Proteasomal Activity in Skeletal Muscle: A Matter of Assay Design, Muscle Type and Age

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RESULTS:

ASSAY SETUP:

10 µg of total skeletal muscle lysate result in a stable signal

In general, reliable results of proteasomal activity measurements are related to the stability and quality of the luminescence signal. In a first step, we studied the influence of various amounts of protein (1, 5, 10, 25, and 50 µg) on signal intensity, signal stability, and unspecific activities (i.e., total peptidase activity divided by unspecific background activity). Using a protein extract from skeletal muscle with a total amount of 5 or 10 µg of protein resulted in a fairly stable luminescence signal between 50 and 70 min. In contrast, 25 or 50 µg of protein yielded a marked and distinct loss of signal intensity, whereas 1 µg of protein resulted in only a very weak signal at the baseline.

SUMMARY AND CONCLUSIONS

Since biochemical analyses are often hampered by the limited amount of available tissue, we established and validated a luminescence-based proteasomal activity assay applicable to 5-mg quantities of skeletal muscle tissue. This novel assay allows to distinguish between unspecified background activities and specific proteasomal activity. We demonstrate that the specific proteasomal activity differs in individual muscle groups and decreases with aging. This proteasomal activity assay enables systematic analyses of the proteasome function in tissue specimens derived from animal models and human pathologies.