02.2-02 COMPARISON OF THE STRUCTURES OF KDPG ALDOLASE AND TRIOSE PHOSPHATE ISOMERASE. By L. Lebioda, M.H. Hatada, I. Mavridis and A. Tulinsky, Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, U.S.A.

The structure of 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase has been extended to 2.8 A resolution using MIR methods. The final MIR phases were improved further with 3 cycles of refinement via electron density modification procedures coupled with inverse fast Fourier transform phase angle calculations. With the complete sequence of the enzyme now available (Suzuki, N. and Wood, W.A., J. Biol. Chem., 255, 3427 (1980)), a Kendrew model was constructed corresponding to the electron density of the final cycle. All 225 residues have been accounted for in the model. The main chain density is generally good except in the region 189-197 where it is weak and discontinuous; of the side chains, 139 residues have good density, 36 residues have only partial, and 29 residues have no side chain density, while 21 residues are Gly.

Of the approximately 50 residues not located in the 3.5 A resolution map, about 40 have been found in the interior of the molecule as part of 8 strands of a β-barrel. The overall folding of KDPG aldolase is similar to that of triose phosphate isomerase (TIM) with adjacent strands of β-structure linked by α-helical segments. However, the content of regular secondary structure is lower: α helix 29 and 55%, β sheet 16 and 23% for KDPG aldolase and TIM, respectively. The similarity between the structure of KDPG aldolase and TIM was originally recognized by Richardson (Biochem. Biophys. Res. Comm., 90, 285 (1979)) at the 3.5 A resolution level. This led her to propose 4 chain reconnections to create 4 additional β-strands and thus an 8 strand β-barrel. Richardson's proposed reconnections are necessarily approximately correct topologically, however, since most of the additional residues of the 2.8 A resolution map were found in the interior as part of the β-barrel, the details of the barrel are different. The very prominent electron density of the helical periphery of the molecule probably affected the generally weaker interior density adversely at lower resolution.

The C-C_α coordinates of KDPG aldolase have been measured with the aid of a surveyor's transit and a cathetometer. The C_α structure is being compared to that of TIM. The primary structures and the active site regions of KDPG aldolase and TIM are also being compared. Depending upon the degree of significance of correlations, a comparison of the side chain structure might also be carried out.

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02.2-03 TWO ANALYSES OF PROTEIN STRUCTURES : 1. THE ROLE OF THE AMINO AND CARBOXY TERMINI. 2. SALT BRIDGES AND THE DISTRIBUTION OF CHARGED GROUPS. By J. Thornton and L. Chakauya, Department of Crystallography, Birkbeck College, Malet Street, London, WC1E 7HX, UK.

1. A survey of the N and C terminal regions in proteins of known structure has been performed. We have considered conformation, accessibility, proximity and specific interactions between termini, relationship to the active site and functional regions, domain linking and dimerisation and the asymmetry of the termini. The results suggest that the termini fulfil certain specific roles in maintaining the stable native conformation.

2. The distribution of salt bridges and charged groups in proteins of known structure has been analysed. The survey includes residue type, conformation, acessibility and function. The role of salt bridges and charged groups in proteins is considered.

02.2-04 CALCULATING MOLAR VOLUMES OF PROTEINS FROM ATOMIC COORDINATES : PROBLEMS OF VOLUME PARTITIONING AND WATER-PROTEIN INTERACTIONS. By B. J. Gellatly and J. L. Finney, Crystallography Dept, Birkbeck College, Malet Street, London, WC1E 7HX, England.

An ability to calculate accurate molar volumes of proteins is a strong test of a model of solvent-protein interactions, and is desireable if we are to understand pressure effects. Moreover, increasing use of low-angle neutron scattering measurements of radius of gyration require a knowledge of excluded volume for full interpretation. However, attempts to perform such calculations have been so far unsuccessful (Richards, J. Mol. Biol. 82, 1(1974); Finney, ibid, 96, 721(1975)), because of (a) technical problems of physically reasonable space partitioning, and (b) an inadequate knowledge of where to position bounding solvent. Richards' use of a surrounding ordered water lattice resulted in calculated volumes greater than experiment. Finney used a variety of surface treatments and managed to obtain a range of results which encompassed the experimental value. However the use of van der Waals surfaces was unjustified with respect to hydrogen bonding interactions. The present availability of high resolution structures including much of the first shell water suggests further progress can now be made in solving this problem. We report here the initial results of studies on several small but well-defined proteins.

(1) Volume partitioning between protein and the surroundings was performed using the Voronoi construction, which places dividing planes midway between atom pairs, and the Radical Plane generalisation (Fisher et al, Neues Jahrb. Min. Monat. 227(1971)), in which the dividing plane is drawn at a distance which is a function of the sizes of the two atoms concerned (cf. Richards' "method B"). Its application to protein packing will be

described elsewhere.

(2) The placing of surface points to allow the partitioning of volume between protein and surroundings is critical and should be based on physical reality. Approaches based on van der Waals radii were rejected, as they fail to reflect the difference in approach distance of a water molecule to a hydrogen donor (eg NH) or acceptor (eg CO) group. Two other possibilities were examined :

(a) The use of "solvated radii" obtained from small molecule hydrate structures as described previously (Finney et al, Biophys.J, 32, 17(1980)). The procedure raises queries as to the transferability of solvent approach distances from small systems, and also takes no account of likely dispersion in these values. In favourable cases, actual solvent positions can be used where the data is adequate (eg insulin, APP). There are further problems concerning the subsequent placing of the dividing plane between solvent and the relevant protein group: the Voronoi construction will, because the plane falls midway, misallocate volume to a degree depending upon the particular interaction. Use of the radical plane would appear to overcome this, but unfortunately introduces additional problems of self-consistency at certain points near the surface.

(b) Alternatively, we could use the Voronoi construction but in conjunction with artificially adjusted "solvent radii" to ensure the dividing plane occurs in a physically-reasonable position for each interaction. This appears the most satisfactory procedure, although geometrical problems do arise particularly near highly-exposed groups. The extent of this problem depends strongly upon the radii chosen, and underlines the need to use a physically-reasonable set of water approach distances. Possible ways of overcoming these remaining problems are discussed.

02.3-01 A NEW STRUCTURE MODEL FOR COLLAGENS.
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A new structure pattern for collagens has been deduced. Each polypeptide chain, at least in its regular Gly-X-Y part, is a 1-residue-per-turn helix, with an essentially straight axis. These chains, with parallel axes, are grouped into "3-stacks", with each amino acid residue hydrogen-bonded laterally to two other residues -- one in each of the other chains of the 3-stack. The side chains extend laterally from each 3-stack, forming crosslinks, largely by hydrogen-bonding, to neighboring 3-stacks. This type of structure is in much better agreement with the fundamental principles of molecular and crystal structure than are the coiled coil structures that have been in vogue for about 30 years. Insofar as it has been tested, the new structure appears to be in at least as good agreement with pertinent experimental data as the older models. Attempts to decide between alternative patterns for packing of the 3-stacks for different types of collagen are in progress. Possible axial shifts (staggering) of the chains within each 3-stack and of the 3-stacks in larger units are being considered. When the packing patterns are known, the kinds and locations of the crosslinks and of noncrosslinking side chains should be determinable, using the known residue sequences. Some structural details will doubtless require further experimental research, using x-ray and other techniques, by other scientists. Other aspects of the structure will be dealt with, if time permits.

02.3-02 THE CRYSTAL AND MOLECULAR STRUCTURE OF A COLLAGEN-LIKE POLYPEPTIDE $(Pro\text{-}Pro\text{-}Gly)_{10}$.
By Struther Arnott, Masao Kakudo, Kenji Okuyama, Kaoru Okuyama and Motowo Takayanagi, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

$(Pro\text{-}Pro\text{-}Gly)_{10}$ forms orthorhombic $(P2_12_12_1)$ single crystals (a = 2.693 nm, b = 2.642 nm, c = 10.04 nm) providing X-ray diffraction data to 0.22 nm resolution. In the crystals the polypeptides form triplexes which aggregate end-to-end in quasi-infinite, collagen-like helices with axial translation per tripeptide h = 0.287 nm and the corresponding rotation t = -102.9°. This structure with 7_5 screw symmetry may represent the first of a series of allomorphs of collagen which heretofore has been reported only to have 10_7 screw symmetry. The 7_5 allomorphic structure has been refined by the linked-atom least-squares method (Arnott and Wonacott, Polymer (1966) 7, 157-166; Smith and Arnott, Acta Cryst. (1978) A34, 3-11). In addition three water molecules per tripeptide have been detected by Fourier difference synthesis. One of them forms an intra-chain hydrogen-bonded bridge $O(Pro_2)\text{---}W\text{---}O(Gly)$. Within the triplex there are also inter-chain hydrogen bonds $(Gly)NH\text{---}O(Pro_1)$.

02.3-03 X-RAY DIFFRACTION STUDY OF DEAD SEA SCROLLS
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The parchments of the Dead Sea Scrolls were prepared from animal skins, mainly sheep and goats. They were copied and hidden some 2000 years ago in caves near the Dead Sea, where they were discovered in the late 1940's, and after passing through various hands, they were deposited in the Israel Museum. Visual inspection of the Scrolls shows lighter and darker areas, the latter generally close to the edges in areas of poorer preservation. With the aid of X-ray diffraction, we have been able to identify a major chemical change associated with this deterioration, estimate it semi-quantitatively, and gain some understanding of how and when it occurred.

The parchments consist mainly of collagen, which was evidently lightly tanned and limed. At moderately elevated temperatures and humidities collagen denatures to form gelatin. Collagen and gelatin have distinct X-ray powder patterns, and those of the various Dead Sea Scrolls were found to be combinations of these. By taking densitometer scans of powder photographs and measuring ratios of the slopes of the 10Å and 5Å peaks, we have been able to derive a collagen:gelatin index that correlates well with the appearance of the Scrolls as observed by optical and scanning electron microscopes. The X-ray measurements are relatively unaffected by contaminating inorganic materials and are, of course, non-destructive, so they can be used to monitor possible further deterioration of parchments under various conditions. Measurements of D-aspartic acid contents of the Scrolls indicate that appreciable racemisation occurs in gelatin but not collagen, and that this slow process must have started many centuries ago.