Short Communications

Small Sub-units of Myosin

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Several reports have recently appeared of low-molecular-weight components dissociated from rabbit skeletal myosin. Denaturation in 5M-guanidine hydrochloride gave a light component of mol.wt. 46000 (Dreizen, Hartshorne & Stracher, 1966), whereas treatment with 0.1M-Na₂CO₃ at pH 11.0 produced a fraction of mol.wt. 20000 that gave three bands on cellulose acetate electrophoresis (Gershman, Dreizen & Stracher, 1966). Acetylation (Locker & Hagyard, 1967*b*) or succinylation (Oppenheimer, Barany, Hamoir & Fenton, 1967) also gave low-molecular-weight components. Some of these might, of course, be adsorbed impurities in the myosin preparations, but Locker & Hagyard (1967*b*) have shown that a fraction (AcMII) of mol.wt. about 20000 could be obtained in yields of up to 15% of the total material by DEAE-cellulose chromatography of the low-molecular-weight material from myosin purified by either (NH₄)₂SO₄ fractionation (Kielley & Harrington, 1960) or DEAE-cellulose treatment (Takahashi, Hashimoto & Tonomura, 1963). Further, Locker & Hagyard (1967*a*) obtained low-molecular-weight material from myosin by carbonate or tripolyphosphate treatment, or heat coagulation, that gave, after acetylation, the three electrophoretic components characteristic of fraction AcMII. The question arises whether these components are similar in amino acid sequence. I now describe some experiments that suggest that this may be so.

A 'light alkaline component' was prepared from rabbit skeletal myosin (Perry, 1955) by treatment with 0.1M-Na₂CO₃-0.4M-KCl at pH 10.4 at 2° (Gershman *et al.* 1966). The high-molecular-weight material was precipitated by dilution at neutral pH, and the supernatant fractionated on DEAE-cellulose (Whatman DE-52) in 0.04M-tris-chloride buffer, pH 8.0, with a gradient up to 0.4M-KCl. Three peaks emerged and further elution with 2.0M-KCl did not remove any more protein.

The first two peaks were rechromatographed in a shallower KCl gradient and samples examined, after oxidation with performic acid, by electrophoresis in

8M-urea, pH 8.3, on cellulose acetate. Fraction I contained a major component, *a*, and a trace component, *b*; fraction II contained three components, *a*, *b* and *c*; fraction III was almost entirely a single component, *c*.

Analytical ultracentrifugation of fractions I and III showed no high-molecular-weight material, and both gave sedimentation coefficients (*S*₂₀) of about 1.6s. Their amino acid compositions were somewhat similar except that fraction III contained much less proline, but both were appreciably different from whole myosin. The cysteic acid content, measured by amino acid analysis of the performic acid-oxidized proteins, was 6.14 moles/10⁵g. for fraction I and 6.32 moles/10⁵g. for fraction III.

Fractions I and III were each incubated with dithiothreitol to ensure that all the thiol groups were reduced, and then treated with a large excess of [³⁵S]cystamine (Weeds, 1967). The radioactivity incorporated showed that over 93% of the total thiol groups had reacted. The labelled fractions were digested with trypsin for 5 hr. at 37° at pH 8.0, with an enzyme/substrate ratio 1:50 (w/w), and the peptides were fractionated by paper electrophoresis and chromatography. Fig. 1 shows the ninhydrin-positive peptides and those containing tyrosine, plus the major radioactive components. Peptide A was the most strongly radioactive spot in fraction I and the only strongly radioactive spot in fraction III. The tyrosine-containing peptides in both fractions appear to be identical and the major radioactive component is the same. Most of the ninhydrin-staining peptides are also common to both fractions I and III, but fraction I contains additional basic and neutral peptides, whereas fraction III contains a few additional acidic peptides. This suggests that fractions I and III have much amino acid sequence in common.

To confirm this conclusion, peptide A was purified by the 'diagonal' technique (Weeds & Hartley, 1967). Both fractions IA and IIIA had *N*-terminal methionine sulphone and identical amino acid compositions, and their chymotryptic and peptic 'fingerprints' were identical. The amino acid sequences of the oxidized peptide IA

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Fig. 1. Two-dimensional 'fingerprints' of peptides from fraction I (in *a*, *c* and *e*) and fraction III (in *b*, *d* and *f*). (*a*) and (*b*) show peptides basic at pH 6.5 chromatographed at right angles in butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.) (abbreviated BAWP). (*c*) and (*d*) show peptides neutral at pH 6.5 further submitted to electrophoresis at pH 3.5 and then chromatographed in butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.). (*e*) and (*f*) show peptides acidic at pH 6.5 submitted to electrophoresis at pH 2.0 at right angles. All buffers were as described by Ambler (1963). ○, Weak ninhydrin-staining; ◐, strong ninhydrin-staining; ●, tyrosine-staining; ◐, radioactive.

and fragments derived from it were determined, after amino acid analysis, by 'dansyl-Edman' degradation (Gray & Hartley, 1963) and carboxypeptidase A treatment. A chymotryptic digest of peptide IA gave four peptides (IAC1-IAC4). Peptide IAC1 was MetSO₂*-Lys-Glu-(Glu₃,Ala,Val,Leu) and gave the fragments MetSO₂-(Lys,Glu₃)-Val-Glu-Ala-Leu and Ala-Leu after digestion with pepsin. Peptide IAC2 was MetSO₂-Ala-Gly-Gln-(Glu,Asp,Ser,Asp,Gly)-Cya-Ile-Asn-Tyr, which is

* Abbreviations (in amino acid sequences): MetSO₂, methionine sulphone; Cya, cysteic acid.

identical with peptide 4 isolated by Weeds & Hartley (1967) from a peptic digest of cystine-exchanged myosin, except for a deaminated asparagine, which is perhaps due to the original alkaline treatment of the myosin. Peptide IAC3 was Glu-Ala-Phe, and peptide IAC4 was Val-Lys; a peptic fragment Phe-Val-Lys from peptide IA shows that these overlap. The *N*-terminus of the whole peptide IA was MetSO₂-Lys-Glu-Glu-Glu and hence the total sequence is:

Further, a mixture of fractions I and III was treated with carboxypeptidase A. After 2 min. digestion 0.59 mole of isoleucine/25000g. of protein was found, with less than 0.19 mole of any other amino acid. After 15 min. and 4 hr. digestion the isoleucine recoveries were 0.87 and 0.93 mole/25000g. respectively. Hence isoleucine appears to be quantitatively the *C*-terminal group of these light alkaline components, confirming the qualitative observations of Gershman *et al.* (1966).

Are fractions I and III contaminants in the original myosin or genuine parts of the myosin molecule? Purification by chromatography on cellulose phosphate, which removes adenylate deaminase (Harris & Suelter, 1967), followed by DEAE-Sephadex chromatography (Richards, Chung, Menzel & Olcott, 1967), produced a myosin that still gave low-molecular-weight material after treatment with Na₂CO₃ or reaction with maleic anhydride. Moreover, the thiol sequences of myokinase (mol.wt. 20000) are not consistent with the sequence of peptide A (A. G. Weeds, unpublished work).

The yield of peptides from whole myosin containing this thiol sequence should indicate the number of moles of this low-molecular-weight component/mole of myosin. In a peptic digest of [³⁵S]cystine-exchanged myosin, Weeds & Hartley (1967) found only three radioactive peptides that arose from this sequence. The ratio of radioactivity in these peptide spots to the total radioactivity incorporated in the myosin showed that there were 2-3 moles of this thiol group/mole of myosin. Hence there appear to be at least 2 moles of this sub-unit/mole of myosin, which makes it unlikely that it is an adventitious component in the preparation. In studies on the *C*-terminus of myosin, Sarno, Tarendash & Stracher (1965) observed the release of 4 moles of isoleucine/530000g. of myosin, which is consistent with the hypothesis that there are four polypeptide chains in myosin with the same *C*-terminal residue. Thus the model that best fits all these data is a molecule containing two

large polypeptide chains of mol.wt. about 240000, and two smaller ones of mol.wt. about 20000.

There remains, however, the problem of the heterogeneity of these low-molecular-weight sub-units. Studies on myosins obtained from 'fast' and 'slow' muscles of pigeon (Maddox & Perry, 1966) have shown differences in their adenosine triphosphatase activities and their susceptibility to tryptic cleavage. These differences may reflect differences in the amino acid sequences of these proteins. Thus the heterogeneity in the low-molecular-weight components obtained from rabbit skeletal-muscle myosin could also be attributed to the presence of different myosins in the preparation. However, it is equally possible that the electrophoretic differences result from the high-pH treatment on the protein, e.g. by deamidation of a single precursor. Such deamidation has already been observed in peptide A. But, since the same electrophoretic components are produced by different disaggregating procedures (Locker & Hagyard, 1967a), this deamidation must occur consistently with all these procedures, and should produce components of identical molecular weights. Alternatively, heterogeneous components of different molecular weight could arise if the sub-units represented different cleavage products from the large polypeptide chains of myosin produced, e.g. by catheptic activity (Gordon & Zak, 1963), during the extraction or purification procedures. This is unlikely in view of the claim of Richards, Chung, Appel & Olcott (1967) that small sub-units can be produced from myosin prepared in the presence of cathepsin inhibitors.

Thus the small sub-units produced from myosin by alkaline treatment that appear heterogeneous both on ion-exchange chromatography and electro-

phoresis on cellulose acetate show remarkable similarity in their peptide 'fingerprints', and contain identical thiol sequences. This suggests that they may be derived from a single precursor.

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