On the relationship between myosin and tropomyosin A

Serological reactions provide a valuable tool for the study of interrelationships among the "contractile" proteins of muscle. It is important to clarify such relationships in view of increasing evidence that myosin, the principal contractile protein of muscle, must be considered a heterogeneous protein[1]. Myosin can be easily broken down into smaller components[2,3,4] which show striking resemblances to other fibrous proteins of muscle[5,6,7]: tropomyosin B, tropomyosin A and actin.

In this paper the immunization of rabbits against actin, tropomyosin A and tropomyosin B is described and the cross-reactions of the resulting immune sera with these proteins and myosin is reported. Previously, KESZTYÜS, NIKODEMUSZ AND SZILÁGYI[8] showed that actin is isoantigenic. KLATZO, HORVÁTH AND EMMART[9] recently noted that myosin is not species specific.

Tropomyosin A was prepared from the adductor muscle of the clam (*Venus mercenaria*) as described by LAKI[10]. The minced muscle tissue was washed with cold 0.4 % $NaHCO_3$ and dried with acetone. From the KI extract of the dried mince, tropomyosin A was crystallized and the crystalline material used for immunization. Tropomyosin B was prepared from chicken-breast muscle by the method of BAILEY[11]. Actin was prepared from rabbit muscle according to MOMMAERTS[12]. The myosins were prepared from rabbit muscle and cat muscle, according to the method of SZENT-GYÖRGYI[13]. The homogeneity of these preparations was checked by analytical ultracentrifugation.

Antisera against actin, tropomyosin A and tropomyosin B were produced in rabbits. 5 mg of antigen were injected in 1–3 ml volume intravenously, two or three animals being immunized with each antigen. Injections were given every other day for 2 weeks. 5–7 days after the last injection 10–40 ml blood was taken from each animal, and the immunization course was repeated. Sera from animals in each group were pooled. Myosin antisera were already available from the rabbits immunized with human myosin, as described by KLATZO, HORVÁTH AND EMMART[9].

Precipitin reactions were carried out, testing each antiserum against each antigen. An equal volume of antigen solution was added to serial, 2-fold dilutions of antisera. Dilutions of the antisera were made with 0.15 *M* NaCl, containing 0.02 *M* sodium phosphate buffer, pH 7. All antigens, except myosin, were diluted with the same buffer to a concentration of 1 mg/ml. Since myosin is precipitated at low ionic strengths, it was diluted with 0.5 *M* NaCl to the same concentration. The final salt concentration in all tubes containing myosin was 0.32 *M*, in all others 0.15 *M*. In some instances precipitin rings were observed immediately, but final readings were routinely taken after overnight standing in the cold. The amount of precipitate was graded 0 to 3.

Each antiserum gave a precipitate with its homologous antigen. However, the reaction of rabbit actin with anti-rabbit-actin serum deserves comment. At the time that KESZTYÜS, NIKODEMUSZ AND SZILÁGYI showed actin to be iso-antigenic[8], the best available actin was only 50 % pure. The present results, obtained with purified actin, rule out the impurities and establish actin as the iso-antigenic protein.

Although the muscle proteins actin, myosin and tropomyosin B reacted with their respective homologous antisera, none of them exhibited a precipitin reaction with antisera to the other two antigens. This finding may be interpreted as indicating that each protein preparation was uncontaminated by either of the other two.

The antiserum to tropomyosin A gave a very strong reaction with its homologous antigen and also precipitated both rabbit myosin and cat myosin. Conversely, tropomyosin A was precipitated by human myosin antiserum, prepared in the rabbit.

It is noteworthy that antisera to myosin and tropomyosin B did not cross-react, nor did tropomyosin A antiserum cross-react with antiserum to tropomyosin B to a significant extent.

These observations indicate an immunological difference between tropomyosin A and tropomyosin B. They also suggest a close relationship in immunological properties between myosin and tropomyosin A.

It was also found that tropomyosin A antiserum precipitates the lighter fragment of myosin, L-meromyosin. This finding suggests that the site of cross reaction with tropomyosin A antiserum is that portion of the myosin molecule which, on tryptic digestion, yields L-meromyosin.

The site of cross-reaction can further be localized on the "crystalline fraction of denatured myosin". When myosin is denatured with alcohol and ether, the addition of trypsin brings into solution 25 % of myosin[14]. This soluble part of myosin readily crystallizes out of solution, and has the chemical and physico-chemical properties of tropomyosin A. This protein is precipitated by the tropomyosin A antiserum to the same extent as tropomyosin A itself. Since this crystalline component of myosin is identical with the "crystalline fraction of L-meromyosin" (representing 60 % of L-meromyosin)[15], the site of cross reaction appears to be this component of L-meromyosin.

The close relationship of a segment of myosin to tropomyosin A removes a gap between vertebrate and invertebrate muscles that came to light with the discovery of tropomyosin A. While it was found that the muscles of invertebrates contained quite large amounts of tropomyosin A easily extractible[16,17], no tropomyosin A could be extracted from vertebrate muscles. The explanation seems now at hand. In vertebrate muscle tropomyosin A is built into the myosin

molecule[17]. In invertebrate muscles where the amount of myosin is usually less, tropomyosin A occurs in the free, unbound form. The difference between vertebrate and invertebrate muscles is thus a quantitative rather than a qualitative one.

K. LAKI
B. HORVÁTH*
I. KLATZO*

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland (U.S.A.)

1 S. F. VELICK, *Biochim. Biophys. Acta*, 20 (1956) 228.
2 J. GERGELY, *J. Biol. Chem.*, 200 (1953) 543.
3 E. MIHÁLYI, *J. Biol. Chem.*, 201 (1953) 197.
4 A. G. SZENT-GYÖRGYI, *Arch. Biochem.*, 42 (1953) 305.
5 D. R. KOMINZ, A. HOUGH, P. SYMONDS AND K. LAKI, *Arch. Biochem. Biophys.*, 50 (1957) 148.
6 K. LAKI, *J. Cell. Comp. Physiol.*, 49 (1957) 249.
7 D. R. KOMINZ, F. SAAD AND K. LAKI, *Nature*, 179 (1957) 206.
8 L. KESZTYÜS, S. NIKODEMUSZ AND T. SZILÁGYI, *Nature*, 163 (1949) 136.
9 I. KLATZO, B. HORVÁTH AND E. W. EMMART, *Proc. Soc. Exptl. Biol. Med.*, 97 (1958) 135.
10 K. LAKI, *Arch. Biochem. Biophys.*, 67 (1957) 240.
11 K. BAILEY, *Biochem. J.*, 43 (1948) 271.
12 W. F. H. M. MOMMAERTS, *J. Biol. Chem.*, 188 (1951) 559.
13 A. G. SZENT-GYÖRGYI, *J. Biol. Chem.*, 192 (1951) 361.
14 K. LAKI, *Transactions of the Conference on the Chemistry of Muscular Contraction, Tokyo 1957*, in the press.
15 C. COHEN AND A. G. SZENT-GYÖRGYI, *J. Am. Chem. Soc.*, 79 (1957) 248.
16 K. BAILEY, *Biochem. J.*, 64 (1956) 9P.
17 D. R. KOMINZ, F. SAAD AND K. LAKI, *Transactions of the Conference on the Chemistry of Muscular Contraction*, Tokyo, 1957, in the press.

Received March 14th, 1958

Preliminary Notes

An aminosugar nucleotide from *Carcinus maenas*

It has been demonstrated that uridine pyrophosphate (UPP) derivatives can act as glycosyl donors for the enzymic synthesis of both disaccharides and polysaccharides[1,2]. (For recent reviews see refs [3,4].) GLASER AND BROWN[5] have shown that the incorporation of ^{14}C-labelled N-acetylglucosamine into chitin by cell-free extracts of *Neurospora crassa* involves ^{14}C-labelled uridine-pyrophosphate-N-acetylglucosamine (UPPAG) as an intermediate. Attempts are now being made to decide whether uridine nucleotides are also concerned in the biosynthesis of chitin by arthropods. The present communication reports the isolation of what appears to be a new UPP derivative from the hypodermis of the shore crab, *Carcinus maenas*.

A crude extract was obtained from hypodermis of 20 crabs (supplied by the Marine Biological Laboratory, Plymouth) in the following way. The fresh tissue was transferred immediately after removal to 100 ml of icewater, and was rapidly minced in a Waring blendor. The minced tissue was boiled for 1 min, filtered through muslin and the residue re-extracted with a further 50 ml of boiling water. The combined filtrates were stirred with unground acid-washed[6] moist Nuchar C (about 40 g) until the liquid became colourless. The filtered charcoal was then washed with water (150 ml) and the nucleotides were extracted repeatedly with 50% aqueous ethanol containing 1% (v/v) 0.88 *N* ammonia until the extracts become pale straw coloured. The combined extracts, concentrated under reduced pressure (temp. 35°) to about 100 ml were applied to a Dowex-1 × 2 (Cl^-) column (200–400 mesh, 20 cm × 0.64 cm^2). The nucleotides were separated in 10-ml fractions by stepwise elution at room temperature at a flow rate not exceeding 40 ml/h.

The following elutants were used: I, 0.01 *N* HCl containing 0.01 *N* NaCl; II, 0.01 *N* HCl containing 0.03 *M* NaCl; III, 0.01 *N* HCl containing 0.06 *M* NaCl; IV, 0.01 *N* HCl containing 0.10 *M* NaCl; V, 0.01 *N* HCl containing 0.20 *M* NaCl.

* National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. (U.S.A.).