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BDM (2,3-butanedione monoxime), an inhibitor of myosin-actin interaction, suppresses myofibrillogenesis in skeletal muscle cells in culture

Abstract During the initial phase of myofibrillogenesis in developing muscle cells, the majority of thin filaments lie parallel to, and exhibit correct polarity and spatial position with thick filaments, as in mature myofibrils. Since myosin is known to function as an accelerator of actin polymerization in vitro, it has been postulated that myosin-actin interaction is important in the initial phase of myofibrillogenesis. To clarify further the role of actin-myosin interaction in myofibril formation during development, BDM (2,3-butanedione 2-monoxime), an inhibitor of myosin ATPase, was applied to primary cultures of skeletal muscle to inhibit myosin activity during myofibrillogenesis, and myofibril formation was examined. When 10 mM BDM was added to the myotubes just after fusion and the cultures were maintained for a further 4 days, cross-striated myofibrils were scarcely observed by fluorescence microscopy when examined by staining with antibodies to actin, myosin, troponin and α-actinin, whereas in the control myotubes not exposed to BDM, typical sarcomeric structures were detected. Electron microscopy revealed a disorganized arrangement of myofilaments and incomplete sarcomeric structures in the BDM-treated myotubes. Thus, formation of cross-striated myofibrils was remarkably suppressed in the BDM-treated myotubes. When the myotubes cultured in BDM-containing media were transferred to control media, sarcomeric structures were formed in 2–3 days, suggesting that the inhibitory effect of BDM on myotubes is reversible. These results suggest that actin-myosin interaction plays a critical role in the early process of myofibrillogenesis.

Key words Myofibril assembly · Actin · Myosin · 2,3-Butanedione 2-monoxime (BDM) · Skeletal muscle · Culture · Chicken

Introduction

In sarcomeres of myofibrils in striated muscles, actin and myosin filaments are organized in a hexagonal lattice with correct polarity and ordered spatial position (Fischman 1967; Shimada and Obinata 1977). Actin filaments are anchored in Z-disks, and myosin filaments are linked to M-bands to maintain the spatial organization. It has been suggested that the spatial information required for the formation of bipolar contractile units of myofibrils with a hexagonal arrangement of actin filaments around myosin filaments is provided by myosin filaments themselves. In an in vitro study, the polymerization of actin in the presence of myosin filaments led to the formation of an ordered hexagonal arrangement of actin filaments (Hayashi et al. 1977). Myosin filaments are known to function as an accelerator of actin polymerization in vitro even in the presence of an inhibitory factor of actin polymerization (Abe and Obinata 1989). Myosin-dependent actin polymerization or actin-myosin interaction seems to be involved in determining the arrangement of the two filaments in the sarcomeric structure during muscle cell development. In the initial phase of myofibrillogenesis in developing muscle cells, the majority of thin filaments lie parallel to thick filaments and exhibit the correct polarity and spatial position with regard to thick filaments (Shimada and Obinata 1977).

Three troponin components, TnT, TnI and TnC, are synthesized and assembled along actin filaments from the beginning of myofibrillogenesis (Obinata et al. 1979b) and the troponin on nascent actin filaments is functionally active (Obinata et al. 1974). Therefore, the actin-myosin interaction which forms the bundles of thin and thick fila-
ments in muscle cells is probably controlled by troponin in a Ca\textsuperscript{2+}-dependent manner.

BDM (2,3-butanedione 2-monoxime) is a potent inhibitor of the contraction of skinned as well as intact muscle of vertebrates. It acts directly on myosin molecules and inhibits actomyosin ATPase and actin-myosin interaction in vitro (Horiuti et al. 1988; Higuchi and Takemori 1989; Yagi et al. 1992; Osterman et al. 1993; McIllopin et al. 1994). Its inhibitory action is not influenced by the troponin-tropomyosin system or the concentration of calcium ions. Therefore, BDM seems to be an appropriate agent for inhibiting actin-myosin interaction during myofibrillogenesis in developing muscle cells.

In this study, to clarify the role of actin-myosin interaction in myofibril formation, BDM was applied to skeletal muscle cultures at early stages of myofibril formation. We observed that the formation of cross-striated myofibrils was remarkably suppressed by BDM. These results indicate that actin-myosin interaction plays an important role in the early process of myofibrillogenesis.

**Materials and methods**

**Cell culture**

Chicken mononucleated myogenic cells from pectoral muscles of 12-day-old chicken embryos were separated by mechanical dissociation (Ii et al. 1982) and plated on collagen-coated glass coverslips in 60-mm-tissue culture dishes at a density of 1 \times 10\textsuperscript{6} cells. The culture medium consisted of 81% MEM (Nissui, Tokyo) supplemented with 2 mM L-glutamine, 15% horse serum, and 4% chick embryo extract. Cultures were maintained in an atmosphere of 5% CO\textsubscript{2} and 95% air at 37°C. For treatment with BDM (Sigma Chemical Co., St. Louis, MO), the culture medium containing 10 mM BDM was prepared just before use by adding 1/500 volume 5 M BDM which had been dissolved in dimethylsulfoxide (DMSO) and stored at –20°C in the dark. The BDM-containing medium was replaced with fresh medium every day after the 2nd day of culture. The control culture medium without BDM contained 0.2% DMSO.