in the destination of its endings in the neuropil. I_2 inhibits fast flexor muscle, so it is possible that the bilateral distribution helps to synchronize muscular contraction in the abdomen. They found that I_2 has a characteristic branching pattern, repeated in each individual. As they point out, their technique determines the areas of neuropil which are innervated by particular categories of neurone.

Remler, Selverstein and Kennedy have used the same technique to locate the cell bodies of the lateral giant fibres of the crayfish (*Science*, **162**, 281; 1968). The giant fibres are known to consist of an interneurone in each segment, connected together by unpolarized electrical septa at each end. Each interneurone also sends endings to the neuropil of its own ganglion, makes electrical synapse with a motor giant neurone and forms a commissural synapse with its contralateral partner. Micro-electrode recordings, however, from cell somata in the abdominal ganglia have not detected any physiologically identifiable interneurones. On injection of Procion-Yellow from the rostral end of a giant fibre in one segment the dye diffuses into a soma on the contralateral side of the segment. The cell body stained in each case lies caudally on the ventral surface of the abdominal ganglion, near the exit of the second nerve root. When recordings are made simultaneously from the soma and the contralateral giant fibre small electrotonically decaying somatic potentials can be observed. These are always in a fixed time relation to the axonal spike and will follow repetitive axonal stimulation up to 100 Hz, although spikes recorded from the motor neurone somata will only follow to 10 or 15 Hz. There seems to be no doubt that the somata of the interneurones making up lateral giant fibres have been found and that they are capable neither of spike propagation nor of initiating spikes in their axons. This is supported by the thinness of the neurite linking the soma to the giant fibre and by the failure of depolarization by as much as 80 mV to cause spikes to appear in the axon.

MOLECULAR BIOLOGY

Lysozyme Interactions

from our Molecular Biology Correspondent

If it is generally true that X-ray determinations of protein structures have failed to reveal in unequivocal terms how the molecule works, the results on lysozyme certainly come nearest to fulfilling this objective. The work of Phillips and his group on lysozyme-inhibitor complexes has led to some convincing inferences about the mechanism of action. These have in turn provoked many investigations of enzyme-inhibitor and substrate interactions in solution, partly with the aim of encompassing a wider variety of substrates, many not amenable to crystallographic study, and partly as a proving ground for new physical methods.

Some of the most promising results have come from proton magnetic resonance studies. The latest of these, reported by Dahlquist and Raftery (*Biochemistry*, **7**, 3269; 1968), reveal more closely the interaction between lysozyme and the monosaccharide inhibitor, N-acetylglucosamine (NAG). This molecule exists in two anomeric forms, α and β, and both are found to bind to the active site. On binding, some protons in the inhibitor (acetamido methyl protons) undergo a chemical shift, which can be used to follow the binding process. The magnitude of this shift is different for the α and β forms, and consequently, in the presence of the enzyme, the contributions of the two can be resolved. Measurements of chemical shifts and line-widths as a function of concentrations provide enough data in principle to determine association constants, and also the line-widths for each complex in the pure state. The latter differ by a factor of about two between bound α and β-NAG. It follows that their environments on the active site are not equivalent. There is also a two-fold difference in the binding constants, and it is suggested that the corresponding small increment in binding energy arises from an interaction of the sugar hydroxyl, which is the site of the anomeric difference between the two forms. This is a safe inference, for Phillips and his co-workers showed in 1967 that the sugar ring orientations of the two anomers on the enzyme were indeed different. Dahlquist and Raftery suggest that the difference in shift of the two forms involves the anisotropy of the active site tryptophan-108.

In a second paper (*ibid.*, 3277) the same authors describe the *p*H-dependence of the association of lysozyme with β-methyl-NAG. The binding constant is diminished within a *p*H range which implicates an ionizing group of pK_{app} of 6·1. This is thought to be a carboxyl group, for titration data indicate the existence of one such, with anomalously high pK, and other nuclear magnetic resonance evidence excludes the solitary histidine residue from any contact with the inhibitor. The group in question could be asp-52 or glu-35, and the latter is a better candidate because of its non-polar surroundings. The chemical shifts of the acetamido methyl protons of the inhibitor undergo changes centred on about *p*H 4·7 and *p*H 7, the second only being accompanied by a change in binding constant. The first shift is therefore apparently a purely vicinal effect, and is tentatively attributed to the ionization of asp-103. Now a trimeric inhibitor is known from X-ray work to interact with a further site, involving asp-101, and, from the *p*H profile of the interaction, this can be assigned a pK_{app} of 4·2. Thus a considerable proportion of the important ionizing groups may already be allocated individual pK values.

The concept of subsites which interact with various groups of substrates has been developed by a group of workers at the Weizmann Institute, and a current conference report (Pollock *et al.*, *Israel J. Chem.*, **6**, 112P; 1968) gives new data on the interaction of several saccharides with lysozyme, derived from fluorescence spectra of the protein. When three sites, *A*, *B* and *C*, are occupied, the introduction of a substrate group, to interact with a further site, *D*, leads to considerable fluorescent quenching. It is surmised that a local conformational distortion alters the relation between the important try-108 residue and glu-35, which, as the closest charged group, is its probable quencher. Addition of two further saccharide units, binding at subsites *E* and *F*, partly reverses this effect, and the position of glu-35 is perhaps again disturbed so as to assist protonation of the glycosidic oxygen between residues at *D* and *E*, or introduce strain energy at this position. A second report (*ibid.*, 120P) gives the free energy contributions for the binding of successive sugar residues. A destabilization of the residue at *D* is again indicated; this suggests that the degree of strain at this point is instrumental in determining the rate of fission of different saccharides by the enzyme.