structure (Blake *et al.*, 1965), we accepted the identification of these residues by Jollès and his colleagues. As will be seen, this was a mistake.

The model was completed in time for it to form the centrepiece at Bragg's 75th birthday party at the Royal Institution on 31 March 1965, and a description was submitted to *Nature* at about the same time (Blake *et al.*, 1965). One of the difficulties at this stage of development of the subject was that computer graphics had not yet been developed to the stage at which illustrations of protein structures could be produced with any degree of facility. Consequently, we were most grateful that Sir Lawrence Bragg enthusiastically drew the model freehand. His drawing provided the main illustration for the published paper: the original sketch is reproduced in Fig. 26.1.3.11.

During the early summer of 1965, we rebuilt the model, taking care to ensure that the components fitted the electron density as well as could be judged by eye, and that the contacts between them were fully consistent with current understanding of van der Waals forces and hydrogen bonds. The model components were fixed firmly in position with small brass clamps, designed and constructed by Bruce Morris in the Royal Institution workshop, so that at the end of the process we had an unrealistically rigid model that incorporated all the information available to us at the time. At this stage it became clear that the Canfield sequence (Fig. 26.1.3.10) was to be preferred in every respect.

A stereo-photograph of this model (Blake *et al.*, 1967) is shown in Fig. 26.1.3.12 in a view that shows the most striking feature of the molecule. The molecular face in the foreground of this view is crossed by a deep cleft, roughly parallel to the *c* axis of the crystal unit cell.

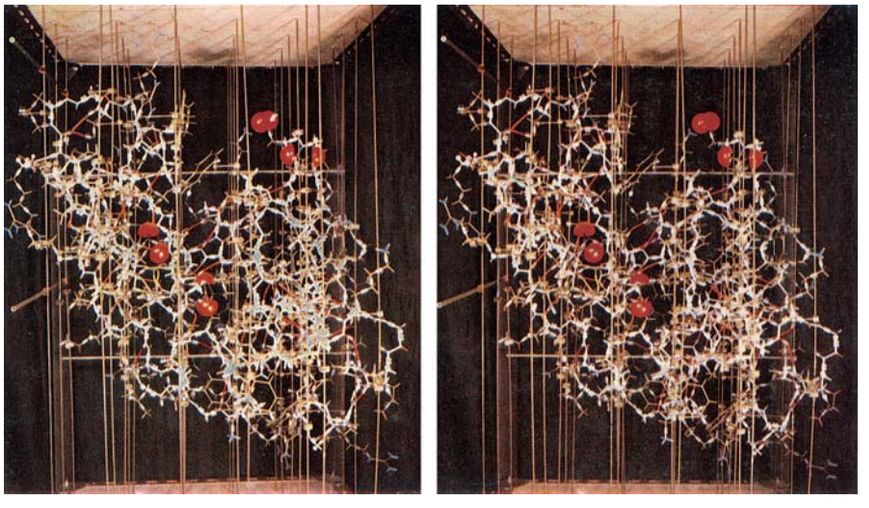


Fig. 26.1.3.12. Stereo-photographs of a model of the lysozyme molecule to a scale of 2 cm to 1 Å. The main polypeptide chain is painted white, and nitrogen, oxygen and sulfur atoms are indicated by blue, red and green sleeving, respectively. Some hydrogen bonds are shown by red connections. Oxygen atoms of the acid side chains near the cleft, Glu 35, Asp 52, Asp 101 and Asp103, are shown by red hemispheres (Blake *et al.*, 1967).

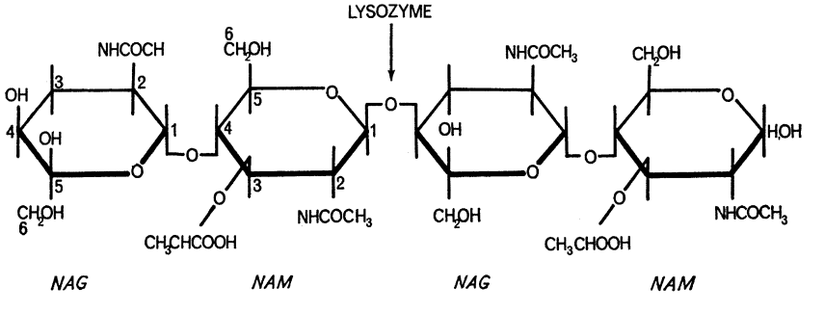


Fig. 26.1.4.1. The cell-wall tetrasaccharide with the $\beta(1\rightarrow4)$ glycosidic bond that is hydrolysed by lysozyme indicated (Blake *et al.*, 1967).

26.1.4. Structural studies on the biological function of lysozyme

26.1.4.1. *Lysozyme substrates*

By the summer of 1964, the results for the structure determination of lysozyme were sufficiently promising for us to consider diffraction studies on the biological function of lysozyme. At about the same time, the Royal Society invited Max Perutz to organise a Discussion Meeting on lysozyme at The Royal Institution in early 1966, to which the leading workers worldwide would be invited. This provided an additional spur to our efforts.

Simultaneously with his discovery of lysozyme in 1922, Fleming had found a gram-positive species of bacteria, *Micrococcus lysodeikticus*, that was particularly susceptible to lysis by lysozyme (Fleming, 1922). During the 1940s and 1950s, further work by a number of authors had shown that lysozyme exerted its biological effects through hydrolysis of the bacterial cell wall. These studies led to a definitive description of the polysaccharide component of the bacterial cell wall, as shown in Fig. 26.1.4.1.

The structure is composed of alternating sugars, linked by $\beta(1\rightarrow4)$ glycosidic links, of *N*-acetylglucosamine (GlcNAc, abbreviated by us at the time as NAG) and *N*-acetylmuramic acid (MurNAc, previously abbreviated as NAM). The 3-hydroxyls of the MurNAc residues are attached to short peptides, and the peptides themselves are cross-linked to provide an extensive and rigid two-dimensional proteoglycan network. Lysozyme cleaves the $\beta(1\rightarrow4)$ glycosidic bonds between MurNAc and GlcNAc residues, thus leading to the dissolution of the bacterial coat and lysis of the bacterium.

Kinetic studies on the activity of lysozyme were hampered by the lack of a suitable small-molecular-weight substrate. The turbidometric assay in use at the time, and still in use today, followed the change in optical density of a suspension of *Micrococcus lysodeikticus* cells as the cells were lysed by lysozyme. The assay could work reliably but it was sensitive to physical parameters such as ionic strength, the method by which the cells were suspended and product inhibition. In 1962, Wenzel *et al.*, in an effort to obtain a small-molecular-weight substrate, had reported that lysozyme promoted the cleavage of the trimer of GlcNAc, tri-*N*-acetylchitotriose, releasing dimer and monomer sugars, and that the monomer, GlcNAc, was an inhibitor of lysozyme (Wenzel *et al.*, 1962). Compounds such as glucose or cellobiose that lacked the *N*-acetyl group did not inhibit.

John Rupley extended this work. Rupley, a chemist from the University of

Arizona, Tucson, played a crucial role in developing the mechanisms for lysozyme catalysis. In results that were summarized in 1964 and 1967 (Rupley, 1964, 1967) and made available to workers at the Royal Institution in 1965, he showed that the rate of hydrolysis of GlcNAc homopolymers increased by 30 000 as the chain length increased from trimer to hexamer, and that there was no further increase in rate for substrates of greater length than the hexamer. Furthermore, the cleavage patterns for the smaller polymers were complex, but the optimal substrate, the hexamer, was cleaved to a tetrasaccharide and a disaccharide.

Thus by the time we began structural studies, the nature of the substrate and the specificity of lysozyme were established. GlcNAc was the only compound that was available commercially. This seemed a good starting point. It was anticipated that as a competitive inhibitor it would bind to and allow the identification of the catalytic site.

26.1.4.2. *The crystal structure of GlcNAc*

During the first two years of her graduate work (1962–1964), LNJ determined the crystal structure of GlcNAc. β-D-*N*-Acetylglucosamine was crystallized from a methanol–water mixture, and diffraction data were recorded on film with a modified Weissenberg camera. Intensities were estimated visually from comparison with a scale of fixed-time exposures of the attenuated main beam. The structure was solved manually using a sharpened Patterson function and application of the minimum function of Buerger (1959). The structure revealed a standard glucopyranose ring in the chair conformation with the plane of the *N*-acetyl group normal to the ring (Johnson & Phillips, 1964; Johnson, 1966). The structure of glucosamine hydrochloride had been solved by Cox & Jeffrey in 1939 (Cox & Jeffrey, 1939), a remarkable early achievement.

By 1963, about ten glucopyranose structures were available, including that of cellobiose (Jacobson *et al.*, 1961). An analysis by Ramachandran *et al.* (1963) showed that the glucopyranose ring is remarkably uniform in its conformation and may be regarded as a rigid structure.

The final difference-Fourier synthesis of β-GlcNAc revealed an additional peak adjacent to the C1 carbon atom in the position of a hydroxyl group in the α configuration. Peak heights suggested that there could be a mixture in the crystal of approximately 80% β-*N*-acetylglucosamine and 20% α-*N*-acetylglucosamine. Refinement indicated that a mixture of the two anomers could be accommodated in the crystal lattice.

Tests on the optical rotation of the crystalline sample after dissolution, compared with the starting material, also added support to the notion that the crystal contained a mixture of α and β anomers, probably as a result of mutarotation during crystallization. Although a later and more precise structure determination of GlcNAc indicated a lower proportion of the α anomer (Mo & Jensen, 1975), mixtures of α and β sugars in crystals have been observed for other compounds (Jeffrey, 1990). The consideration of the α and β anomers of GlcNAc meant that we were keenly aware of the importance of configuration at the C1 atom. This turned out to be essential when we were interpreting the results with lysozyme.

26.1.4.3. *Low-resolution binding studies of lysozyme with GlcNAc and other sugars*

In the first binding studies of lysozyme with GlcNAc, crystals were soaked overnight in solutions of 0.15 *M* GlcNAc in the standard crystallization medium, and a 15° precession photograph was recorded by LNJ. The changes in intensities of reflections compared to a native lysozyme photograph were extremely small, much smaller than those observed with the heavy-atom derivatives, but were sufficiently promising to encourage us to collect three-dimensional data to 6 Å resolution. Data were collected on the linear diffractometer using the three counters in the flat-cone setting over a period of about 20 h, as described for the heavy-atom derivatives. The data were processed using the programs of ACTN and VRS on the Elliott 803B computer. The 6 Å difference-Fourier map, using the phases from the improved set of heavy-atom derivatives, was obtained at the end of October 1964. It showed a single rather elongated peak which, when superimposed onto the 6 Å model, was located in the cleft in the enzyme surface between the two domains (Johnson & Phillips, 1965).

The power of the difference-Fourier technique in protein crystallography was immediately demonstrated by this first 6 Å electron-density difference map for the lysozyme–inhibitor complex, as had also been demonstrated earlier in the work (Stryer *et al.*, 1964) on the binding of azide to sperm-whale myoglobin. Once a protein structure had been solved, it was apparent that ligand-binding sites could be established with ease. Following the GlcNAc result, 6 Å binding studies were repeated with a number of other compounds (Blake *et al.*, 1967). Kinetic studies using the turbidometric assay were carried out with each of the compounds in order to establish the mode of inhibition of lysozyme activity. We were fortunate in being able to use the skills of JWHO, a carbohydrate chemist who had been at the Royal Institution for many years. JWHO had synthesized 6-iodo-6-deoxy-*N*-acetyl β-methylglucosaminide, a compound which was found to inhibit more powerfully than GlcNAc itself. The low-resolution binding study showed a stronger and more compact peak at the catalytic site than that observed with GlcNAc, but it was not possible to resolve the iodine and hence identify the six positions of the sugar.

By January 1965, we had been given a sample of the disaccharide di-*N*-acetylchitobiose, $(GlcNAc)_2$, sent by John Rupley. Efficiency of data collection and map production had increased. Data collection was started on 19 January and the map was obtained by 28 January. Other results followed with *N*-acetylmuramic acid (a gift from R. W. Jeanloz) and the disaccharide *N*-acetylglucosamine β-(1,4)-*N*-acetylmuramic acid (GlcNAc-MurNAc – a gift from N. Sharon) (Fig. 26.1.4.2).

As part of these studies, penicillin V and *p*-iodophenoxymethyl penicillin potassium salt were also investigated. They were observed to inhibit lysozyme. Crystallographic studies showed that the penicillins did indeed bind to lysozyme in the catalytic cleft, but at a site remote from the GlcNAc binding site (Johnson, 1967). As established shortly after this result was obtained in 1965, penicillin exerts its potent antibiotic activity by inhibition of the enzymes responsible for the biosynthesis of the peptide cross-linking component of the bacterial cell wall (Tipper & Strominger, 1965; Wise & Park, 1965). An original suggestion that penicillin might resemble MurNAc turned out to be incorrect (Collins & Richmond, 1962). The interactions of penicillin with lysozyme are probably fortuitous, but were not fully investigated.

By the end of May 1965, we were ready to move on to 2 Å data collection, a formidable task that required fourteen crystals and more than two weeks continuous data collection on the multiple-counter linear diffractometer. Data for the lysozyme–GlcNAc complex were completed first, and the map was available around October 1965. The electron density was puzzling, and an interpretation was not possible until the high-resolution results with the trisaccharide were available.

26.1.4.4. *Binding studies of lysozyme with tri-N-acetylchitotriose, $(GlcNAc)_3$, at 2 Å resolution*

John Rupley visited the Royal Institution in July 1965. He brought with him a sample of tri-*N*-acetylchitotriose (Fig. 26.1.4.2), previously abbreviated as tri-NAG and now as $(GlcNAc)_3$. The lysozyme–$(GlcNAc)_3$ complex turned out to be the crucial structure for understanding activity. When lysozyme crystals were soaked in

0.05 *M* $(GlcNAc)_3$ solution, they turned opaque and became difficult to mount for X-ray studies. The problem was overcome by soaking the crystals already mounted in a capillary tube. By 17 July 1965, a 6 Å map of the lysozyme–$(GlcNAc)_3$ complex was obtained and showed a peak occupying the whole top part of the cleft on the enzyme surface.

Rupley had also succeeded in co-crystallization of lysozyme with $(GlcNAc)_3$. Data collected from these crystals gave an identical difference-Fourier synthesis to that obtained with crystals soaked in $(GlcNAc)_3$. Data from another co-crystallized crystal, which were collected ten days later, also gave an identical result, indicating that there had been no hydrolysis of the trisaccharide in the crystal during this time. The way was open for high-resolution data collection with $(GlcNAc)_3$. Rupley set up co-crystallizations of lysozyme with $(GlcNAc)_3$ (with concentrations 1:1.1 molar ratio under standard conditions) at the Royal Institution during the hot August of 1965. The crystals grew well and provided us with a plentiful supply of crystals for the 2 Å data collection, which was carried out by LNJ following the same procedures as for the native crystals described above.

The 2 Å difference-electron-density map arrived on Thursday 6 January 1966, but there was a mistake. After correction and the laborious task of contouring by hand and transferring the contours to transparent sheets had been completed, serious model building by DCP, LNJ, ACTN and other members of the team began on 19 January 1966.

The interpretation of the difference map for $(GlcNAc)_3$ was straightforward. Three sugars could readily be identified in the density. The first occupied a site at the centre of the cleft at a position similar to that occupied by GlcNAc by itself and labelled site C. The acetamido group was visible and fitted neatly into a pocket where there were complementary hydrogen bonds between the NH and CO of the acetamido group and the main-chain carbonyl oxygen of residue 107 and the amido group of residue 59. Lysozyme has three tryptophan residues at the catalytic site. One of these, Trp108, was at the bottom of the acetamido pocket and made van der Waals contacts to the methyl group of the acetamido. The precise and extensive contacts to the acetamido group explained immediately the specificity of lysozyme for *N*-acetyl sugars. The free reducing group of the sugar in site C pointed down (towards lower *z*). The second sugar could be fitted to the density above the first (in the direction of increasing *z*), linked to the O4 of the sugar in site C. It was clear that a second tryptophan, Trp62, stacked against the glucopyranose ring for this sugar in site B, and that there was a shift of this tryptophan towards the sugar. This and other conformational changes could explain the tendency of the lysozyme crystals to become disordered when soaked in $(GlcNAc)_3$ solutions.

In cellobiose, whose crystal structure had been determined in 1961 by Lipscomb and colleagues (Jacobson *et al.*, 1961), the conformation about the $\beta(1\rightarrow4)$ glycosidic link rotates the second sugar about 180° with respect to the first, and there is an intramolecular hydrogen bond between the O3 hydroxyl of one sugar and the O5 of the adjacent sugar. This relative orientation of the two adjacent sugars was found to fit the density for sugars C and B and also to agree with models proposed by Carlstrom (1962) for chitin and the analysis of Ramachandran *et al.* (1963). The lobes representing the acetamido and C6 alcohol groups for site B provided further confirmation for this orientation. The third sugar in site A appeared less well located, but nevertheless could be placed in density and was observed to make satisfactory contacts with the protein.

The interpretation of the difference-Fourier map was made by one worker without reference to the protein structure, while a second worker fitted the model into the protein structure. It was most exciting and satisfying to observe the contacts when the skeletal models of the sugars were fitted into the skeletal model of the protein. Shifts in atomic coordinates of selected protein groups were estimated from the gradient of electron density in a difference map divided by the curvature of the electron density at that point, as described by Lipson & Cochran (1968). Using this formula, the shift in Trp62 was calculated to be 0.75 Å, a value that turned out to be accurate when the lysozyme–$(GlcNAc)_3$ complex was refined by least-squares methods many years later (Cheetham *et al.*, 1992). It was also noted that there were two acidic residues on either side of the catalytic cleft, Glu35 and Asp52, which were some distance from the $(GlcNAc)_3$ position. There was a further acidic residue, Asp101, near the top of the cleft.

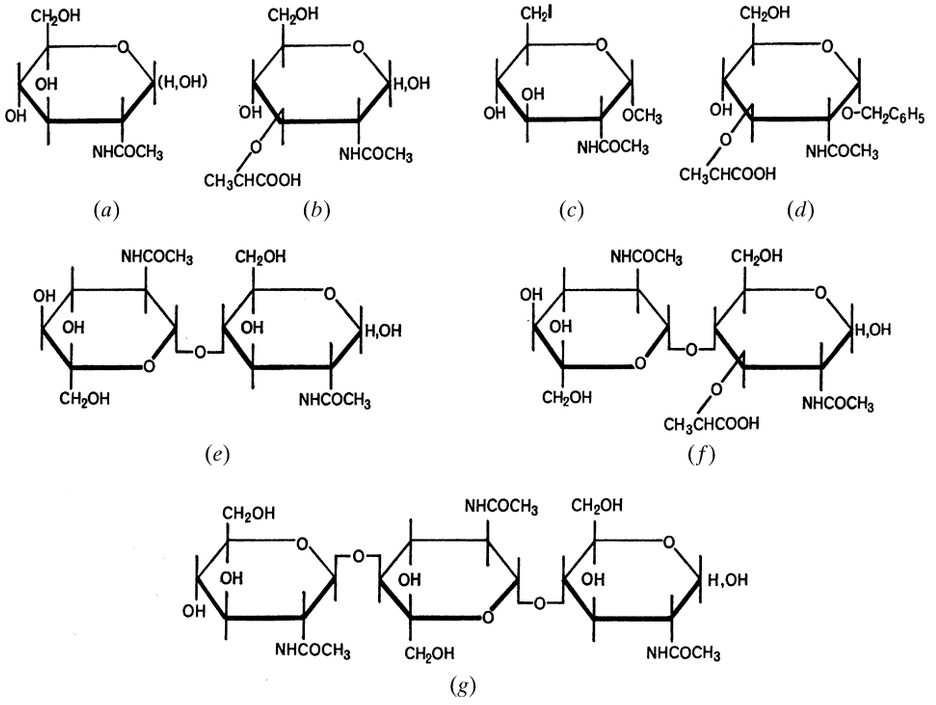
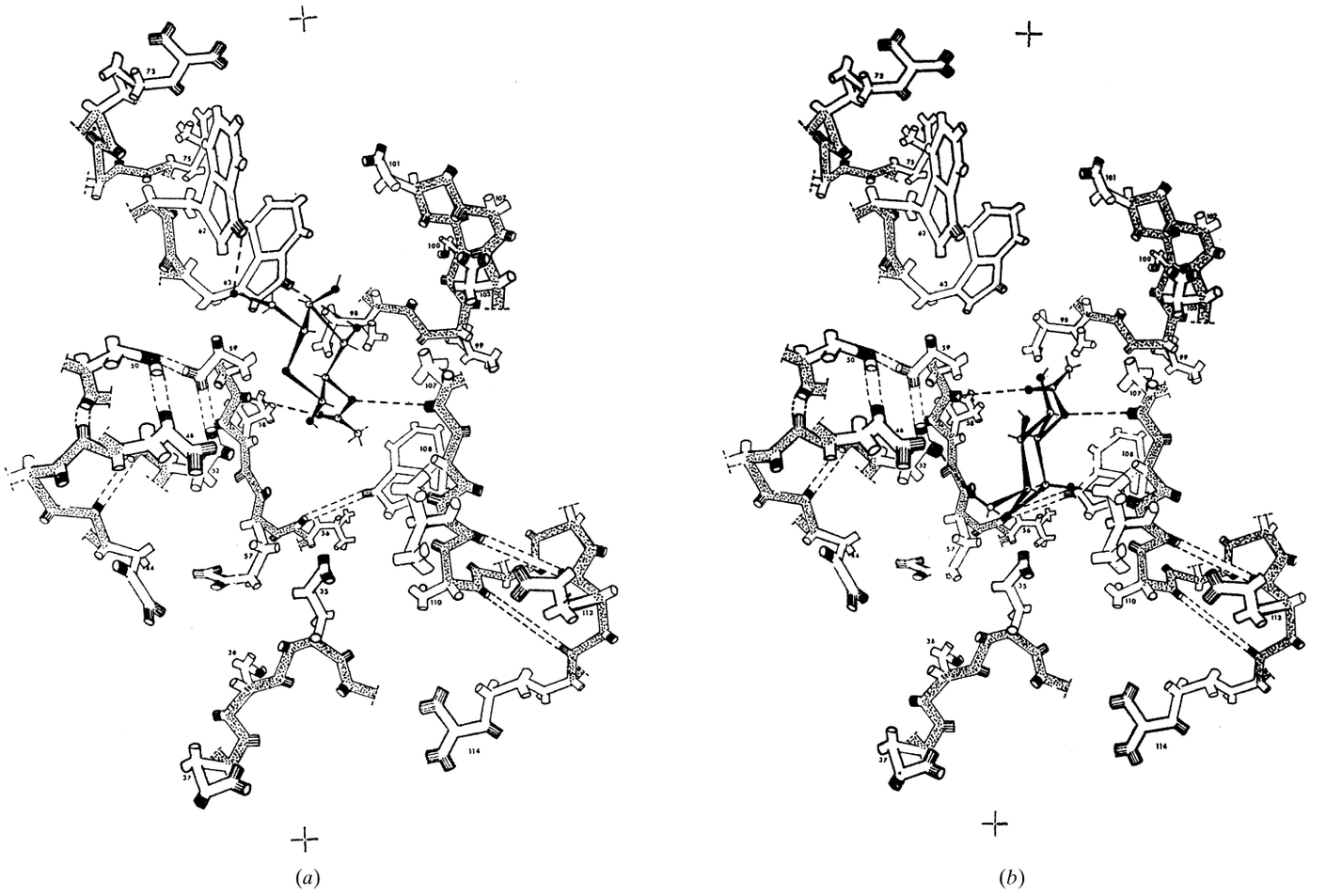


Fig. 26.1.4.2. Inhibitor molecules of lysozyme (Blake *et al.*, 1967). (*a*) *N*-acetylglucosamine; (*b*) *N*-acetylmuramic acid; (*c*) 6-iodo-α-methyl-*N*-acetylglucosaminide; (*d*) α-benzyl-*N*-acetylmuramic acid; (*e*) di-*N*-acetylchitobiose; (*f*) *N*-acetylglucosaminyl-*N*-acetylmuramic acid; (*g*) tri-*N*-acetylchitotriose.

The structure of the $(GlcNAc)_3$–lysozyme complex allowed an interpretation of the GlcNAc result. It was apparent that GlcNAc bound to site C in one or other of two distinct but closely related ways, depending upon whether it is in the α or β enantiomeric form (Fig. 26.1.4.3).

The GlcNAc molecule had evidently undergone mutarotation in solution to produce an equilibrium mixture of α and β forms, both of which bound to lysozyme, although of course the specificity of the enzyme for longer oligosaccharides is for β-linked sugars. Both binding modes exploited the specificity of the *N*-acetyl group and its interactions with the enzyme.

One mode was characteristic of β-GlcNAc, as observed for the terminal



(*a*) (*b*)

Fig. 26.1.4.3. Contemporary drawings of the binding to lysozyme of: (*a*) β-*N*-acetylglucosamine and (*b*) α-*N*-acetylglucosamine (Blake *et al.*, 1967).

sugar of $(GlcNAc)_3$. The other mode was characteristic of α-GlcNAc and involved a rotation about the hydrogen bonds of the acetamido group to the protein. This finding could explain the result for 6-iodo-6-deoxy-*N*-acetyl α-methylglucosaminide, which could not fit into the α-GlcNAc site because of the additional methyl group but could only be accommodated in the 'β-site'. The dual mode of binding for the terminal residue in the α or β configuration could also explain the bifurcated peak observed for the disaccharides GlcNAc-MurNAc and $(GlcNAc)_2$. When the terminal sugar is in the α configuration, the second sugar is placed out of the main run of the cleft, while in the β configuration the second residue is as in site B of the trisaccharide complex.

26.1.4.5. *Proposals for the catalytic mechanism of lysozyme*

A Royal Society Discussion Meeting had been arranged for 3 February 1966 under the organization of Max Perutz. CAV, from University College, London, who had worked on mechanisms for the non-enzymic hydrolysis of glycosides in solution, had been invited to contribute to this meeting. In mid-January (the exact date is not recorded) he visited the Royal Institution to discuss the possible reaction mechanism of lysozyme in the light of the crystallographic evidence. At that stage, the crystallography had shown, from low-resolution studies, the inhibitor binding sites within a surface cleft lined with several acidic residues that included Glu35 and Asp52. CAV's work had shown that the hydrolysis of methyl-α-D-glucopyranoside proceeded *via* a ring-closed carbonium-ion intermediate (*i.e.* a carbocation) and that the rate-determining step was the heterolysis of the C1 carbon–oxygen bond (reviewed in Vernon, 1967). Rupley's studies with ^{18}O water had shown that hydrolysis of oligosaccharide substrates by lysozyme also proceeded through cleavage of the C1—O bond (Rupley, 1967). Whereas in free solution the carbocation intermediate is presumed to be stabilized by interaction with solvent, in the catalytic site of lysozyme it was suggested that the carbonium-ion intermediate might be stabilized by the nearby ionized carboxylate of one of the acidic residues in the catalytic site.

The interpretations of the $(GlcNAc)_3$ and the GlcNAc complexes were finished by Monday 31 January 1966. The date is significant. The Royal Society Discussion Meeting had been arranged for the following Thursday. The results with the non-hydrolysable complex with $(GlcNAc)_3$ had identified the catalytic site and had provided immediate explanations for the specificity requirement for the enzyme. However, information on how a true substrate might bind was missing. This problem was solved in one day. DCP, noting that Rupley's work had shown the optimal substrate to be a hexasaccharide, constructed a hexasaccharide substrate in sites A–F, using the position of the experimentally determined trisaccharide in sites A–C and model building those sugars in sites D–F. Noting also the specificity of lysozyme for bacterial-cell-wall substrates, where the polysaccharide is composed of alternating $\beta(1\rightarrow4)$ linked GlcNAc and MurNAc residues, and the bond cleaved is that between MurNAc and GlcNAc residues, but not that between GlcNAc and MurNAc residues, he also examined sites likely to be specific for MurNAc. It was apparent that, for steric reasons, sites A, C and E could not accommodate MurNAc residues, but that sites B, D and F could do so. Thus the site of cleavage must be between sites D and E (or sites A and B, but

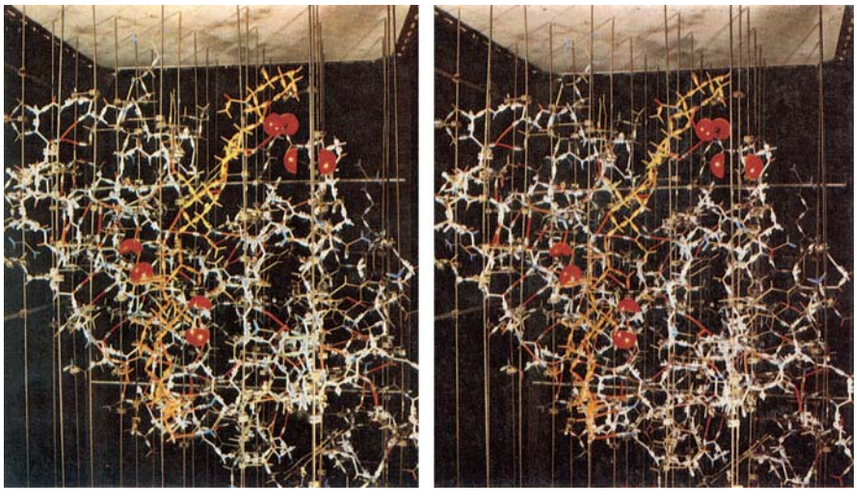


Fig. 26.1.4.4. Stereo-photographs of a model of the lysozyme molecule showing how a hexasaccharide substrate may bind to the enzyme. The yellow trisaccharide model shown at the top of the cleft is in the position occupied by tri-*N*-acetylchitotriose in the crystals of its complex with lysozyme. The orange trisaccharide in the lower part of the cleft has been added by model building alone. The fourth sugar residue from the top, the uppermost orange one, is distorted from the chair conformation (Blake *et al.*, 1967). The glycosidic linkage that is broken is the one between the fourth and fifth sugar residues from the top. It lies between the side chains of Glu35 (to the right) and Asp52 (to the left), the oxygen atoms of which are marked by red hemispheres. Asp101 and Asp103, near the top of the cleft, also have their oxygen atoms marked.

this could be discarded because the trisaccharide complex was stable).

Close to the bond between sites D and E were two acid residues (Fig. 26.1.4.4). As noted previously by members of the team, one of these, Glu35, was in a non-polar environment and shielded by Trp108, Val109 and Ala110. In this environment, the carboxylic acid was likely to have a raised p*K* and to be protonated at pH 5, the optimal pH for activity against homopolymers of GlcNAc. The other acid group, Asp52, was also buried, but in an environment that was largely polar and at the centre of a hydrogen-bonding network. Hence it might be predicted that its p*K* was likely to be similar to that of a carboxyl group in an aqueous environment, and the group would therefore be mostly ionized at pH 5. There was one other important factor. The sugar in site D could not be accommodated in the usual chair conformation of a glucopyranose ring. In order to avoid overcrowding between the C6O6 atoms of the alcohol group and the protein atoms at site D, it was necessary to distort the sugar ring from the conventional chair conformation to a sofa conformation. This relieved the overcrowding by bringing the C5—C6 bond axial and retained the links from C4—O4 and C1—O4′ equatorial, so that the adjacent sites of C and E were not perturbed by the distortion of the sugar in site D (Fig. 26.1.4.4). CAV visited the Royal Institution on a second occasion between the completion of the model and the Royal Society meeting, probably on 1 February. When shown the hexasaccharide substrate complex with two acid residues on either side of the susceptible bond and the distortion of the sugar in site D, CAV explained that this was exactly what he would expect from his own work for a carbonium-ion mechanism with transition-state distortion of the substrate.

The mechanism was presented for the first time at the Royal Society meeting on 3 February 1966. It was noted that the nearest oxygen of the protonated Glu35 was about 3 Å from the glycosidic oxygen between residues D and E (the O1 atom of residue D and the equivalent O4 atom of residue E). On the other side of the cleft, the nearest oxygen of the ionized Asp52 was about 3 Å from the C1 atom of residue D and about the same distance from the O5 of that residue. The distances were, of course, subject to some error from experimental model building. It was suggested (Blake *et al.*, 1967; Phillips, 1967) that:

(1) Asp52 carries a negative charge that promotes the formation of a carbonium ion at C1 of residue D and stabilizes it when formed;

(2) distortion of residue D from the chair conformation into the sofa conformation would contribute to stabilization of a carbonium ion at C1 by favouring a conformation in which the charge at C1 could be shared with the ring oxygen atom (Lemieux & Huber, 1955), and hence contribute to the consequent weakening of the C1—O1 bond; and

(3) Glu35 could act as a proton donor, facilitating the formation of a hydroxyl group with the bridge oxygen atom (O1), and release of residues E and F.

Fig. 26.1.4.5 shows a sketch of the proposed lysozyme–hexasaccharide substrate complex prepared by Irving Geis for a *Scientific American* article published later that year (Phillips, 1966).

As Perutz commented in his closing remarks at the Royal Society meeting: 'For the first time we have been able to interpret the catalytic activity of an enzyme in stereochemical terms' (Perutz, 1967). The scheme for catalysis has been subjected to numerous experimental tests and has been investigated further in crystallographic experiments. The mechanism provided a satisfactory explanation for the body of evidence already in existence at the time of the proposals and has been substantiated by the results of new experiments designed to test it (see, for example, Imoto *et al.*, 1972; Ford *et al.*, 1974; Strynadka & James, 1991; Hadfield *et al.*, 1994; Jolles, 1996).

Acknowledgements

This work would not have been possible without the continuous support and encouragement of Sir Lawrence Bragg and the Medical Research Council, UK, for which we were and are most grateful. We are also grateful to our colleagues Robert Canfield, Pierre Jolles, Gordon Lowe, John A. Rupley, Nathan Sharon and others who shared their results on the catalytic mechanism with us before publication. The successful outcome also depended greatly upon the skilled work of the craftsmen in the Royal Institution's mechanical and electronic workshops, Messrs T. H. Faulkner, S. B. Morris, J. E. T. Thirkell and A. R. Knott. Finally, we acknowledge gratefully the unstinting contributions made by our team of research assistants, Mrs W. J. Browne, Mrs A. Hartley, Mrs K. Sarma, Miss D. Glass, Mrs R. Arthanari, Mrs S. J. Cole, Mrs J. A. Conisbee and Miss M. Hibbs. Clearly, thirty years ago protein-structure analysis was still very labour-intensive, despite the advances in computing and diffractometry described above. This was largely because we were still designing and building our own apparatus and drawing maps and building models by hand. This situation has been transformed chiefly by the availability of more powerful computers and X-ray detectors, by the developments of computer graphics, and by the exploitation of synchrotron radiation. The review has used the original figures prepared during the 1960s with a few exceptions. We acknowledge with special thanks the skills of Stephen Lee in scanning and reproducing these figures.

references