH 7.4 (Beynon & Kay, 1978; Carney *et al.*, 1978). In an analogous fashion to the tissue-distribution studies described above for the Ca^{2+} -activated proteinase, the trypsin-like type of activity has been isolated from rat pancreas (J. Kay, C. R. Elliss & J. A. Greweling, unpublished work), and increased amounts of a trypsin-like serine proteinase have been measured in whole homogenates of diseased human muscle, e.g. in dystrophy and polymyositis (Kar & Pearson, 1980). Degradation of myosin (from smooth and skeletal muscles) by this type of trypsin-like serine proteinase was found to proceed at a rate approx. 20 times faster than that observed with bovine trypsin (Kay *et al.*, 1982). The ability of smooth-muscle myosin to combine with actin in the presence of Ca^{2+} to hydrolyse ATP was destroyed very rapidly.

Thus we decided to examine the degradation of myofibrils and individual myofibrillar proteins by this type of proteinase and to compare the results with the known effects of the Ca^{2+} -activated proteinase and with bovine pancreatic trypsin (as representative of the most active of extracellular neutral proteinases towards native protein substrates Kay *et al.*, 1977).

Materials and methods

Bovine pancreatic trypsin (Type III) and soya-bean trypsin inhibitor were purchased from Sigma. All other buffers, salts and chemical reagents were of analytical grade.

The concentration of the trypsin-like serine proteinase in rat intestinal muscle is very low (Beynon & Kay, 1978), so that it is difficult to obtain sufficient enzyme at a high stage of purity. A proteinase with very similar enzymic properties has been found in much larger amounts in rat pancreas. The proteinase from this tissue was purified to homogeneity by a procedure (Kay *et al.*, 1982) involving chromatography on DEAE-cellulose and affinity chromatography on benzamidine-agarose. This preparation was used in these studies, and the activity of this trypsin-like serine proteinase was stopped instantly and completely by the addition of an equimolar amount of soya-bean trypsin inhibitor.

The thiol proteinase activated by millimolar concentrations of Ca^{2+} was purified from bovine heart muscle as described previously (Szpacenko *et al.*, 1981).

Myofibrils were prepared by the method of Siemankowski & Dreizen (1978). Rabbit skeletal

muscle was ground in a mincer and homogenized (12 ml/g) in 0.1 M-KCl/7 mM- KH_2PO_4 /8 mM-EGTA/1 mM- MgCl_2 /1 mM-dithiothreitol, pH 7.0, containing 1% (v/v) Triton X-100. After centrifugation (2000 g for 10 min), the sedimented pellet was resuspended in the above buffer and homogenized again. This centrifugation and washing procedure was repeated for a further five cycles, with omission of the Triton in the last two washes. The myofibrils thus obtained were resuspended finally and washed twice in the buffer to be used experimentally. The protein concentration was determined by the method of Lowry *et al.* (1951) after dilution of the myofibril preparation 10-fold with 1 M-KOH and leaving it at room temperature for 30 min. Bovine serum albumin was used as standard.

Actin was prepared from rabbit skeletal muscle essentially as described by Spudich & Watt (1971). The G-actin obtained was repolymerized to F-actin by adjusting the ionic strength to 0.1 M in KCl containing 500 μM - MgCl_2 . α -Actinin was obtained from rabbit skeletal muscle by the method of Arakawa *et al.* (1970). Tropomyosin was prepared as described by Greaser & Gergely (1973). The final concentrations of these protein preparations were determined relative to bovine serum albumin as described above.

Different conditions were used for proteolysis of myofibrils and the individual purified proteins, and so are described for each experiment in the Results section.

Samples for analysis by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis were removed from the proteolytic incubation mixtures at appropriate times, and the digestions were stopped by the addition of an excess of soya-bean trypsin inhibitor or EDTA to the trypsin-like or Ca^{2+} -dependent proteinases respectively. For whole-myofibril incubations, samples were centrifuged at 100 000 g for 20 min, and the pellets, after washing with 2×5 ml of 0.1 M-NaCl, were resuspended in 0.1 M-NaCl.

Samples were removed and boiled for 45 min in 1% sodium dodecyl sulphate. All samples for analysis were loaded on polyacrylamide gels (8% or 5–17% gradient gels) as described previously (Kay *et al.*, 1982). Electrophoresis was carried out at 100 V until the tracking dye (pyronin Y) had migrated to the bottom of the gels. After removal, gels were stained overnight by the method of Fairbanks *et al.* (1971).

The effects of the proteinases on the ultra-structure of the myofibrils were examined in the electron microscope. Digested myofibrils (and controls) after washing with NaCl as described above were fixed in 2.5% glutaraldehyde followed by 1% OsO_4 . Embedding was in Epon/Araldite after graded acetone dehydration. Sections were stained

with 2% uranyl acetate in methanol, post-stained with lead citrate and examined in a Philips EM200 microscope.

Results

Rabbit skeletal myofibrils were incubated in 0.1 M-Tris/acetate buffer (pH 7.4)/0.1 M-KCl containing 5 mM- Ca^{2+} and 5 mM-mercaptoethanol with the rat pancreatic trypsin-like proteinase and with the bovine heart Ca^{2+} -activated proteinase at substrate-protein/proteinase ratios (w/w) of 3000:1 and 200:1 respectively. After 75 min at 25°C, the reactions were stopped. The suspensions had cleared considerably and the residual material had undergone an apparent aggregation. After centrifugation at 100 000 g for 20 min, the supernatants were removed, and from the A_{280} values obtained (0.67 and 0.36 respectively; average of four separate determinations) it appeared that the trypsin-like proteinase (with 15 times less enzyme) had solubilized approximately twice as much protein as the Ca^{2+} -activated thiol proteinase, although these values represent less than 10% of the total myofibrillar protein. Little synergistic effect was apparent, since the A_{280} of the supernatant obtained after incubation of myofibrils with both proteinases (concurrently or consecutively) rose only to 0.71. Control incubations in which soya-bean trypsin inhibitor and EDTA were added before the respective proteinases showed no increase in A_{280} of the supernatants relative to the control myofibrils to which no proteinases had been added.

The pellets obtained after incubation and centrifugation were washed and samples were dissolved in sodium dodecyl sulphate for analysis by gel electrophoresis. Fig. 1 shows that the troponin proteins (T and I) were very sensitive to both proteinases (even with so little trypsin-like proteinase added), whereas tropomyosin and actin both appeared to be resistant. The intensity of the α -actinin band in myofibrils treated with the Ca^{2+} -activated proteinase was decreased, consistent with previous observations (Dayton *et al.*, 1976b) that one of the major effects of this enzyme is to release α -actinin from the Z-disc. The α -actinin band also appeared to have been removed by the rat serine proteinase, although a band apparently of slightly higher molecular weight was generated in the digestion with this proteinase (see below for digestion of purified α -actinin). It has been shown previously (Kay *et al.*, 1982) that the heavy chain of myosin is degraded very rapidly by this trypsin-like proteinase [within 20 min at a myosin/proteinase ratio (w/w) of 30 000:1]. Under the conditions of digestion of the myofibrils (Fig. 1), where almost 10 times as much rat proteinase was used, the myosin heavy chain was degraded into smaller fragments, and it seems likely that the band

Fig. 1. Products of digestion of myofibrils from rabbit skeletal muscle by the Ca^{2+} -dependent and trypsin-like proteinases

Myofibrils (10 mg, final vol. 2 ml) were incubated at 25°C in 0.1 M-Tris/acetate buffer, pH 7.4, containing 0.1 M-KCl, 5 mM-mercaptoethanol and 5 mM- Ca^{2+} with the Ca^{2+} -dependent and trypsin-like proteinases. Each digestion was stopped after 75 min by the addition of EDTA or soya-bean trypsin inhibitor, respectively. A third sample that had been treated with the rat enzyme and inhibited by soya-bean trypsin inhibitor was incubated for a further 60 min with the Ca^{2+} -dependent enzyme. All samples were centrifuged at 100 000 g for 20 min. After washing, the pellets were resuspended in 0.1 M-NaCl and portions were taken for sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Tracks: 1, myofibrils without added proteinase; 2, plus Ca^{2+} -dependent proteinase; 3, plus rat trypsin-like proteinase; 4, plus both proteinases. Abbreviations: TnT, TnI, troponins T and I.

appearing with a molecular weight slightly greater than that of α -actinin was probably a breakdown fragment from the myosin heavy chain. In keeping with earlier findings (Dayton *et al.*, 1976b), myosin was not degraded by the Ca^{2+} -activated thiol proteinase. The myofibrils that had been exposed to both proteinases (Fig. 1, track 4) showed an electrophoretic pattern very similar to those that had been incubated with the serine proteinase alone, suggesting that the predominant effect was being exerted by this proteinase.

The digested myofibrils (and controls) were washed as described above, and prepared for examination in the electron microscope. The control myofibrils (Plate 1a) were structurally in good condition, exhibiting the typical ultrastructure of mammalian myofibrils, with a well-defined Z-line in the centre of the I-band and a defined H-zone within the A-band. In keeping with previous observations

(Dayton *et al.*, 1976b), treatment with the Ca^{2+} -activated thiol proteinase (Plate 1b) resulted in the loss of the intense Z-line, but otherwise the normal ultrastructure of the sarcomere was not disturbed. Incubation with the rat proteinase also resulted in Z-line removal, but, in addition, the regularity of the ultrastructure appeared to have been disrupted to a greater extent (Plate 2). Many sections were examined of myofibrils that had been treated with this proteinase, but it was not possible to detect sarcomeres with the defined structure that was readily observed after treatment of the myofibrils with the Ca^{2+} -activated proteinase.

Thus incubation of myofibrils with the trypsin-like serine proteinase appeared to produce greater disruption of the ultrastructure of the contractile unit. In a separate experiment (results not shown) it was found that degradation by this proteinase proceeded equally rapidly whether the incubations with myofibrils were carried out in a contracting buffer (containing $25\mu\text{M}\text{-CaCl}_2$ and $1\text{mM}\text{-Mg}^{2+}\text{-ATP}$) or in a relaxing medium (with $7.5\text{mM}\text{-EGTA}$ and $1\text{mM}\text{-Mg}^{2+}\text{-ATP}$).

It was therefore decided to examine whether the disruptive effect on the myofibrils was being achieved through the alterations in the myosin molecule described previously (Kay *et al.*, 1982) or whether the proteinase had a demonstrable effect on other individual myofibrillar proteins. Since many reports in the past have utilized bovine pancreatic trypsin for investigations into structure-function relationships of muscle proteins (see, e.g., Goll *et al.*, 1971), a comparison was made of the susceptibilities of purified myofibrillar proteins to digestion by bovine trypsin and by the trypsin-like proteinase from the rat.

F-actin was incubated in $2\text{mM}\text{-Tris/HCl}$ buffer, pH 8.0, containing $100\text{mM}\text{-KCl}$ and $500\mu\text{M}\text{-MgCl}_2$ with the rat and bovine trypsin at substrate/proteinase ratios (w/w) of 430:1 and 60:1 respectively. G-actin was incubated with the two proteinases under the same conditions except that the Tris buffer contained only $200\mu\text{M}\text{-Ca}^{2+}\text{-ATP}$. F-actin (Fig. 2) was totally resistant to the rat proteinase (and to bovine trypsin) even after incubation for 19 h. By contrast, G-actin was degraded by the rat enzyme such that after 19 h, very little of the original (42000 mol.wt.) protein band remained. Similar results (not shown) were obtained with bovine trypsin, except that the rate of disappearance of the original (42000 mol.wt.) subunit was approximately one-half of that observed with the rat proteinase. Thus proteolysis of G-actin by the rat enzyme appears to proceed about 10–15 times more rapidly than by an equimolar amount of trypsin. This is a similar rate-enhancement effect to that observed with myosin (Kay *et al.*, 1982).

α -Actinin was incubated in $1\text{mM}\text{-KHCO}_3/5\text{mM}$ -

Fig. 2. Extent of digestion of rabbit skeletal-muscle actin by the rat trypsin-like enzyme

F-actin (3 mg, final vol. 1.0 ml) was incubated at 25°C in $2\text{mM}\text{-Tris/HCl}$ buffer, pH 8.0, containing $100\text{mM}\text{-KCl}$ and $0.5\text{mM}\text{-Mg}^{2+}$ with the rat proteinase. G-actin was incubated in $2\text{mM}\text{-Tris/HCl}$ buffer, pH 8.0, containing $200\mu\text{M}\text{-Ca}^{2+}\text{-ATP}$ but otherwise identical conditions, and samples were removed from both digestions at the indicated times and added to sufficient soya-bean trypsin inhibitor to inactivate the proteinase completely and instantaneously. Loadings corresponding to $30\mu\text{g}$ of F-actin (a) and of G-actin (b) were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

mercaptoethanol with rat proteinase and bovine trypsin at substrate/proteinase ratios (w/w) of 300:1 and 60:1 respectively. Samples were removed for gel electrophoresis at appropriate times, and Fig. 3 shows that α -actinin was degraded very rapidly by the rat proteinase, with the native protein subunit (mol.wt. 95000) being removed within about 20 min. Proteolysis of the native α -actinin subunit by bovine trypsin proceeded at a rate approximately one-half of that observed with the rat proteinase, and a slightly different pattern of fragments was apparent on the gels of the samples taken from the later stages of digestion. Thus, with the higher amount of bovine trypsin that had to be used in order to obtain proteolysis of the intact α -actinin molecule, further degradation of some of the breakdown fragments may have taken place and to a greater extent than

EXPLANATION OF PLATE 1

Electron micrographs of longitudinal sections of myofibrils from rabbit skeletal muscle
Untreated myofibrils (a; magnification $\times 31\,000$) and myofibrils treated with the Ca^{2+} -activated proteinase (b; magnification $\times 27\,000$) were fixed, sectioned and stained.

EXPLANATION OF PLATE 2

*Electron micrograph of a longitudinal section of rabbit skeletal myofibrils treated with the rat serine proteinase
(magnification $\times 31\,000$)*

Fig. 3. Extent of digestion of rabbit skeletal-muscle α -actinin by the rat trypsin-like proteinase

α -Actinin (1 mg, final vol. 0.2 ml) was incubated at 25°C in 1 mM-KHCO₃/5 mM-mercaptoethanol with the rat proteinase. Samples were removed at the indicated times and added to an excess of soya-bean trypsin inhibitor. Loadings corresponding to 30 μ g of original α -actinin protein were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Fig. 4. Extent of digestion of rabbit skeletal-muscle tropomyosin by the rat trypsin-like proteinase

Tropomyosin (1 mg, final vol. 1 ml) was incubated at 25°C in 1 mM-KHCO₃/5 mM-mercaptoethanol with the rat proteinase. Samples were removed at the indicated times and added to an excess of soya-bean trypsin inhibitor. Loadings corresponding to 30 μ g of original tropomyosin protein were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

was possible with the lower concentration of rat proteinase. However, once again proteolysis of a native myofibrillar protein by the rat trypsin-like enzyme appears to proceed at a rate approximately

one order of magnitude faster than is observed with bovine trypsin.

Since rabbit skeletal tropomyosin had appeared to be relatively resistant to the rat enzyme (and the Ca²⁺-activated thiol proteinase) in the whole-myofibril digestion experiments, purified tropomyosin was incubated (in 1 mM-KHCO₃/5 mM-mercaptoethanol) with the rat proteinase and with bovine trypsin at substrate/proteinase ratios of 250:1 (w/w) for both enzymes. Samples were removed at appropriate times for analysis by gel electrophoresis, and it was found that even after 80 min incubation with the rat proteinase (Fig. 4) considerable quantities of protein still remained in the position of the original tropomyosin subunit. However, with bovine trypsin, all of the protein in the tropomyosin band had been degraded into low-molecular-weight fragments by the time the first sample was analysed after 2 min incubation (results not shown). Thus, in complete contrast with all of the other protein substrates that have been tested (Beynon & Kay, 1978; Kay, 1980; Kay *et al.*, 1982), rabbit skeletal tropomyosin appears to be more readily degraded by bovine trypsin than by the trypsin-like type of activity from the rat.

Discussion

It has been suggested that the Ca²⁺-activated proteinase in muscle is involved in initiating the disassembly and turnover of myofibril proteins (see, e.g., Dayton *et al.*, 1981). However, it would appear that the trypsin-like type of proteolytic activity has a much greater effect than the Ca²⁺-activated proteinase on whole myofibrils. Little evidence was found *in vitro* that would support a co-operative involvement of both types of proteinase in the turnover of myofibril proteins *in vivo*, e.g. disassembly by the Ca²⁺-activated enzyme followed by extensive degradation by the trypsin-like proteinase. The latter seems well able to initiate disassembly by itself. The apparent ease with which the trypsin-like proteinase is able to digest myofibrillar proteins *in vitro* suggests that, if this type of activity does have access to these proteins within the muscle, it could have a profound effect on the structure of the contractile unit *in vivo*. The present study has confirmed our earlier investigations (Kay *et al.*, 1982) that myosin is degraded very rapidly by tiny amounts of this type of proteinase. By contrast, actin was much more resistant to proteolysis. At a concentration of proteinase 40 times that which degraded myosin within a few minutes, breakdown of G-actin required about 20 h, and F-actin was almost totally resistant to proteolysis. Since this type of proteinase is the most active known towards native proteins at neutral pH (Kay, 1980), it would seem likely that depolymerization of F-actin might

have to be a prerequisite for turnover of this thin-filament protein. Indeed, it has been found that an $F \rightleftharpoons G$ equilibrium is in existence in non-muscle cells, with a substantial proportion of the total actin in the monomeric G-form (Korn, 1978), whereas in muscle cells the equilibrium favours polymerization to the filamentous F-form.

The α -actinin protein was degraded very readily by the trypsin-like proteinase. It has been shown previously (Dayton *et al.*, 1975) that the Ca^{2+} -activated proteinase does not digest this protein. Thus, although both enzymes remove Z-discs from myofibrils, it is likely that this effect is accomplished in different ways. The Ca^{2+} -activated proteinase solubilizes α -actinin (intact) from its site of attachment in the Z-discs of the myofibril, whereas the trypsin-like enzyme degrades the protein as well.

G-actin and α -actinin were both degraded by the rat proteinase at a rate approx. 10 times faster than that with bovine trypsin. By contrast, tropomyosin was broken down more readily by bovine trypsin than by the rat enzyme. The latter is anionic (C. R. Elliss & J. Kay, unpublished work) and, since the rod-like tropomyosin molecule has one of the lowest isoelectric points (approx. pH 5) of the myofibrillar proteins, it may be that at neutral pH the electrostatic interactions between the negatively charged tropomyosin and the anionic enzyme are sufficiently strong to diminish the high intrinsic activity of the rat trypsin-like proteinase to a value less than that of the cationic enzyme, bovine trypsin.

These results emphasize how the nature and the conformation of the substrate protein can influence its rate of degradation at neutral pH. The action of the Ca^{2+} -activated proteinase on the contractile proteins is limited, but nevertheless the activity of this enzyme appears to be under strict control. A specific inhibitor has been characterized (Otsuka & Goll, 1980), and a further level of control is thought to exist through interconversion between forms of the enzyme active at micromolar and millimolar concentrations of Ca^{2+} (Szpacenko *et al.*, 1981; Dayton *et al.*, 1981). By comparison, then, since the trypsin-like serine proteinase seems to be much more effective in degrading most of the contractile proteins, at least *in vitro*, this type of activity would have to be regulated very carefully indeed. A specific inhibitor of this type of proteinase has been isolated from smooth muscle and characterized (Carney *et al.*, 1980). However, little immunocytochemical information is available at present to indicate the cellular location of either of the proteinases or of their respective inhibitors. It is clearly of importance to establish not only where these proteins are located but also to examine whether both enzymes coexist within the same cell and if they have ready access to the myofibrillar and cytoplasmic proteins. This should provide a clearer understanding of their

relative importance and involvement (co-operatively or exclusively) in the non-lysosomal pathway of intracellular protein degradation.

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References

- Arakawa, N., Robson, R. M. & Goll, D. E. (1970) *Biochim. Biophys. Acta* **200**, 284–295
- Beynon, R. J. & Kay, J. (1978) *Biochem. J.* **173**, 291–298
- Carney, I. T., Beynon, R. J., Kay, J. & Birket, N. (1978) *Biochem. J.* **175**, 105–113
- Carney, I. T., Curtis, C. G., Kay, J. & Birket, N. (1980) *Biochem. J.* **185**, 423–433
- Dayton, W. R., Goll, D. E., Stromer, M. H., Reville, W. J., Zeece, M. G. & Robson, R. M. (1975) in *Proteases and Biological Control* (Reich, E., Rifkin, D. B. & Shaw, E. N., eds.), pp. 551–577, Cold Spring Harbor Laboratory, Cold Spring Harbor
- Dayton, W. R., Goll, D. E., Zeece, M. G., Robson, R. M. & Reville, W. J. (1976a) *Biochemistry* **15**, 2150–2158
- Dayton, W. R., Reville, W. J., Goll, D. E. & Stromer, M. H. (1976b) *Biochemistry* **15**, 2159–2167
- Dayton, W. R., Schollmeyer, J. V., Chan, A. C. & Allen, C. E. (1977) *Biochim. Biophys. Acta* **584**, 216–230
- Dayton, W. R., Schollmeyer, J. V., Lepley, R. A. & Cortes, L. R. (1981) *Biochim. Biophys. Acta* **659**, 48–61
- Dean, R. T. (1979) *Biochem. J.* **180**, 339–345
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Goll, D. E., Robson, R. M., Temple, J. & Stromer, M. H. (1971) *Biochim. Biophys. Acta* **226**, 433–452
- Greaser, M. L. & Gergely, J. (1973) *J. Biol. Chem.* **248**, 2125–2133
- Grinde, B. & Seglen, P. O. (1980) *Biochim. Biophys. Acta* **632**, 73–86
- Guroff, G. (1964) *J. Biol. Chem.* **239**, 149–155
- Jobsis, F. F. & O'Connor, M. J. (1966) *Biochem. Biophys. Res. Commun.* **25**, 246–252
- Kar, N. C. & Pearson, C. M. (1976) *Clin. Chim. Acta* **73**, 293–297
- Kar, N. C. & Pearson, C. M. (1980) *Biochem. Med.* **24**, 238–243
- Katunuma, N. & Kominami, E. (1977) in *Proteinases in Mammalian Cells and Tissues* (Barrett, A. J., ed.), pp. 151–180, North-Holland, Amsterdam
- Kay, J. (1978) *Biochem. Soc. Trans.* **6**, 789–797
- Kay, J. (1980) *Protein Degradation in Health and Disease; CIBA Found. Symp.* **75**, 219–225
- Kay, J., Carney, I. T. & Beynon, R. J. (1977) *Acta Biol. Med. Germ.* **36**, 1637–1644

- Kay, J., Siemankowski, R. F., Siemankowski, L. M. & Goll, D. E. (1982) *Biochem. J.* **201**, 267–278
- Knowles, S. E. & Ballard, F. J. (1976) *Biochem. J.* **156**, 609–617
- Korn, E. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 588–599
- Kubota, S.-I., Suzuki, K. & Imahori, K. (1981) *Biochem. Biophys. Res. Commun.* **100**, 1189–1194
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Marban, E., Rink, T. J., Tsien, R. W. & Tsien, R. Y. (1980) *Nature (London)* **286**, 845–849
- Mellgren, R. L. (1980) *FEBS Lett.* **109**, 129–133
- Otsuka, Y. & Goll, D. E. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2044
- Pennington, R. J. T. (1977) in *Proteinases in Mammalian Cells and Tissues* (Barrett, A. J., ed.), pp. 515–543, North-Holland, Amsterdam
- Poole, B., Okhuma, S. & Warburton, M. (1977) *Acta Biol. Med. Germ.* **36**, 1777–1788
- Puca, G. A., Nola, E., Sica, V. & Bresciani, F. (1977) *J. Biol. Chem.* **252**, 1358–1370
- Riebow, J. F. & Young, R. B. (1980) *Biochem. Med.* **23**, 316–323
- Shaw, E. N. & Dean, R. T. (1980) *Biochem. J.* **186**, 385–390
- Siemankowski, R. F. & Dreizen, P. (1978) *J. Biol. Chem.* **253**, 8648–8658
- Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **246**, 2125–2133
- Szpacenko, A., Otsuka, Y., Goll, D. E. & Stromer, M. H. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2044
- Szpacenko, A., Goll, D. E., Kay, J. & Otsuka, Y. (1981) in *Proteinases and Their Inhibitors; Structure, Function and Applied Aspects* (Turk, V. & Vitale, Lj., eds.), pp. 151–161, Pergamon Press, Oxford
- Vedeckis, W. V., Freeman, M. R., Schrader, W. T. & O'Malley, B. N. (1980) *Biochemistry* **19**, 335–343
- Wildenthal, K., Wakeland, J. R., Ord, J. M. & Stull, J. T. (1980) *Biochem. Biophys. Res. Commun.* **96**, 793–798