MUSCLE CROSSBRIDGE POSITIONS FROM EQUATORIAL DIFFRACTION DATA: AN APPROACH TOWARDS SOLVING THE PHASE PROBLEM

John Squire and Jeffrey Harford

Biopolymer Group, Imperial College, London SW7, England

ABSTRACT
Following a discussion of the problems involved in the analysis of X-ray diffraction data from muscle, a description is given of a possible procedure for solving the phase problem in the case of equatorial diffraction data. The approach involves the use of the Patterson Function which can be determined unambiguously from the observed diffracted intensities. The method is tested using five different muscle-like model density distributions for which the correct phases can be calculated directly. It is then applied to the equatorial X-ray diffraction data from relaxed frog sartorius muscle where it selects a phase set which is also the most likely to be correct on the basis of other available data on frog muscle. This phase set gives rise to a Fourier synthesis map in which the crossbridges form a uniform shelf of density around the myosin filament backbones. Possible lateral movements of the crossbridges from this relaxed configuration in active and rigor muscle are discussed. The approach to solving the phase problem is now being applied to data from fish muscle, insect flight muscle and crab muscle. It should also have its application to other fibrous materials apart from muscle.

INTRODUCTION
X-ray diffraction studies of muscle clearly have the potential to reveal the nature of the myosin crossbridge movements which are involved in force generation. Changes have been observed in the diffracted intensity from muscles in different static states (e.g. relaxed and rigor) or while active (e.g. Huxley and Brown, 1967; Yu, Hartt and Podolsky, 1979; Huxley et al., 1980; 1981) and these changes are thought to be largely due to crossbridge movements. However, in the past, the problems involved in interpreting the observed diffraction patterns, or the changes between them, have been formidable. In the case of vertebrate skeletal muscle this has been for two main reasons. First, there
is the problem inherent to all X-ray diffraction studies that when the
diffraction pattern is recorded only one-half of the information necessary
to reconstruct an image of the diffracting object is available. As
described below, both the diffracted amplitudes and the relative phases
of the diffracted beams are needed to do this, but only the amplitudes
can be determined experimentally. This is the well-known phase problem
in X-ray diffraction. Second, there has been the problem in vertebrate
muscle that the ‘unit cell’ (including the symmetry and three-
dimensional arrangement of the myosin filaments) in the overlap region
of the A-band where force is actually generated has until recently been
ill-defined. Attempting to understand the diffraction patterns from ver-
tebrate muscles in detail has therefore been akin to trying to solve the
structure of a globular protein using X-ray crystallography but without
knowing the basic symmetry of the unit cell of the protein crystal.

Recently we have been able to define the three-dimensional
geometry of two types of vertebrate skeletal muscle; frog muscle with a
myosin filament superlattice (Luther and Squire, 1980) and fish muscle
with a simple myosin filament lattice (Luther, Munro and Squire, 1981).
We have also provided strong evidence that vertebrate myosin filaments
have 3-fold rotational symmetry (Luther et al., 1981; a result consistent
with data from other sources (e.g. Maw and Rowe, 1980; Stewart et aI.,
1981). More recently, Drs. P.K. Luther (this laboratory) and A.R. Crowther
(MRC Laboratory of Molecular Biology, Cambridge) have confirmed
directly, by three-dimensional reconstruction of tilted A-band cross-
sections of fish muscle, that these myosin filaments have the symmetry of
the Dihedral Point Group 32 (i.e. a 3-fold rotation axis along the filament
axis, perpendicular to three 2-fold axes in the plane of the middle of the
M-band (M1; Luther, Crowther and Squire, 1982). Thus the symmetry of
the vertebrate A-band unit cell is now becoming much clearer. For this
reason it is timely to turn to the other basic problem in X-ray diffraction
studies; that of solving the phase problem. This paper discusses one pos-
sible approach to the solution of the phase problem in the case of cen-
trosymmetric structures. It has particular application to the equatorial
X-ray diffraction data from muscle.

**Equatorial Diffraction Studies**

If a diffraction pattern is recorded from a structure with order in
three dimensions, then each observed diffraction peak hkl will have an
intensity I(hkl) which, after suitable correction, can be used to yield a
structure amplitude |F(hkl)| \(= (I(hkl))^{1/2}\). The structure factor F(hkl)
is in general a complex quantity given by F(hkl) = |F(hkl)| \(\exp(\alpha)\) where
\(\alpha\) is the relative phase of that particular diffracted beam. Since the
intensity of the peak (I(hkl)) is the only thing recorded experimentally
and this is equal to |F(hkl)|^2, no information is available directly from the
diffraction pattern about the value of the phase angle \(\alpha\). This is the phase
problem. It is a problem because, in order to reconstruct the electron
density \(\rho(xyz)\) in the diffracting object, it is necessary to know F(hkl) in
both amplitude and phase since: