Myosin Head Rotation in Muscle Fibers Measured Using Polarized Fluorescence Photobleaching Recovery

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The technique of polarized fluorescence photobleaching recovery (PFPR) has been applied for the first time to investigation of the rotational correlation time of the myosin head in muscle fibers. This is a novel application of PFPR because it is the first time PFPR has been applied to a sample which is not cylindrically symmetric about the optical axis. Therefore we present a method for analysis of PFPR results from an oriented sample such as the muscle fibers aligned perpendicularly to the optical axis used here. Control experiments performed on fluorescently labeled myosin heads in solution demonstrate that, under some conditions, our PFPR apparatus can easily measure a rotational correlation time of less than 200 μs. Validity of this application of PFPR to muscle fibers is provided by the agreement of our results with published results from a variety of other spectroscopic techniques. In particular, using glycerinated rabbit psoas muscle fibers, we find that for relaxed fibers and isometrically contracting fibers, the myosin heads undergo high-amplitude rotations on the submillisecond time domain. For fibers in rigor the myosin heads are highly oriented and nearly immobile. For fibers in ADP the myosin heads are highly ordered in a distribution quite different from that in rigor, and they are slightly more mobile than in rigor.

KEY WORDS: Muscle contraction; cross-bridge rotation; polarized fluorescence photobleaching recovery.

INTRODUCTION

The molecular mechanism of muscle contraction involves the relative movement of myosin and actin filaments driven by the hydrolysis of ATP in the myosin head or cross-bridge. It is widely accepted that the cross-bridge, or a large part of it, undergoes a rotation while attached to actin to impel one filament relative to the other and that the impulsive force is from transduction of chemical energy liberated in ATP hydrolysis. For some time now we have used spectroscopic probes to investigate the steady-state orientation of specific sites on the cross-bridge and we have determined the path and extent of cross-bridge rotation in the course of its activated cycle.¹⁻⁴ In this work we introduce for the first time the application of the time-resolved technique of polarized fluorescence photobleaching recovery (PFPR) to the investigation of the rotational dynamics of the cross-bridge. Our goal is to characterize the time scale and range of cross-bridge rotation in four physiological states of the muscle fiber including isometric contraction.

Rotation of the myosin head in muscle fibers has been investigated on the submillisecond time scale with EPR⁵⁻⁷ and phosphorescence.⁸⁻⁹ These methods are limited to submillisecond investigations due to the fixed lifetimes of the signals generated from the probes. PFPR does not depend on the fluorescence lifetime and therefore can investigate arbitrarily long time scales. PFPR has been used to detect rotation on the time scale of microseconds¹⁰⁻¹¹. A previous investigation
Fig. 1. Polarized fluorescence photobleaching recovery curves for 5-
IATR-labeled S1 in 70% glycerol at T = -30°C. The photobleaching 
pulse was 5 μs in duration and occurred at t = 0.3 ms. The bleach 
polarization was parallel to the illumination polarization for the filled 
squares and perpendicular for the open circles.

on the millisecond time scale used fluctuations of polarized 
fluorescence to measure myosin head rotation in 
fibers.[12]

This study is the first application of PFPR to myosin 
head rotation in muscle fibers. Therefore control 
experiments were performed on samples of the rhoda-
mine-labeled myosin head, R-S1, tumbling in solution 
which demonstrate that, under some conditions, our 
PFPR apparatus can easily measure rotational correlation 
times for the myosin head of less than 200 μs. Addi-
tional control experiments were performed on rhodamine 
B immobilized in silicone vacuum grease.

This is also the first time that PFPR has been 
applied to a sample which is not either completely isotropic 
or cylindrically symmetric about the optical axis. There-
fore we present a method for analysis of PFPR results 
obtained from an oriented sample such as the muscle 
fibers in the present study are an oriented sample. 
We give a brief description of the PFPR technique, fol-
lowed by considerations for oriented samples.

PFPR is used to measure the rotational correlation 
time of molecules or small particles labeled with a flu-
orescent probe. This is accomplished by using a brief 
intense flash of polarized light to photobleach preferen-
tially fluorescent probes whose absorption dipoles are 
parallel to the optical axis. Relaxation of the resulting 
anisotropic distribution of bleached fluorophore is measured by monitoring the postbleach flu-
orescence excited by a constant low-intensity polarized 
illumination. Two types of experiments are used, with 
the photobleach polarization parallel and perpendicular 
to the illumination polarization, yielding postbleach flu-
orescences F\parallel(t) and F\perp(t). An emission polarizer is ori-
ented parallel to the illumination polarization.

Figure 1 shows PFPR results for rhodamine-labeled 
myosin subfragment 1 (R-S1) in 70% glycerol at -30°C 
and a photobleaching duration of 5 μs. For the parallel 
photobleach, the fluorophores whose absorption dipoles 
are aligned with the illumination polarization are prefer-
entially photobleached. Thus the initial postbleach flu-
orescence for the parallel photobleach is deeper than for 
the perpendicular photobleach, which preferentially pho-