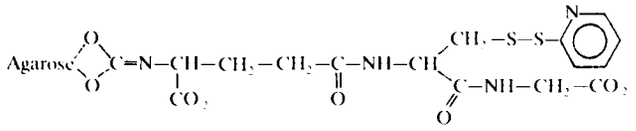
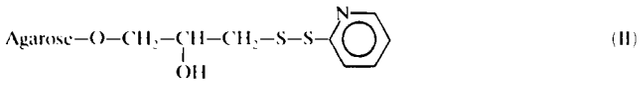
Our model of chymopapain M reveals differences from papain in the S_2-subsite (see [18, 19] for schematic diagrams), where P68 and W69 in papain are respectively E68 and S69 in chymopapain M. These differences may affect substrate binding. It is interesting also that Y61, Y67, E62 and H81, suggested as possible candidates for modulators of papain reactivity and activity [18], are conserved in chymopapain M, and pH dependence studies on this enzyme will be of value in the study of these possibilities. The differences in the structures of the S_1 subsites of papain and chymopapain M discussed in [8] could account for the observed specificity of the latter enzyme for a glycine residue at P_1 and for our findings that chymopapain M can be separated from the other chymopapains by using the agarose–glutathione-2-pyridyl disulphide gel (I). Like all other cysteine proteinases evaluated thus far, chymopapains A and B react with gel (I) and may be isolated by covalent chromatography [20]. Chymopapain M uniquely fails to react and is contained in the unbound fraction. We are investigating (*a*) the possibility of bonding chymopapain M to less sterically demanding gels such as gel (II) and (*b*) the nature of binding site-catalytic site signalling mechanisms in this enzyme by using substrate-derived 2-pyridyl disulphides as reactivity probes, as described for papain in [21].



(I)



(II)

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A model for the solution conformation of rat IgE

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Through their Fab and Fc portions antibodies recognize and interact with both antigen (via Fab) and host effector systems (via Fc), leading to the clearance of invading material. Crystallographic studies on antibodies have used hingeless mutants, isolated Fab and Fc fragments, as well as Fab/antigen complexes, but no high-resolution structure is available for intact immunologically active antibodies. Attempting to assess the average solution conformation of intact antibodies, we have collected solution data on antibodies (sedimentation coefficients and radii of gyration) and then tried to reproduce the experimental parameters from models which incorporate as many of the known structural and immunological properties of antibodies as possible.

Immunoglobulin E (IgE) is found in monomeric form in serum and possesses five immunoglobulin domains in its heavy chain as well as being glycosylated at various sites. Two different Fc receptors have been identified, a low-affinity receptor found on monocytes, macrophages, lymphocytes, eosinophils and platelets, and a high-affinity receptor found on mast cells and basophils. While the physiological role of the low-affinity receptor is not clear, the allergic response is triggered when IgE bound to the high-affinity receptor is cross-linked by antigen [1].

Rat IgE was used in sedimentation velocity experiments on an MSE Centriscan analytical ultracentrifuge, and radii of gyration were obtained from small-angle X-ray scattering experiments undertaken at the Synchrotron Radiation Source, Daresbury. From the concentration dependence the following values were obtained: $s^0_{20,w} = 7.92\ (+0.10)$ S, and radius of gyration, $R(g) = 5.07\ (\pm 0.16)$ nm. In using interactive sphere theories to produce low-resolution bead

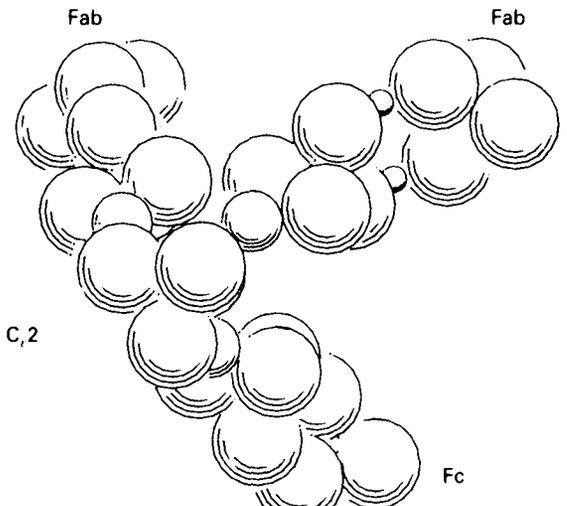

Fig. 1. *A view of a model of Rat IgE that fits the experimental data*

The Fab arms in the upper part of the illustration point away from the observer, the Fc portion in the lower part also points away from the observer. This projection has the $c_\epsilon 2$ region nearest the observer indicating how 'accessible' this region is.

models of biopolymers, two main problems become apparent — uniqueness and hydration effects. Uniqueness arises when an experimental parameter is reproduced by more than one arrangement of a given set of beads. Hydration effects describe both the 'chemical' water/biopolymer interactions and the influence that non-chemically bound water can have on biopolymer hydrodynamics. These difficulties have been overcome in this case by representing each immunoglobulin domain as two beads and then arranging the beads to reproduce the domain arrangement indicated in the low-resolution (6.5 Å) structure of the hingeless mutant antibody protein Mcg [2]. Then using M_r and partial specific volume values derived from a consensus primary sequence, calculations to reproduce the measured sedimentation coefficient of Mcg were repeatedly performed with different sized beads until the measured value was obtained. Beads of 1.1 nm radius were found to be appropriate. Therefore, implicit in the modelling is the assumption that hydration effects in all molecules made up of immunoglobulin domains are similar to those of Mcg. Beads of radius 0.32 nm were included in the Fab fragment as the elbow region, also included were three beads of 0.74 nm radius to allow for lengths of peptide and the polysaccharide moieties arranged around the $c_\epsilon 2$ region linking it to the Fc and Fab fragments. In each of the models analysed, an elbow angle of 160 degrees was included, this emerged as an average value from the reported Fab structures examined. Arranging the beads into the conventional T shape used frequently to describe antibodies, the calculated values were $s^0_{20,w} = 7.26$ S and $R(g) = 6.77$ nm, which do not reproduce the experimental data. To reproduce the experimental values the whole molecule assumes a 'cusp-like' shape, as the Fc and Fab arms move towards each other. The model shown in Fig. 1 produces values of the measured parameters of $s^0_{20,w} = 7.93$ S and $R(g) = 5.08$ nm which are within experimental uncertainty. As a 'bent' model is required to reproduce the experimental data, this investigation indicates that IgE is not a planar molecule. This model which effectively 'exposes' the $c_\epsilon 2$ region and the $c_\epsilon 2/c_\epsilon 3$ interface area is consistent with the proposal that this area contains the site recognized by the high-affinity Fc_ϵ receptor [3].

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Structure of rodent urinary proteins

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There is great interest in the prediction of protein tertiary structure. Over the last decade the number of protein sequences derived from DNA/mRNA sequencing has increased dramatically. In addition, the number of protein structures determined by X-ray crystallography has also increased, but at a slower rate. These advances, coupled to a rapid increase in computational power, have made the modelling of sequences homologous to known structures a possibility.

Our work has concentrated on a superfamily of proteins, the lipocalycins (α_2-urinary globulin family). We are interested in their role as ligand-binding and transport molecules. The structures of retinol-binding protein (RBP), β-lactoglobulin (BLG), and insecticyanin, have already been determined by X-ray crystallography [1]. However, sequences for several other members of the family are also known [2].

Abbreviations used: BLG, β-lactoglobulin; α_{2u}g, α_2-urinary globulin; MUP, major urinary protein; RBP, retinol-binding protein.

The work presented here focuses on two members of the family, both from rodent urine. α_2-Urinary globulin (α_{2u}g) from male rat urine is of interest because of its role in certain renal toxicity effects [3]. Major urinary protein (MUP) from mouse urine is well studied genetically, yet its function *in vivo* is still unclear [4]. It would seem likely that the proteins are used to carry marker substances (pheromones) to mark territory or help identify mates/family. A knowledge of the tertiary structure of both proteins may help to understand their physiological roles, and also the molecular interactions which produce the rat renal toxicity effects.

Work has already begun on the purification of both proteins and their subsequent crystallization for crystallographic analysis. The determination of a high-resolution crystal structure will take some time to complete; therefore, this has afforded the opportunity to use molecular modelling to determine possible structures for the proteins with the eventual aim of comparing the modelled and crystal structures. Being members of a large superfamily, in which some structures are already known, made the construction of start-