myosin light chains has been demonstrated in embryonic pectoralis muscle myosin[16,17]. This heterogeneity may be explained either by the presence of different myosin isoenzymes or by the existence of molecules characteristic of embryonic tissue. The structure of the embryonic tissue molecules could be composed of fast and slow adult light chains associated with special heavy chains. The heavy chains may be made up of stretches of sequences similar to adult fast heavy chains interspersed with sequence-stretches typical of adult slow heavy chains. The fact that in the map of embryonic heavy chains only a few components peculiar to the slow myosin digest are present (one would have expected all the slow myosin components if myosin in embryonic tissue was made by a mixture of fast and slow isoenzymes) supports the hypothesis of the existence of a hybrid myosin.

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2 N. Rubinstein, F. Pepe and H. Holtzer, Proc. natl Acad. Sci. USA *74*, 4524 (1977).
3 G. Pelloni-Muller, M. Ermini and E. Jenny, FEBS Lett. *70*, 68 (1976).
4 R. Roy, F.A. Sreter and S. Sarkar, Devl Biol. *69*, 15 (1979).
5 N. Rubinstein and H. Holtzer, Nature *280*, 323 (1979).
6 J.F.Y. Hoh, FEBS Lett. *98*, 267 (1979).
7 R.G. Whalen, G.S. Butler-Brown and F. Gros, J. molec. Biol. *126*, 415 (1978).
8 R.G. Whalen, K. Schwartz, P. Bouveret, S.M. Sell and F. Gros, Proc. natl Acad. Sci. USA *76*, 5197 (1979).
9 L. Dalla Libera, Cell Biol. int. Rep. *5*, 112 (1981).
10 J.F.Y. Hoh and G.P.S. Yeoh, Nature *280*, 321 (1979).
11 F.A. Sreter, M. Balint and J. Gergely, Devl Biol. *46*, 317 (1975).
12 G. Huszar, Nature New Biol. *240*, 260 (1972).
13 J.I. Rushbrook and A. Stracher, Proc. natl Acad. Sci. USA *76*, 4331 (1979).
14 T. Masaki and C. Yoshizaki, J. Biochem., Tokyo *76*, 123 (1974).
15 M. Cantini, S. Sartore and S. Schiaffino, J. Cell Biol. *85*, 903 (1980).
16 T. Obinata, T. Masaki and H. Takano, J. Biochem., Tokyo *87*, 81 (1980).
17 L. Reeburg-Keller and L.P. Emerson, Proc. natl Acad. Sci. USA *77*, 1020 (1980).
18 G.F. Gauthier, S. Lowey and A.W. Hobbs, Nature *274*, 25 (1978).
19 M. Barany and R.I. Close, J. Physiol. *213*, 455 (1971).
20 L. Dalla Libera, A. Margreth, I. Mussini, C. Cerri and G. Scarlato, Muscle Nerve *1*, 280 (1978).
21 U. Carraro, C. Catani and L. Dalla Libera, Exp. Neurol. *72*, 401 (1981).
22 L. Dalla Libera, S. Sartore and S. Schiaffino, Biochim. biophys. Acta *581*, 283 (1979).

Photodynamic studies on acid ribonuclease from pea cotyledons

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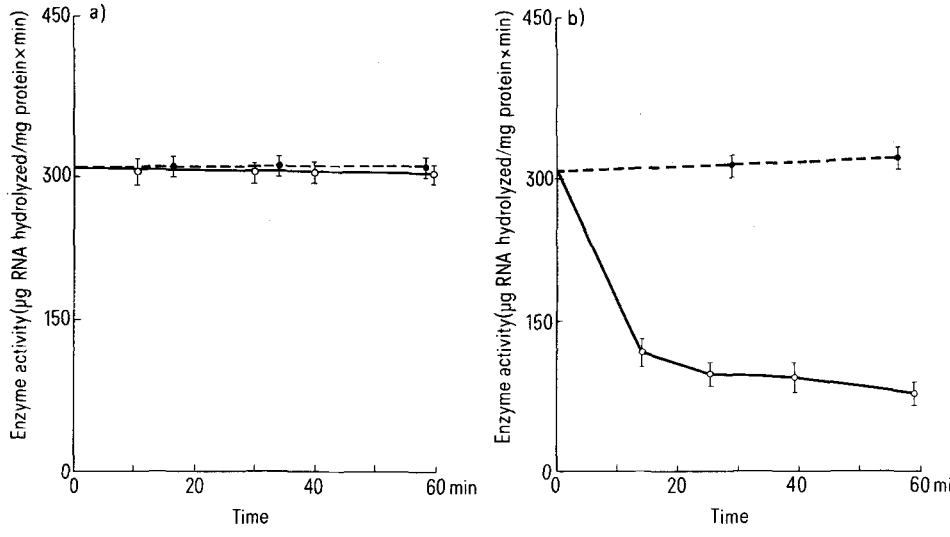
Summary. In crude extracts, pea cotyledon acid ribonuclease is not inactivated by photodynamic treatment, but after 150-fold purification it is markedly inactivated when illuminated in the presence of rose bengal at pH 7.1. Data suggests that histidine photo-oxidation reduces catalytic activity.

Only 5 amino acids, cysteine, histidine, methionine, tryptophan and tyrosine are modified by photodynamic treatment[1]. By careful control of the reaction conditions and selection of an appropriate oxidizing dye, photo-oxidation may be limited to a single amino acid species[2]. It is therefore possible to determine the importance of the amino acids which are susceptible to dye-sensitized photo-oxidation for the activity of the enzyme. For the great majority of enzymes, most of the amino acid residues are involved in the maintenance of catalytic activity, and modification of any amino acids species leads to loss of activity.

It has been demonstrated[3] that a small fragment of pea cotyledon acid ribonuclease (EC. 2.7.7.16), with a mol. wt of 3100 retains some catalytic activity. The retention of activity in such a small fragment suggests that a large part of the amino acid sequence may not be directly involved in maintaining the integrity of the active site. Acid ribonuclease is therefore an interesting subject for study by photodynamic techniques.

Experimental. Pea seeds (*Pisum sativum* L., cv Feltham First) were surface sterilized in Na hypochlorite solution, washed in running tap water for 4 h, planted in moist vermiculite and allowed to germinate at 22–25 °C. The seedlings were harvested after 5 days. The cotyledons were homogenized in 0.2 M Na acetate buffer, (pH 5.4) with a pestle and mortar at 3 °C. The homogenate was centrifuged at 34000 × g for 15 min at 4 °C. Differential precipitation of acid ribonuclease with $(NH_4)_2\,SO_4$, followed by gel filtration on columns of Sephadex G-50 (fine) results in a purification of about 150-fold (2.1 ± 0.2 µg RNA hydrolyzed/mg protein/min in crude extracts; 312 ± 105 µg RNA hydrolyzed/mg protein/min in partially purified extracts). During irradiation the samples were maintained at 1–5 °C. Aliquots were removed at 15-min intervals for assay of acid ribonuclease[4]. Control samples containing dye were kept in darkness. Aliquots from control samples were taken at zero time and after 60 min. For experiments in which irradiation was performed under alkaline conditions, enzyme samples were prepared in 0.05 M Tris-HCl buffer. The pH was adjusted to 5.4 after irradiation. Samples of enzyme preparations were irradiated with visible light from an Osram 1500-W tungsten bulb (120 mm distance from sample) in the presence of dye.

Results and discussion. Pea cotyledon acid ribonuclease, in crude supernatants, is completely insensitive to photodynamic action over a range of pH (5.6–8.1). In contrast, acid phosphatase in the same crude supernatants is extensively inactivated by photodynamic action. The figure (a and b) shows the effects of irradiation of the partially purified enzyme preparation in the presence of rose bengal at pH 5.6 and 7.1. It is clear that at pH 5.6 irradiation in the presence of rose bengal and crystal violet, has no effect on the activity of acid ribonuclease. Irradiation, at pH 8.1, in the presence of rose bengal has no effect on the enzyme activity. In contrast, irradiation of the enzyme at pH 7.1



Effect of irradiation in the presence of rose bengal on the activity of acid ribonuclease. *a* At pH 5.6; *b* at pH 7.1. Data are the mean of 6 determinations. Vertical bars are standard errors of the mean. O—O, Experimental; ●---●, controls.

causes a marked decrease in activity over the first 30 min. The control samples (pH 7.1) show a very slight increase in activity during a 60-min incubation and it is thus likely that the total extent of inactivation is even greater than that recorded in the figure (b).

The resistance of the enzyme to photodynamic treatment at pH 5.6 is interesting. Methionine, cysteine and to a lesser extent, tryptophan, are photo-oxidized at this pH[2], and the results therefore suggest that these amino acids do not contribute directely to the enzyme activity. In pancreatic ribonuclease photo-oxidation of methionine lying on the surface of the enzyme molecule causes a significant reduction of activity[5]. The fact that this does not happen with pea cotyledon ribonuclease supports earlier findings that a small fragment of the native enzyme retains catalytic activity[3]. When the methionine residue in position 13 of pancreatic ribonuclease, is photo-oxidized the activity of the enzyme decreases by 80%[5]. This is because this particular methionine residue lies in the active site, adjacent to a histidine residue which participates in the enzyme reaction[6,7]. Our data thus suggest that methionine does not occupy such a position in pea ribonuclease.

The acid ribonuclease from pea cotyledons is very markedly inactivated by photo-oxidation at pH 7.1. At this pH, histidine is by far the most vulnerable amino acid, and cysteine, methionine and tryptophan are also photo-oxidized to a significant extent. The marked inactivation at pH 7.1 (taken with the lack of inactivation at pH 5.6) may thus be attributed to photo-oxidation of histidine. Cysteine is efficiently photo-oxidized by crystal violet in acidic media[8]. Tryptophan photo-oxidation is more rapid as the pH of the solution is raised from neutral[2]; the lack of inactivation at pH 8.1 suggests that this amino acid is not involved in the active site. Histidine, therefore, appears to be directly involved in the active site. The mode of action of pea cotyledon ribonuclease is thus likely to be very similar to that of pancreatic ribonuclease. In pancreatic ribonuclease, the histidine residues at positions 12 and 119 lie on opposite sides of the groove which forms the active site[6,9] and actually participate in the reaction. Modification of either of these residues by photo-oxidation[10], or by iodoacetate[9], causes a marked loss of enzyme activity.

1 D. Spikes and M.L. MacKnight, Ann. N.Y. Acad. Sci. *171*, 149 (1970).
2 J.S. Bellin and C.A. Yankus, Archs Biochem. Biophys. *123*, 18 (1968).
3 J.A. Bryant, S.C. Greenway and G.A. West, Planta *130*, 141 (1976).
4 B.I.S. Srivastava, Biochim. biophys. Acta *169*, 534 (1968).
5 G. Jori, G. Galiazzo, A.M. Tamburro and E. Scoffone, J. biol. Chem. *245*, 3375 (1970).
6 D.G. Smyth, W.H. Stein and S. Moore, J. biol. Chem. *238*, 227 (1963).
7 D. Findlay, D. Herries, A.P. Mathias, B.R. Rabin and C.A. Ross, Biochem. J. *85*, 152 (1962).
8 G. Jori, Int. Protein Res. *1*, 289 (1969).
9 A.M. Crestfield, W.H. Stein and S. Moore, J. biol. Chem. *238*, 2421 (1963).
10 F.M. Richards and H.W. Wyckoff, in: The Enzymes, vol. IV, p. 647. Ed. P.D. Boyer. Academic Press, New York 1971.

Effects of larval firefly extracts on molluscan cardiac activity[1]

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Summary. Larval firefly midgut extracts and larval firefly hemolymph extracts were found to produce a potent inhibitory effect when applied directly to the heart of a terrestrial mollusc. It is suggested that such substances could comprise an important part of the paralyzing toxin which is reportedly injected by larval fireflies into their prey.

The larvae of fireflies, bioluminescent members of the beetle family Lampyridae, have been reported to subdue their prey, much larger invertebrates such as terrestrial annelids, arthropods, and molluscs, by injection of a paralyzing toxin via their hollow fang-like mandibles[2]. A tubular canal is found within each mandible and this canal extends from near the mandible's tip, where it exits, to the mandible's base, where it opens into the mouth. Several