NATIVE CONFORMATION OF M-PROTEIN

M.-F. LANDON and C. ORIOL

Laboratoire de Biochimie Cellulaire, Collège de France, 75231 Paris Cedex 05, France

Received October 30,1974

The purification of the M-Protein isolated from a myofibrilar M-line extract is described. Its molecular weight at low ionic strength calculated by YPHANTIS method is equal to 160 000. Optical rotatory dispersion and circular dichroism spectra indicate the presence of about 30 % β-structure and a low α-helical content.

INTRODUCTION

The M-band of skeletal muscle is a structural region of myofibrils, located in the center of the sarcomere. It has been suggested that the function of M-line is to maintain the thick filaments in proper alignement during shortening of sarcomere length when the inter-filament distance increases. It is known that the M-line is composed of three proteins. One of these has a subunit molecular weight of 40 000 daltons and it has been purified by MORIMOTO and HARRINGTON (1) and later identified as MM - creatine kinase by TURNER et al. (2). Another protein of 100 000 daltons was observed by MASAKI and TAKAITI (3) and also by EATON and PEPE (4). These latter authors reported that this 100 000 daltons component is complexed with creatine kinase in the total M-line extract. ETLINGER and FISHMAN (5), and also MASAKI and TAKAITI (3), have described a third protein whose polypeptide chain determined by SDS gel electrophoresis is 165 000. However, MASAKI and TAKAITI, who named this component "M-Protein", used a urea method for its purification.

In this paper, we report a method for the purification of homogeneous M-Protein and the determination of its native molecular weight and conformation.

METHODS

Myofibrils from rabbit skeletal muscle were prepared

in 100 mM KCl, 20 mM borate, 5 mM EDTA, pH 7.0 and the packed myofibrils were then extracted in 10 volumes of 5 mM Tris-HCl, pH 8.0 for 30 min, as described by EATON and PEPE (4). At this stage, the removal of M-line was checked by electron microscopy. The extract was concentrated and precipitated with 18 % of crystallized ammonium sulphate. The precipitate was dissolved in 0.5 mM ATP, 1 mM β-mercaptoethanol, 0.2 mM $CaCl_2$, 2 mM Tris, pH 8.0, and after dialysis against this buffer, applied to a DEAE-cellulose column. The protein was eluted with a 0 to 0.4 M KCl linear gradient and each fraction was analyzed by SDS gel electrophoresis according to NEVILLE (6).

Protein concentration was determined by the Folin or microbiuret method using bovine serum albumin as standard.

All physical measurements were performed in 5 mM Tris-HCl, pH 8.0.

RESULTS and DISCUSSION

Purification

After ammonium sulphate precipitation, the myofibrilar extract contained three proteins whose molecular weights calculated by SDS gel electrophoresis are 165 000, 100 000 and 43 000 (actin) (Fig. 1 a). The binding of the mixture to the DEAE-cellulose column was performed using conditions where actin is depolymerized (7). Pure M-Protein was obtained in the fraction eluted with 0.16 M KCl. The elutions of actin and 100 000 component began at 0.175 M and 0.2 M KCl, respectively. It is probable that MASAKI and TAKAITI could not separate the two components of 165 000 and 100 000 daltons because they began the elution of their QAE-Sephadex at 0.2 M KCl.

In Fig. 1 b and c are shown the SDS and native gel electrophoresis of the purified M-Protein.

Structure

The native molecular weight of this M-Protein was evaluated to 160 000 daltons by high-speed equilibrium (YPHANTIS, 1964) (8) taking $\bar{v}$ = 0.74 ml / g, as calculated from amino acid composition (3). Plot of log absorbance at 290 nm versus the square of the distance from the center of rotation gave a straight line. This linearity is consistent with a

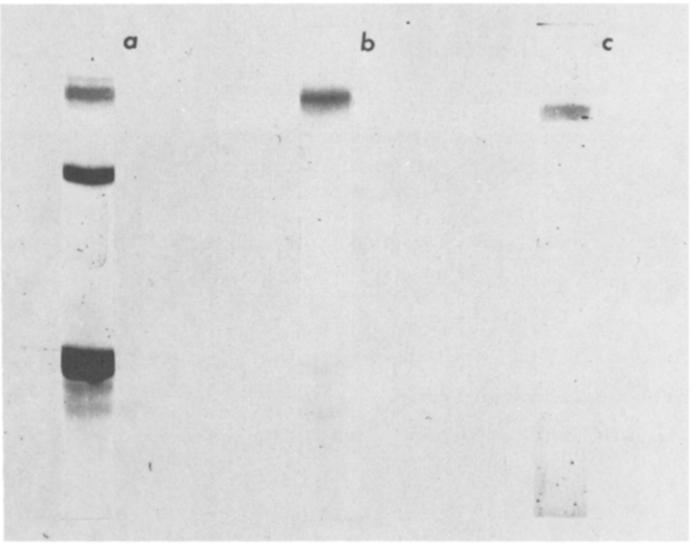

Fig. 1 a and b : SDS gel electrophoresis of the 18 % ammonium sulphate precipitate (a) and of the purified M-Protein (b). Acrylamide concentration : 11.1 %. Molecular weights were determined using myosin, C-protein, phosphorylase A, bovine serum albumin and actin as standards.
c : gel electrophoresis of the M-Protein at 7.5 % acrylamide concentration in conditions described by DAVIS (12).

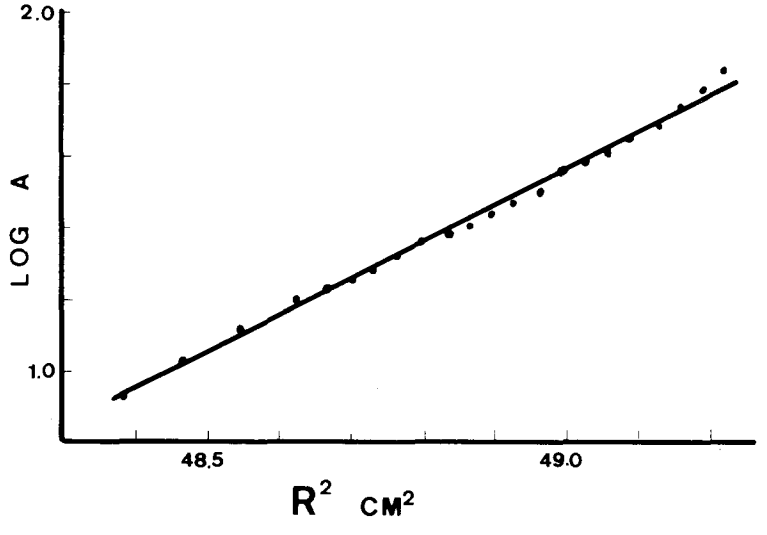

Fig. 2 : high-speed equilibrium of M-Protein in 5 mM Tris-HCl, 1 mM β-mercaptoethanol, pH 8.0, at 5°C. The rotor speed was 16 000 rpm for 32 h.

homogeneous preparation (Fig. 2).

The ORD curve displaid in the far UV region a shallow negative trough at 228-230 nm. The reduced mean residue rotation was $[m']_{229} = -3000 \pm 100°$. This Cotton effect indicated a low α-helical content and the presence of β-structure (9).

The circular dichroism spectrum of M-Protein is shown in Fig. 3. The spectrum differed from that of a typical polypeptide chain in the α-helical conformation as described by HOLZWARTH and DOTY (10). The first negative dichroic band at 222 nm which is characteristic of an α-helix, was shifted to 217-218 nm and the minimum, which had a mean molar residue ellipticity of -6400 ± 300 deg.cm^2.dmol^{-1}, suggested the presence of β-structure. The ellipticity value at 208 nm was $[\theta]_{208} = -7600 \pm 700$ deg.cm^2.dmol^{-1} and a α-helical content of 12 % was calculated at this wavelength from the equation given by GREENFIELD and FASMAN (11). In the spectral zone below 205 nm, the absorbance/ellipticity ratio was unfavorable and the spectrum is not illustrated but it was possible to see that the ellipticity reached approximately a zero value near 190 nm. The comparison of the shapes and amplitudes of GREENFIELD and FASMAN's computed curves for varying percentages of α-helix and β-structure suggested that the M-Protein

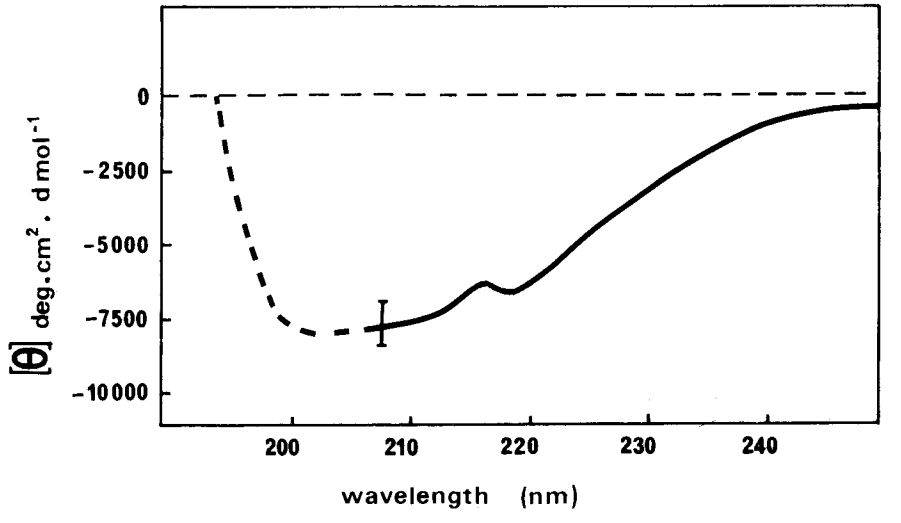

Fig. 3 : CD spectrum of M-Protein in 5 mM Tris-HCl buffer, pH 8.0.

contains approximately 10 % α-helix and 30 % β-structure. These calculations are based on several simplifying assumptions made to evaluate the conformations of proteins from the known structures of polypeptides and these estimations have to be taken only as approached values.

Nevertheless, both ORD and CD spectra agree well with a very low helical conformation and a higher β-structure content.

Experiments directed toward the localization of this M-Protein inside the M-line and the elucidation of its function during muscular contraction are now being carried out.

ACKNOWLEDGEMENTS

We are deeply indebted to Dr. Nguyen van THOAI (Directeur de recherche au Centre National de la Recherche Scientifique) for his interest and encouragement. We are grateful to Dr. H.F. HILDEBRAND for electron microscopy, and to Mrs. Véronique LE COMTE for technical assistance.

This work was supported by a grant from Délégation Générale à la Recherche Scientifique et Technique (A.C. 72 7 0400), and by a grant from Centre National de la Recherche Scientifique (G.R. 6).

REFERENCES

1. MORIMOTO, K. and HARRINGTON, W.F. (1972) J. Biol. Chem. 247, 3052-3061.
2. TURNER, D.C., WALLIMANN, T. and EPPENBERGER, H.M. (1973) Proc. Nat. Acad. Sci. USA, 70, 702-705.
3. MASAKI, T. and TAKAITI, O. (1974) J. Biochem. 75, 367-380.
4. EATON, B.L. and PEPE, F.A. (1972) J. Cell Biol. 55, 681-695.
5. ETLINGER, J.D. and FISCHMAN, D.A. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 511-523.
6. NEVILLE, D.M. (1971) J. Biol. Chem. 246, 6328-6334.
7. REES, M.K. and YOUNG, M. (1967) J. Biol. Chem. 242, 4449-4458.
8. YPHANTIS, D.A. (1964) Biochemistry, 3, 297-317.
9. YANG, J.T. (1967) in "Conformation of Biopolymers" (RAMACHANDRAN, G.N., ed.), Vol. 1, pp. 157-172, Academic Press, New York.
10. HOLZWARTH, G. and DOTY, P. (1965) J. Amer. Chem. Soc. 87, 218-228.
11. GREENFIELD, N. and FASMAN, G.D. (1969) Biochemistry, 8, 4108-4126.
12. DAVIS, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404-427.