# 26. A HISTORICAL PERSPECTIVE

## 26.1. How the structure of lysozyme was actually determined

BY C. C. F. BLAKE, R. H. FENN, L. N. JOHNSON, D. F. KOENIG, G. A. MAIR, A. C. T. NORTH, J. W. H. OLDHAM, D. C. PHILLIPS, R. J. POLJAK, V. R. SARMA AND C. A. VERNON

### 26.1.1. Introduction

For protein crystallographers, the year 1960 was the spring of hope. The determination of the three-dimensional structure of sperm-whale myoglobin at 2 Å resolution (Kendrew *et al.*, 1960) had shown that such analyses were possible, and the parallel study of horse haemoglobin at 5.5 Å resolution (Perutz *et al.*, 1960) had shown that even low-resolution studies could, under favourable circumstances, reveal important biological information. All seemed set for a dramatic expansion in protein studies.

At the Royal Institution in London, two of us (CCFB and DCP) had used the laboratory-prototype linear diffractometer (Arndt & Phillips, 1961) to extend the myoglobin measurements to 1.4 Å resolution for use in refinement of the structure (Watson *et al.*, 1963), and we had begun a detailed study of irradiation damage in the myoglobin crystals (Blake & Phillips, 1962). Meanwhile, David Green, an early contributor to the haemoglobin work (Green *et al.*, 1954), and ACTN had initiated a study of $\beta$-lactoglobulin (Green *et al.*, 1956) and worked together on oxyhaemoglobin before Green went to the Massachusetts Institute of Technology (MIT) in 1959 on leave for a year. At roughly this time, many of the participants in the myoglobin and haemoglobin work at Cambridge went off to other laboratories to initiate or reinforce other studies. Thus, Dick Dickerson went with Larry Steinrauf to the University of Illinois, Urbana, to start a study of the triclinic crystals of hen egg-white lysozyme.

RJP went to MIT from the Argentine as a post-doctoral fellow in 1958 and worked initially with Martin Buerger. In 1959 he transferred to Alex Rich's laboratory and there he soon came into contact with a number of veterans of the myoglobin and haemoglobin work. In addition to David Green were Howard Dintzis, who had discovered a number of the important heavy-atom derivatives of myoglobin (Bluhm *et al.*, 1958) and was now on the staff at MIT, and David Blow, who had first used multiple isomorphous replacement and anomalous scattering to determine haemoglobin phases (Blow, 1958) and was on leave from Cambridge. The influence of these people, combined with lectures by John Kendrew and then by Max Perutz on visits to MIT, soon convinced RJP that working on the three-dimensional structures of proteins was the most challenging and fruitful research that a crystallographer could undertake. Dintzis, in particular, persuaded him that preparing heavy-atom derivatives was no great problem, and Blow urged him to look for commercially available proteins that were known to crystallize. This soon focused his attention also on hen egg-white lysozyme (Fleming, 1922), but in the tetragonal rather than the triclinic crystal form. He quickly learned to grow crystals by the method described by Alderton *et al.* (1945) and then found that precession photographs of crystals soaked in uranyl nitrate showed intensities that differed significantly from those given by the native crystals. Encouraged by these results, he asked Max Perutz whether he could join the Cambridge Laboratory, but Max, having no room in Cambridge, suggested that he write to Sir Lawrence Bragg about going to the Royal Institution. Bragg replied with an offer of a place to work on $\beta$-lactoglobulin with David Green, who had by then returned to London. RJP accepted the offer and left for London late in 1960 – after first discussing what was going on at the Royal Institution with ACTN, who had just arrived at MIT for a year's leave with Alex Rich.

Early in 1961, RJP showed Bragg his precession photographs of potential lysozyme derivatives, and Bragg enthusiastically encouraged him to continue the work, at the same time urging DCP to arrange as much support as possible. This was a characteristic response by Bragg, who was well aware that at least two other groups were already working on lysozyme, Dickerson and Steinrauf at Urbana and Pauling and Corey at Cal Tech (Corey *et al.*, 1952): competition with Pauling was a common feature of his career. In describing his reaction to Bragg's encouragement, RJP recalled Metchnikoff's view of Pasteur. 'He transferred his enthusiasm and energy to his colleagues. He never discouraged anyone by the air of scepticism so common among scientists who had attained the height of their success . . . He combined with genius a vibrant soul, a profound goodness of heart.'

### 26.1.2. Structure analysis at 6 Å resolution

#### 26.1.2.1. *Technical facilities*

In 1961, the Davy Faraday Laboratory was well equipped with X-ray generators. They included both conventional X-ray tubes, operating at 40 kV and 20 mA to produce copper $K\alpha$ radiation, and high-powered rotating-anode tubes that had been built in the laboratory to the design of D. A. G. Broad (patent 1956) under the direction of U. W. Arndt. We had a number of Buerger precession cameras and a Joyce–Loebl scanning densitometer, which had been used in the analysis of myoglobin (Kendrew *et al.*, 1960). In addition, we had a laboratory prototype linear diffractometer (Arndt & Phillips, 1961), which had been made in the laboratory workshop by T. H. Faulkner, and the manually operated three-circle diffractometer that had been used to make some of the measurements in the 6 Å studies of myoglobin (Kendrew *et al.*, 1958) and haemoglobin (Cullis *et al.*, 1961). The diffractometers were used with sealed X-ray tubes, since the rotating anodes were not considered to be reliable or stable enough for this purpose.

At this stage, most of the computations were done by hand, but we did have access to the University of London Ferranti MERCURY computer, usually in the middle of the night. This machine was programmed in MERCURY Autocode. The development of the early computers, their control systems and compilers mentioned in this article have been described by Lavington (1980).

#### 26.1.2.2. *Lysozyme crystallization*

Tetragonal lysozyme crystals were first reported by Abraham & Robinson (1937) and the standard method of preparation was developed by Alderton *et al.* (1945); RJP used this method. Lyophilized lysozyme was obtained commercially and dissolved in distilled water at concentrations ranging from 50 to 100 mg ml$^{-1}$. To a volume of the lysozyme solution, an equal volume of 10% (*w*/*v*) NaCl in 0.1 *M* sodium acetate (pH 4.7) was added. About 1 to 2 ml aliquots of this mixture were pipetted into glass vials and tightly capped. Large crystals, frequently with





references