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Does 2,3-butanedione monoxime inhibit nonmuscle myosin?

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Summary. BDM (2,3-butanedione monoxime) has been used extensively to inhibit nonmuscle myosin. However, recent articles raise the question of what BDM actually does, because of experiments in which BDM did not affect the actin-activated ATPase of nonmuscle myosins. We describe results that indicate that BDM indeed inhibits motility due to nonmuscle myosins: in many different cells BDM has the same effects as anti-actin agents and/or as other anti-myosin agents, and BDM slows or stops the sliding between actin filaments and myosin in vitro. We discuss how the two sets of apparently contradictory results might be resolved, and we suggest possible experiments that might clarify the contradictory interpretations.

Keywords: 2,3-Butanedione monoxime; Myosin; Motility; Actin-activated ATPase.

Introduction

BDM (2,3-butanedione monoxime) has been and is used in cell biological experiments to inhibit the functioning of nonmuscle myosin. BDM inhibits the actin-activated ATPase of skeletal muscle myosin (Higuchi and Takemori 1989) as an uncompetitive inhibitor (Herrmann et al. 1992), which means that it does not compete with ATP for the active site of myosin but rather interacts with a different part of the myosin molecule after ATP has bound to the active site. Because BDM also inhibits the actin-activated ATPase of tissue-purified blood platelet myosin II, tissue-purified chick brain myosin V, and partially purified *Drosophila melanogaster* myosin VI (Cramer and Mitchison 1995), within the same range of concentrations that affect muscle myosin, cell biologists have used BDM to implicate nonmuscle myosins in a variety of cell processes.

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One side of the story: BDM inhibits nonmuscle myosins

BDM and anti-actin agents

BDM has been used in some experiments in conjunction with anti-actin agents: when BDM and the anti-actin agents affect a cellular motile process in the same way, this is taken as evidence that myosin is involved in that process. Experiments of this kind have been performed in numerous cellular systems. In lamella of cultured newt cells, retrograde (rearward) flow of microtubules was blocked with both cytochalasin D (an actin poison) and BDM, implicating actin and myosin in the movements of those microtubules (Waterman-Storer and Salmon 1997). In crane-fly spermatocytes, the actin poisons cytochalasin D and latrunculin (Forer and Pickett-Heaps 1998) altered anaphase chromosome movements in the same way as BDM (Silverman-Gavrila and Forer 2001), and BDM, cytochalasin D, latrunculin, and swinholide, another actin poison, each blocked tubulin flux along kinetochore microtubules (Silverman-Gavrila and Forer 2000), implicating actin and myosin in these functions. In plant cells, directional movements of Golgi stacks were inhibited by cytochalasin D and by BDM (Nebenführ et al. 1999), directional movements of peroxisomes were inhibited by latrunculin and by BDM (Jedd and Chua 2002), movements of mitochondria were inhibited by latrunculin and by BDM (Van Gestel et al. 2002), the induction of spherical

bodies in the cytoplasm was blocked equally by latrunculin, cytochalasin, and BDM (Yokota et al. 2000), and cytoplasmic streaming in *Coleus* spp. was arrested by cytochalasin B and by BDM (Panteris et al. 2004). In human tissue culture cells, cytoplasmic movements of specific secretory organelles were inhibited by latrunculin and by BDM (Manneville et al. 2003), and in sea urchin eggs, cortical contractions were blocked by cytochalasin B and by BDM (Asano and Mabuchi 2001). In all these systems, BDM had the same effects as anti-actin agents, which strongly suggests that BDM indeed interferes with motility due to nonmuscle myosins.

BDM and other myosin inhibitors

BDM has been used in other experiments in conjunction with specific inhibitors of myosin. When both agents have the same effects, this is taken as evidence that myosin motile activity is involved in the processes in question, as in the following examples. In some experiments, BDM had the same effects as ML-7, an inhibitor of myosin light chain kinase: both BDM and ML-7 inhibited the movements of cytoplasmic microtubules in PtK2 cells (Yvon and Wadsworth 2000, Yvon et al. 2001), inhibited protein transport from Golgi bodies to endoplasmic reticulum in cultured cells (Durán et al. 2003), halted growth and then caused collapse of growth cones in *Xenopus laevis* ganglia (Ruchhoeft and Harris 1997), inhibited cytokinesis of crane-fly spermatocytes (Silverman-Gavrila and Forer 2001), blocked the movements of myosin-containing vesicles along actin filaments from clam oocytes (de Pina 2000), inhibited postmitotic nuclear movements and slowed cytoplasmic streaming in algal cells (Oertel et al. 2003), and inhibited cytoplasmic streaming and interfered with cytokinesis in *Tradescantia virginiana* stamen hair cells (Hepler et al. 2002, Molchan et al. 2002). (The last two articles discuss the mode of action of ML-7 in plant cells.) Since in all these systems BDM had the same inhibitory effects as an inhibitor of myosin light chain kinase, BDM appears to have inhibited motility due to nonmuscle myosins. In other experiments, BDM had the same effect as NEM-poisoned myosin subfragment S1 in inhibiting the retrograde flow of actin in neurons (Lin et al. 1996), as a nonfunctional myosin in inhibiting axon retraction (Gallo et al. 2002), as injected anti-myosin antibody and as a specific peptide that inhibits myosin in blocking vesicle transport during cell secretion (Ñeco et al. 2002), and as a peptide that inhibits myosin light chain kinase in blocking the movement of cytoplasmic microtubules (Yvon et al. 2001). In these systems, the effects of BDM were the same as those of other, specific inhibitors of myosin, which indicates that BDM inhibits motility due to nonmuscle myosins.

BDM tested in vitro

BDM has been tested directly in in vitro assays of actin-myosin sliding: it inhibited the sliding of skeletal muscle actin along isolated lily myosin in a concentration-dependent manner (Tominaga et al. 2000), inhibited the sliding of tissue-purified myosin V along actin filaments in a concentration-dependent manner (Uemura et al. 2004), and inhibited the movements of vesicles from clam oocytes along actin filaments (de Pina 2000). These experiments show that BDM directly inhibits motility due to nonmuscle myosins.

The other side of the story: BDM does not inhibit nonmuscle myosin

Dissenting views arose because BDM was reported to have no effect on the ATPases or on the actin-activated ATPases of truncated, baculovirus-expressed nonmuscle myosins I, V, and VI (Ostap 2002). These results contradict the original findings of Cramer and Mitchison (1995) that BDM blocks the actin-activated ATPases of nonmuscle myosins. Ostap (2002) raised the possibility that the difference might be due to his use of recombinant myosins instead of tissue-purified myosins, which had been used by Mitchison and Cramer (1995), but he favored the conclusion that their ATPase assays had "a problem". As a consequence of these results, several publications (Ostap 2002, Titus 2003, Yarrow et al. 2003) questioned whether BDM can be considered an inhibitor of nonmuscle myosins.

These results present us with a contradiction. How can the observations that BDM has no effect on the ATPase activities of nonmuscle myosins I, V, and VI (Ostap 2002) and nonmuscle myosin II (Cheung et al. 2002, Yarrow et al. 2003) be reconciled with the experiments described above that strongly suggest that BDM inhibits motility due to nonmuscle myosins?

Clarifying the contradiction

One possible resolution of the contradiction is that BDM might affect motility but not ATPase. That is, even if the more recent ATPase measurements correctly indicate that BDM does not affect nonmuscle myosin ATPases, BDM might block myosin-induced motility nonetheless, as has been shown with other agents that inhibit myosin-based motility but not its actin-activated ATPase. For example, li-

docaine and tetracaine block actin motility in in vitro sliding assays using skeletal muscle myosin (Tsuda et al. 1996) and 100 μM Ca^{2+} inhibits actin motility in sliding assays using myosin V (Krementsov et al. 2004) but none of these inhibit the actin-activated ATPase activity. These experiments indicate that with both skeletal and nonmuscle myosins, effects on motility can be uncoupled from effects on ATPase activity. Thus, BDM added to cells might affect motility due to nonmuscle myosin and not necessarily its ATPase.

Another possibility is suggested by recent work comparing truncated versus full-length baculovirus-expressed myosin V. Results from experiments using tissue-purified myosin V were inconsistent with those using baculovirus-expressed (truncated) myosin V. The inconsistencies were resolved by comparing a series of different baculovirus myosin V constructs, ranging from a short segment containing only a single-headed active site (plus a short neck with two IQ motifs) to a full-length, double-headed myosin V with attendant regulatory molecules (Krementsov et al. 2004); there were differences in various physiological and motility parameters between tissue-purified myosin and all constructs except the full-length myosin. These observations caution that experimental results obtained with truncated myosins (e.g., Ostap 2002) are not always the same as those obtained with full-length myosins (e.g., Cramer and Mitchison 1995).

In summary, we have discussed possible explanations of the contradictory results and interpretations of the effects of BDM on nonmuscle myosins, including that measurements of truncated myosins can be different from those of full-length myosins, and that the absence of effects on myosin ATPase activity does not necessarily mean that motility is unaffected. The latter point is emphasized by experiments using myosin V that point to crucial roles of regulatory elements (such as calmodulin) and of myosin conformational changes in producing motility, and that indicate that interfering with the function of these elements can prevent motility even with normal ATPase activity (Krementsov et al. 2004). The persuasive evidence that BDM affects nonmuscle myosin motility in vivo and in vitro, and the results that illustrate the physiological differences that exist between truncated and full-length forms of myosin V (Krementsov et al. 2004), together suggest that the contradictory results concerning the effects of BDM might be due to the use of truncated myosins in the recent ATPase assays. We therefore suggest that, before cell biologists give up on BDM as an inhibitor of myosin-induced cell motility, it should be tested with full-length myosins that contain all necessary regulatory elements in assays of both actin-activated ATPase and motility (sliding between actin and myosin).

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