fumarase) by density equilibration in an aqueous sucrose gradient. In this system, monoamine oxidase shows a distinctly lower equilibrium density than the other components. Fumarase, on the other hand, shows an exceptionally high equilibrium density, which depends on the presence of intact RNA.

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The chromatographic behaviour and adenosine triphosphatase activities of the meromyosins

Previous investigations[1] have shown that when L-myosin is chromatographed on diethylaminoethylcellulose the ATPase activities of the eluted fractions vary in a characteristic way. Comparable results have also been obtained in preliminary studies with carboxymethylcellulose[2] and BRAHMS[3] has also reported a fractionation of the ATPase activity. To assess the significance of these indications of the enzymic heterogeneity of chromatographed myosin, L-meromyosin and H-meromyosin have been studied by similar techniques.

H-meromyosin, prepared by the method of SZENT-GYÖRGYI[4] and shown to sediment as a single peak in the ultracentrifuge, was consistently fractionated into two components when applied to a diethylaminoethylcellulose column equilibrated against 0.15 *M* KCl, 20 m*M* Tris–HCl, pH 7.6 or 8.2. Of the total eluted material estimated by its absorption at 280 mμ, 12–13 % passed through the column unheld, whereas the main component was eluted as a symmetrical peak at 0.19–0.20 *M* KCl,

Abbreviations: ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane.

20 m*M* Tris–HCl, pH 7.6. The bulk of the ATPase activity was associated with this peak which consisted of material possessing 230–240 units of specific ATPase activity*, *i.e.* 10–20 % higher than that of the unchromatographed H-meromyosin. The specific ATPase activity was constant in all fractions except for a falling off in the lower regions of the trailing edge of the peak. It is worth noting that the specific activity of some fractions of L-myosin obtained in the earlier study were comparable to those obtained with chromatographed H-meromyosin.

The average specific ATPase activity of the minor peak was about 30–40 % of that of the original H-meromyosin and assay on individual fractions showed a sharp fall in activity from the front of the peak to the trailing edge, where it was either very low or negligible.

L-meromyosin preparations[4] invariably possessed ATPase activity which, when related to absorption at 280 mμ*, was 10–20 % of that of the original myosin. These preparations, which gave single peaks in the ultracentrifuge, were resolved into three fractions on diethylaminoethylcellulose. The first fraction represented about 5-12 % of the original L-meromyosin and was not held in diethylaminoethylcellulose in 0.15 *M* KCl, 20 m*M* Tris–HCl, pH 8.2. This peak possessed only slight, barely significant, ATPase activity whereas there was a characteristic pattern of distribution of specific ATPase activity along the second and main component eluted with its peak at 0.21 *M* KCl, 20 m*M* Tris–HCl, pH 8.2. Highest values were obtained in the first fractions of this peak and the ATPase activity dropped sharply to a fairly constant value of about 15-30 units over the remaining two thirds of the peak.

The trypsin digest of myosin possessed about 50 % of the 5′-adenylic deaminase activity of the original myosin; nevertheless none of this enzyme was present in purified L-meromyosin or H-meromyosin.

An interesting feature of the fractionations reported here was that the ribonucleoprotein invariably found associated with myosin preparations[1,5] was concentrated in the third component isolated from L-meromyosin. This was eluted by the stepwise application of 2 *M* KCl, 20 m*M* Tris–HCl, pH 8.2, after previous removal of the ATPase-containing peak. Under similar conditions H-meromyosin preparations yielded no significant amount of nucleoprotein.

It can be concluded from this investigation that the conventional L-meromyosin and H-meromyosin preparations are chromatographically complex. Further the chromatographic behaviour of the ATPase of H-meromyosin is more compatible with that of a homogenous enzyme preparation than are the results with L-myosin. These findings are of significance in interpreting the results of end-group investigations[6], turnover studies of L-meromyosin and H-meromyosin[7,8], and in the localization of myofibrillar proteins by fluorescent antibody techniques using these myosin derivatives[9].

The investigations are being continued to throw light on the sub-unit structure of myosin.

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* Unit of specific activity[1]: μg P/5 min/1 ml protein of $E_{280}^{1\,cm} = 1$; measured at 25°.

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On the transfer of labeled amino acids from "S-RNA" to purified ribonucleoprotein particles from rat liver microsomes

The incorporation of labeled amino acids into protein by RNP from rat-liver microsomes is enhanced by an enzyme preparation (S-protein[1]) from the soluble liver fraction[2]. The effect of S-protein is increased by GSH or MEA.

The S-protein preparation used in the present experiments corresponded to the purified "S_{50}-protein" of RENDI *et al.*[2], and it had been treated with charcoal for 1 h at 4°. The absence of ribonucleotides in the preparation was ascertained in the following way. S-protein was prepared from rats, treated with 2 mC of [^{32}P]phosphate for 18 h. Carrier RNA was added to the preparation, and ribonucleotides were isolated by chromatography after hydrolysis. No radioactivity was observed in the nucleotide fractions. The activity of the preparations in amino-acid-dependent isotope exchange between [^{32}P]pyrophosphate and ATP (PP–ATP exchange) was slightly variable, but usually small. As a rule no significant formation of [^{14}C]L-leucylhydroxamate could be demonstrated by paper chromatography[3] after incubation of [^{14}C]L-leucine with ATP and NH_2OH in the presence of S-protein.

RNP were prepared by treating mitochondria-free, rat-liver homogenates (in 0.25 *M* sucrose, 0.5 *M* KCl, 0.01 *M* $MgCl_2$ and 0.035 *M* Tris buffer, pH 7.8) with 0.5 % Lubrol W and 1 % sodium deoxycholate, followed by centrifugation through a layer of medium with higher density[2,4]. The incorporation of labeled amino acids into protein by these particles in the presence of S-protein, MEA, ATP, GTP, PEP and pyruvate kinase was amino-acid specific. The incorporation was not inhibited by the presence of other, unlabeled amino acids in a 10-fold excess. Since the S-protein preparations used in these experiments had a quite low activity of amino acid activation, the possibility was considered that certain amounts of amino-acid-activating enzymes were still present in the particles. By use of prolonged incubation periods (60 min) and incubation samples containing 5–7 mg of protein an amino-acid-dependent PP-ATP exchange, amounting to 3.7 %/mg protein, was actually observed in particles of this kind.

Abbreviations: RNP, ribonucleoprotein particles; RNA, ribonucleic acid; ATP, GTP, triphosphates of adenosine and guanosine, respectively; PEP, phosphoenol pyruvate; GSH, reduced glutathione; MEA, 2-mercaptoethylamine; Tris, tris(hydroxymethyl)aminomethane; PP, [^{32}P]pyrophosphate.