A REVIEW OF TECHNIQUES FOR TIME-RESOLVED
X-RAY STUDIES ON MUSCLE

A. R. Faruqi and H. E. Huxley

MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB2 2QH, England

CONTENTS

1. Introduction
2. Muscle Structure and X-ray Diagram
3. Counting Rates and Experimental Requirements
   A. X-ray Detector
   B. Electronic Routing
   C. Data Summation
4. Techniques
   A. Synchrotron Radiation
   B. Position Sensitive Detectors
   C. Data Analysis
5. Examples

1. INTRODUCTION

The molecular structure of muscle is of great importance in modern biology for a variety of reasons but mainly because it allows an insight into the mechanism of conversion of chemical energy into mechanical work. This is a fundamental and general problem encountered not only in muscle but also in other situations, e.g. cell motility, cytoplasmic streaming or flagellar movement. The use of structural methods, viz electron microscopy and X-ray diffraction, has complemented biochemical and physiological techniques in elucidating the structure and function of different types of muscle1,2,3. We will restrict our discussion to vertebrate striated muscles in this paper as they have been most extensively used in structural studies.

Muscle is a dynamic system, the changes in internal structure resulting in external changes, the most important of which is the
generation of force used for performing mechanical work; it is ob-
viously important to find out the nature of these basic structural
changes on a molecular level which are responsible for the generation
of force. Time-resolved X-ray diffraction has proven to be a very
important technique in studying such structural changes during con-
traction because live muscles can be studied in as close to 'natural'
conditions as possible without any structural modifications (such
as heavy metal staining as in electron microscopy). Provided changes
in the X-ray pattern can be recorded sufficiently rapidly during con-
traction, a tool is available, in principle, for finding out the
nature of structural changes.

The main theme of the paper is concerned with the application
of several new techniques (discussed in detail in Section 4) to time-
resolved studies; these include the application of synchrotron radia-
tion, position sensitive detectors and electronic routing systems
along with semi-automatic software for data analysis. A brief intro-
duction to muscle structure and X-ray diagram is given in Section 2
followed by a discussion on the typical counting rates in the X-ray
pattern and the technical requirements that this poses in Section 3.
Finally we show some recent results illustrating the technique in
Section 5.

2. MUSCLE STRUCTURE AND X-RAY DIAGRAM

The aim of this brief introduction to the molecular structure of
vertebrate striated muscle is to give some background information
about those parts of the regular structure which contribute strongly
to the low angle X-ray pattern and to show how changes in this struc-
ture might contribute to changes in the pattern.

The contractile apparatus of the muscle is contained within long,
thin myofibrils, 1 to 2 µm in diameter and several centimetres long.
The myofibrils are packed together to form fibres which have a dia-
meter of ~100 µm. The main protein components are contained in two
partially overlapping filaments, 'thick' filaments which are composed
mainly of myosin and 'thin' filaments consisting mainly of actin
(shown in Figure 1). To give some rough dimensions, the myosin fi-
laments are ~1.6 µm in length and 100-120 Å in diameter and the ac-
tin filaments ~1 µm in length and 50-70 Å in diameter. The actin
filaments are attached to a structure known as the Z-line, which
also delineates a standard unit of the muscle cell known as a "sar-
comere" which is typically ~2.5 µm in resting muscle. Actin fila-
ments on either side of the Z-line have opposite polarity, a fact
that is used in force generation by cross-bridges projecting from
the myosin filaments. Cross-bridges are thought to attach to binding
sites on actin, go through a 'tilting' motion producing a relative
sliding movement between the filaments, and then become detached;
they go repetitively through this cycle in an asynchronous manner,
i.e. different cross-bridges perform the cycle at different times.