A Is For . . .

Calpain

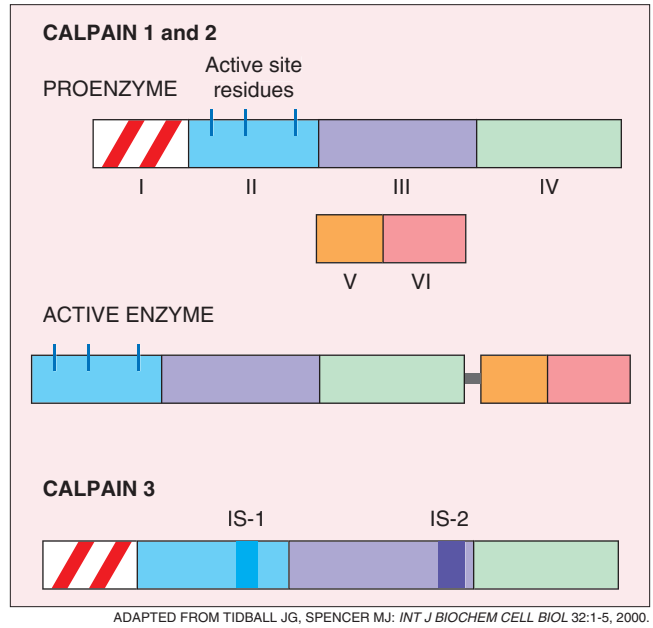
calpain *Any of a large family of intracellular calcium-dependent cysteine proteases, some ubiquitous and others tissue-specific, that are most active at neutral pH; also called calcium-activated neutral protease or CANP.*

The breakdown of proteins into large fragments and ultimately into small peptides or single amino acids is a physiologic function that was historically associated with extracellular sites such as the digestive tract and, inside cells, with lysosomes. The finding of proteolytic activity in the cytosol was sporadically reported as long ago as the 1940s, and attempts to purify the responsible enzymes began in the 1960s, when a calcium-activated protease was described.

Japanese investigators proposed the generic term *calpain* in 1981, "cal" representing calcium and "pain" adapted from papain, a much better known cysteine protease (whose name incidentally is derived, like the protease itself, from the papaya plant). By that time, it had been established that two distinct forms of calpain existed, one requiring millimolar concentrations of calcium and hence called m-calpain (or calpain I) and the other active at micromolar calcium concentrations (μ-calpain or calpain II).

Both enzymes were widely distributed in mammalian cells along with a specific inhibitor, termed calpastatin, but identification of their substrates and therefore their function had not made much progress. For one thing, cytosolic calcium concentrations are ordinarily in the 100-nanomolar range, far too low to produce the enzyme activation that could be shown experimentally, and for another, calcium binding was found to lead almost immediately to autolysis of the protease itself as part of the activation process.

Structurally, m-calpain and μ-calpain were reported to be quite similiar, each isozyme consisting of a large catalytic subunit, in which the active site cysteine was buried until calcium binding induced a conformational change, and a small regulatory subunit that incorporated an additional calcium-binding site. Beginning in 1989, however, other calpains were described that were tissue-specific and that had somewhat different structures, including one in muscle, now called calpain 3, that did not require calcium and was not inhibited by calpastatin.



ADAPTED FROM TIDBALL JG, SPENCER MJ: *INT J BIOCHEM CELL BIOL* 32:1-5, 2000.

"Classical" calpains 1 and 2 are synthesized in two parts, a large subunit comprising domains I to IV and a small subunit made up of domains V and VI. When the protease is activated, the subunits are noncovalently associated. Calpain 3 has additional specific sequences and lacks the small subunit.

In vivo studies of calpain are still handicapped by uncertainty about its substrates, though an emerging consensus favors cytoskeletal proteins and certain membrane-associated proteins as leading candidates. Calpain seems to prefer calmodulin-binding proteins, as might be expected because its own calcium-binding domains are much like calmodulin.

That same handicap prevents the firm association of calpains with disease states; even where tissue levels or activities of calpain or its inhibitor can be shown to be altered, there is no known mechanism linking these changes to a disease. The best case has been made for a variety of limb-girdle muscular dystrophy, type 2A, in which structural proteins at the sarcolemma are all normal (unlike other muscular dystrophies) and the defect is a mutation in calpain 3. Note that the dystrophy is associated with inactivity, not overactivity, of the protease, which means the protease therefore must be vital to muscle function.

RECENT REVIEW

Per-Olof Hasselgren and Josef E. Fischer: Muscle cachexia: Current concepts of intracellular mechanisms and molecular regulation. *Annals of Surgery* 233:9-17, January 2001.