INDEPENDENT SYNTHESIS OF SMALL AND LARGE SUBUNITS OF MYOSIN IN VIVO

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The relative rates of completion of small and large subunits of myosin were determined by comparisons of $^{3}H/^{14}C$ ratios of subunits from a mixture of partially (^{3}H) and uniformly (^{14}C) labeled myosin. Small and large subunits were separated by isoelectric focusing (9 M urea) and SDS electrophoresis in dilute polyacrylamide gels. In all cases the $^{3}H/^{14}C$ ratio of small subunits was significantly higher than that of the large subunits. We interpret these data to indicate that the small subunits were synthesized independently and thus completed and released from polyribosomes more rapidly than the large subunits.

It is now apparent that myosin is composed of subunits which differ in size by an order of magnitude (1). With the accumulation of evidence that the small subunits comprise an essential part of biologically active myosin, it has become important to know if the synthesis of the small subunits (MW 17-30,000) is coordinated with that of the large subunits (MW 200,000). Sarkar and Cooke (3) and Low, Vournakis, and Rich (3) have recently reported results of *in vitro* studies on isolated polyribosomes; they agree that the light chains are assembled on small polyribosomes and the heavy chains are synthesized independently on large polyribosomes. These reports provide convincing evidence for independent synthesis of the myosin subunits on isolated ribosomes *in vitro*, but the possibility remains that intact muscle may conceal some mechanism for coordination of synthesis of the myosin chains. To investigate this possibility, we have adopted the procedure first used by Dintzis (4) to study direction of peptide chain growth in reticulocytes. Our results agree completely with the findings of Low *et al.* (3) and of Sarkar and Cooke (2); there is no apparent coordination of biosynthesis of small and large subunits of myosin in intact muscle.

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Experimental Procedures

Myosin was labeled by injecting 0.1 ml of radioactive leucine (1.0 mCi ^{3}H-L-leucine, 48 Ci/mmole, per embryo for partial labeling; 0.15 mCi ^{14}C-L-leucine, 0.24 Ci/mmole, per embryo for uniform labeling) in both legs of 17-day chick embryos. The eggs were opened at the embryo pole, taking care to minimize damage to blood vessels; the embryo was turned or delicately lifted to expose the legs for injection. The inoculated embryos were incubated for 3 min at room temperature (20-22°) for partial labeling; incubation was for 45 min at 38° for uniform labeling. These incubation times and temperatures were chosen from the results of preliminary time-course experiments in which relative labeling of light and heavy chains was compared. After incubation, myosin was extracted from the embryo leg muscle at pH 6.5 for 15 min as previously reported (5) and purified by treatment with RNase and chromatography on DEAE-Sephadex A-50 (6,7). At this point the two labeled myosin samples were mixed in equal quantities and the double labeled myosin was analyzed by polyacrylamide gel isoelectric focusing (6) and SDS-polyacrylamide gel electrophoresis (8); both procedures have the great advantage of giving simultaneous resolution of the small and large subunits in a single gel. The polyacrylamide cylinders from both methods were fixed in 10% (w/v) trichloroacetic acid, frozen in dry ice-ethanol, and sliced for counting by one of two methods: some were uniformly cut into 1.5 mm slices with a block of razor blades (9), and others were cut manually to give slices corresponding to the small and large subunits revealed by staining with Commassie blue or bromphenol blue. The slices were covered with 0.5 ml NCS (Amersham-Searle) in glass counting vials, incubated at 65° for 2 hours, and processed for counting as described previously (9). Under our conditions, ^{3}H was counted at about 35% efficiency and ^{14}C at 65% efficiency, with backgrounds in both channels in the range 15 to 25 cpm. All samples were counted to a minimum of 4000 total counts and vials exhibiting count rates less than double background were not considered. Details of our external standard

procedure for determining counting efficiencies and computing dpm ^{3}H and ^{14}C are described elsewhere (9).

Results and Discussion

Typical results from the counting of uniformly sliced gels are presented in Figure 1. Unfortunately the 1.5 mm slices were too wide to allow complete resolution of the bands containing the small subunits, and thinner slicing lowered count rates to unacceptable levels. In both IEF and SDS gels, the region of the small subunits exhibited a $^{3}H/^{14}C$ ratio substantially higher than that of the region corresponding to the large subunits. The higher "background" and slightly smaller difference in $^{3}H/^{14}C$ ratio in the IEF gels is probably attributable to some entrappment of myosin within pores

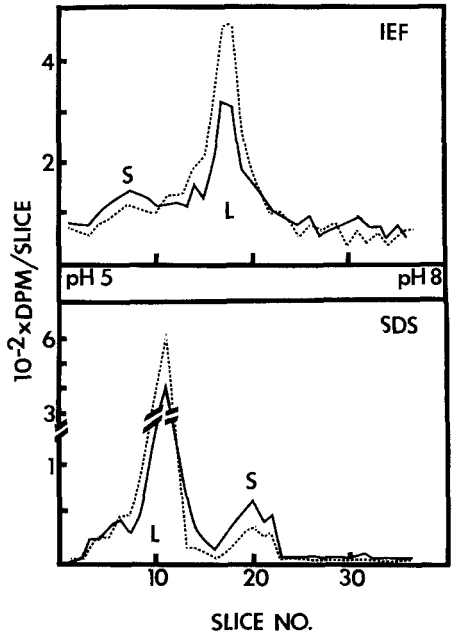

Figure 1. Distribution of ^{3}H and ^{14}C in Myosin Fractionated by Isoelectric Focusing and SDS-Electrophoresis in Polyacrylamide Gels. Myosin was labeled, extracted, and purified as described in the text. Isoelectric focusing (7) was done in 9 M urea, ampholite pH range 5 to 8, and SDS-electrophoresis as described by Paterson and Strohman (8). The gels were fixed in 10% (w/v) trichloroacetic acid and sliced into uniform slices 1.5 mm thick. The slices were counted and absolute radioactivity computed as described in the text. Large and small components of myosin are designated by L and S, respectively; small components are identified by their more rapid mobility in SDS-electrophoresis and by the behavior of isolated small subunits in isoelectric focusing (7). Solid lines indicate ^{3}H and dotted lines ^{14}C.

of the polyacrylamide gel; the sample was present homogeneously throughout the gel during the polymerization process.

Radioisotope determinations in slices of individual bands from the gels confirm the difference between light and heavy chains indicated in Figure 1; data from several analyses are summarized in Table 1. The small subunits exhibited a consistently and significantly higher $^{3}H/^{14}C$ ratio by both methods of analysis.

These results can be interpreted in two ways. In the simpler (and in our opinion, more probable) view, a much greater percentage of small than of large subunit chains were completed, released from polyribosomes, and associated with mature myosin molecules during the short labeling with ^{3}H-leucine. This could not happen if completion of the small subunits were in some way limited to a rate no greater than that of completion of large subunits. This interpretion is in agreement with the reports of Sarkar and Cooke (2) and of Low et al. (3) that the two kinds of subunits are synthesized on separate populations of polyribosomes.

One possible mechanism for coordination of synthesis of large and small subunits is consistent with our data. As Dintzis (4) has shown, the C-terminal portion of newly synthesized polypeptide chains are relatively highly labeled following a short incubation period. Light meromyosin is considered to be at the C-terminal end of the large subunit of myosin (1). Thus if the small components were synthesized as a simple continuation of the peptide chain beyond the light meromyosin terminus (and presumably removed subsequently as in the conversion of proinsulin to insulin), then the small subunits would exhibit the higher $^{3}H/^{14}C$ ratios we observed. Considering the length of the myosin molecule and the ultimate association of the small subunits with heavy rather than light meromyosin (10,11), we regard this as unlikely. However, if the myosin chains grow from C- to N-terminus as suggested by Iwata and Kaji (12) for certain ribosomal proteins, then coordinated synthesis of small subunits could lead to formation of the small subunits at the heavy meromyosin

Table I. Isotope Ratio of the Small and Large Components of Uniformly (^{14}C) and Partially (^{3}H) Labeled Myosin.

Method of Separation	Myosin Preparation	Time of Incubation (in minutes)		Large Subunits	Small Subunits			
					Band 1		Band 2	
		^{3}H	^{14}C	$^{3}H/^{14}C$	$^{3}H/^{14}C$	t test	$^{3}H/^{14}C$	t test
Isoelectric Focusing	1	3	45	0.62±0.09 (4)	1.00±0.62 (4)	$p \leq 0.025$	1.04±0.07 (4)	$p \leq 0.025$
	2	3	45	2.69 (1)	4.85 (1)		5.01 (1)	
	3	5	45	30.7±0.92 (3)	55.5±0.95 (3)	$p \leq 0.005$	52.9±2.77 (3)	$p \leq 0.01$
SDS-electrophoresis	1	3	45	0.55±0.04 (3)	1.07±0.09 (3)	$p \leq 0.025$	1.72±0.24 (3)	$p \leq 0.25$

The $^{3}H/^{14}C$ ratio was obtained by counting slices of gels containing the entire bands of large or small components of double labeled myosin prepared and analyzed under the conditions specified in Figure 13. The small components were numbered arbitrarily; bands 1 and 2 from isoelectric focusing do not necessarily correspond to those obtained by SDS-electrophoresis. The value in parenthesis represents the number of gels from which the mean, S.E.M. and the t test were computed. The t test was performed and evaluated as described by Snedecor and Cochran[14]; p values are for comparisons between small components and the large components in the same gels.

end of the chain. Of course, these single chain models are completely inconsistent with the observations of Sarkar and Cooke (2) and of Low et al. (3); while the possibility exists that their "light chains" could actually be portions of heavy chains synthesized on fragments of polyribosomes formed as artifacts during the isolation procedure, it seems extremely unlikely that such fragments would coincide fortuitously with the size of mRNA for the small subunits normally associated with myosin.

We conclude that our results support the suggestions of Sarkar and Cooke (3) and Low, Vournakis, and Rich (3) that the light chains of myosin are synthesized independently of the heavy chains on a different population of polyribosomes; they are probably produced by genes different from those for the large subunits as recently suggested by Sarkar et al. (13). In addition, our results demonstrate that there is no effective in vivo mechanism for one-to one coordination of biosynthesis of the large and small subunits in embryonic chick muscle.

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