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第 18 回国際結晶学会参加報告

長岡技大・V. Nagarajan

第 18 回目を迎えた IUCr の会合は英国の美しい町 Glasgow で 8/4-8/13 の日程で開催されました。Glasgow は文化的な香りに満ちた保養地としても有名で、最も活気がありまた友好的な市のうちの１つです。学会当日の日中の気温も 18-20℃ぐらいで、とても過ごしやすい気候でした。

会合は Scottish Exhibition and Conference Center(SECC)で開催されました。この建物は、Glasgow の中心を流れる Clyde 川沿いにあって、ここから非常に美しい景観を楽しむことができます。ヨーロッパでも指折りの魅力的で近代的な会議場です。

Durham 大学の Judith Howard 教授（英国結晶学会会長）の世話により開催された会合には、世界各地から 2000 人を越える参加者が集いました。

8/4 水曜日の午後６時に始まったオープニングセレモニーの中では Ewald lecture が行われました。第５回の Ewald prize は G. N. Ramachandran に贈られました。彼の結晶学分野における多大な貢献：異常分散の位相決定への利用、ファイバー、特にコラーゲンの構造解析、特に生体高分子のコンフォメーションにおける基礎的な仕事や 'Ramachandran plot' として知られる生体高分子の構造の評価法（この Ramachandran plot は今日でも有効な構造評価のためのツールとして用いられていますが）など、が評価されての受賞です。IISc Bangalore の Vijayan 教授が Ramachandran 教授の代わりに Ewald prize を受け取り、続いて Ramachandran 教授の講演がありました。

8/5 から 8/13 までの Scientific program は、毎日２つの keynote lecture から始まり、続いて６つの microsymposia が並行して行われました。ポスターセッションは午後に Exihibition hall で行われ、ポスターが展示されているときには会場で昼食もとられました。それからまた６つの microsymposia と２つの keynote lecture が続くという形で毎日のプログラムは進みました。

Monteath-Robertson Symposium

8/7 に J.Monteath Robertoson 教授を偲んで Monteath-Robertson Symposium が開催されました。Robertson 教授は Glasgow 大学の chemistory department の Gardner chair として結晶学の分野でのパイオニア的な仕事をリードした方です。彼の学生や同僚であった著名な結晶学者がこの機に講演を行いました。Robertson 教授のラボで働く機会があった M.G.Rossmann 教授は、「From Aromatic Hydrocarbons to Virus Structures」というタイトルで話をしましたが、その中で 1953 年から今日までの間の結晶学で起こった技術革新の歴史に触れていました。彼は、Cambridge で自家製のコンピュータを使って解かれた最初の低分解能の蛋白質の構造についても話をし、それから位相問題の解決技法の改良とコンピュータの性能アップや放射光の利用などにより、データ測定の質とスピードが進歩することで、蛋白質構造解析が飛躍的に延びたことを述べました。また、1970 年代後半に解かれた最初の球状ウィルスの構造から、今日までの多くのウィルスの構造－ある場合はレセプターとの複合体で、またある場合は非常に巨大な分子量のものを原子レベルで－についても言及していました。

S.C.Abrahams 教授は、J.M.Robertson 教授が新任教授とした Glasgow 大学に着任した年に大学に入学しました。彼は「From Molecules to Physical Properties」という題で話をしました。英国での IUCr の会合の開催場所として Glasgow が選ばれたということから、Glasgow school of crystallography の創始者である Robertson 教授への深い尊敬の念が慮られる、と彼は語っていました。また、教授の指導で行った有機化合物の X 線回折研究中やコンピュータの回折結果へ適用する上で芽生えた興味が多方向へ進化していったことが紹介されました。graphite reactor から中性子が使えるようになったことで、当時は主流であった手動回折データ測定の自動化が必要になり、さらに結果として得られるデータの不確実度についてより深く理解することが求められるようになりました。構造解析は目的あるいは手段かと問うことが、結晶構造と材料の物性との関係を調べることにつながりました。また、彼は、Robertson 教授の影響を受けた大学院生たちが、その後結晶学の広い範囲で活躍していったことも述べていました。

Jack D.Dunitz 教授は「Chemical crystallography: From then till now」というタイトルの話でした。その中で、過去にはどのようにして決定されたすべての結晶構造を知り得たか、誰がそれを行ったか、誰も結晶学的データをカバーできなくなった今日までにその問題がどのように解決されたか、について概略を述べました。彼によれば、Robertson 教授の時代には、化学結晶学者になるためには非常によい記憶力が必要とされたが、今では CSD と PDB が必要だそうです。この変遷に対して Robertson's Glasgow school の果たした役割は大きいことも指摘していました。また、化学の進歩において結晶学の与えた影響についても触れていました。

University of Glasgow, Department of Chemistry の N.W.Isaccs 教授は、Robertson 教授の時代とその後結晶学がいかに繁栄したかについて、また、chemistry department がまだなお結晶学の素晴らしさの中心にあることを述べていました。 Robertson protein crystallography laboratory は human chronic gonadotropin や integral membrane light-harvesting complex の構造の発表をはじめ、これまでに多くの業績を挙げています。

Keynote Lectures

Some of the keynote addresses which were of interest to the author, the talk on ÒNucleosomesÓ by Prof. T.J.Richmond, in which he talked about the X-ray crystal structure of the nucleosome core particle providing substantial insight into chromatic structure–function relationships. In addition to the Protein and DNA components first seen at 2.8• resolution, the atomic structure refined using data to 2.0• permits the reliable location of nearly 1000 water molecules and ions in the electron density map.

Prof. Johann Deisenhofer of the Howard Hughes Medical Institute talked on ÒMembrane protein structure, an exciting FrontierÓ, where in he mentioned about the major challenge that lay ahead for structural biologists in solving the three-dimensional structures of integral membrane proteins. He talked about the technical obstacles and attempts at finding ways to overcome them.

Prof. Jack D. Dunitz talked on the topic ÒPolymorphism: the same yet differentÓ. Polymorphism is the ability of a compound to crystallize in more than one distinct crystal structure. Once regarded as something of a rarity, it is now known to occur extensively and possibly even ubiquitously. This is one of the factors that make crystal structure prediction so precarious. The polymorph obtained by crystallization from the melt or from solution is not necessarily the most stable one under the given condition. Transformation to the stable form may occur spontaneously or may be extremely slow, depending on the presence of seeds or crystal defects

Prof. P.B. Sigler talked on the topic ÒChaperonin Assisted Protein Folding: the final step in genetic expressionÓ. Molecular chaperonins proofread and edit the final step in gene expression, protein folding. Crystallographic structure and functional studies of the bacterial chaperonin GroEL/GroES in various stages of its functional cycle have shown a Òtwo-stroke engineÓ mechanism by which non-native peptides are entrapped, unfolded, refolded in a shielded environment and finally expelled. The recent work

shows the mechanism by which a wide range of misfolded proteins are serviced by GroEL where in the oligo peptide segments with different sequences bind firmly with similar conformation to the same GroEL surfaces.

Prof. Mckay talked on the topic ÒSmall Ribosome structure and MechanismÓ in which he talked about their recent structure of a ÒleadzymeÓ, a Pb^{2+} - dependent ribosome derived from *in vitro* experiments, at 2.7• resolution. Two leadzyme molecules in different conformation are present in the asymmetric unit, with different metal binding properties. Based on the structure a model was proposed for bond cleavage incorporating both the metal binding properties and the apparent flexibility of the cleavage site of the leadzyme molecule.

Prof. Yu Wang talked on the topic ÒCharge density analysis and bond characterization of 3-d transition metal complexesÓ. Bond characterization is ever needed for predicting the physical and chemical properties of the molecule. Charge density analyses have been applied in terms of deformation density distribution, natural bond orbital analysis and the topological analysis on the total electron density. The analysis provides not only the molecular electron density distribution, but also the information such as bond order, bond type and atom domain in molecule. He also described the detailed characterization of metal-ligand as well as intra-ligand bond and also about the recent advance both in experiment and theory that has helped in charge density analysis of molecule in its excited or metastable states.

Prof. S. Cusack talked on the topic ÒAccuracy in translation: tRNA and Amino acid recognition by Amino-acyl synthetasesÓ. Structure analysis was carried out on both native and substrate complexes of five Thermus thermophilus class II synthetases. The native ProRSTT structure and with proline bound have been determined at 2.4• and 2.9• resolution, respectively. Also was presented results on ternary complex of ProRSTT with tRNApro and a prolyl-adenylate analogue. New structure of HisRSTT determined without any substrate bound was presented. As also the structure of AsnRS with an analogue of asparginyl adenylate helped in understanding how discrimination between aspartic acid and asparagine was achieved. The crystal structure helped in shedding further light on the amino-acid recognition, conformational changes associated with amino acid binding, the mechanism of amino acid activation, etc.

Prof. Moffat talked on the topic ÒTime-resolved biological processÓ, in which he explained about structural reactions that can be initiated in the molecule in a single crystal of excellent diffraction quality, for example by illuminating the crystal by a brief laser pulse. The reaction is then followed by subsequent time-dependence of the intensities of all Laue spots and from them, the X-ray structure amplitudes. He also described about the techniques available to conduct such experiments. He also showed some results on myoglobin and on a blue light photoreceptor known as photoactive yellow protein, PYP. PYP is representative of the so-called PAS/LOV domain protein which are widely distributed and participate in biological signaling roles.

Prof. J. Wark talked on the topic ÒPico-second X-ray diffractionÓ. The talk reviewed the historical development of the X-ray sources when several minute exposures were needed to the time when we are finally accessing the sub-Pico second exposures. Bright laser plasma K-a X-ray sources with a few hundred femto second duration have been recently demonstrated, following the development of table-top terawatt laser system. This may allow the observation of electron density during chemical reaction-which has been dubbed ÒWatching molecular moviesÓ. He also discussed about the prospects for the future and certain possible applications.

Microsymposia

The microsymposia dealing with molecular machines and organelles dealt with understanding the ribosomal structure and function with one of the talks focussing on the 5• resolution map of the large sub unit and the other on approaching atomic resolution in ribosomal crystallography.

Another topic of discussion was ÒPreservation and Decay at cryo temperaturesÓ. This symposia had a talk on macromolecular crystal annealing that seem to reduce the mosaicity of flash cooled crystals without affecting molecular structure, where the flash cooled crystal is transferred to a droplet of its original cryo protectant for about 3 minutes and re-flash-cooled to cryogenic temperature. Another talk was on the analysis of the lifetime of the crystals exposed to high intensity x-ray beams from synchrotron sources under cryogenic condition. A systematic study and analysis showed that decreased temperature have only marginal effect on crystal lifetimes. Another talk covered an overview of the process of radiation damage and beam heating. Another interesting talk was on the changes of cell dimension during data collection of a single virus crystal. A single frozen crystal was used for data collection of cricket

paralysis virus. At data processing, it became evident that the data could not be scaled and an analysis had showed that early during the exposure, the crystal had undergone a transition, corresponding to a change in the 'c' axis of 0.8%.

A microsymposia on motor proteins and muscles concentrated on the structural studies of myocin, scallop myosin having at least three conformational states, the structure of an actin cross-linking protein, the unique features of minus end directed kinesin motors revealed from the NCD dimer crystal structure and the time-resolved diffraction studies on muscle using synchrotron radiation.

One other topic of microsymposia was Real time '*in situ*' reaction chemistry. The talks were on the developing field of photo crystallography: Time resolved study of light induced transient species. Use of synchrotron radiation for 'in situ' investigation of the solid state reactions. Analyses of the photo induced metastable structures in several organic crystals. The talks were aimed at understanding the process of a chemical reaction. Coppens and his group are developing methods for time resolved studies of transient species using synchrotron radiation.

Membrane proteins and trans-membrane signaling was the topic of discussion of a microsymposia in which the structure of Outer Membrane Phospholipase A (OMPLA), an integral membrane enzyme located in the outer membrane of gram-negative bacteria was discussed. Also, the structure of ferric enterobactin receptor (FepA), an integral outer membrane protein from *E.Coli.*, that binds ferric enterobactin and transports it into the periplasm solved at 2.4• resolution by MAD was discussed. FhuA protein felicitates ligand-gated transport of ferrichrome-bound iron across *E.coli*. outer membrane, the crystal structure in the presence and absence of ferrichrome revealed two distinct conformations. Also the structure of Bacteriorhodopsin at 1.55• resolution was discussed.

ÒStructural motifs and multi-domain proteinsÓ was another topic of microsymposia in which the structure and several complexes of Carbonyl-phosphate synthetase: an enzyme that catalyses one of the most remarkable biological reactions where by carbamoyl phosphate is assembled from one molecule of bicarbonate, two molecules of Mg2+ATP and one molecule of glutamine was discussed. Also, the structure and function of Botulinum neurotoxin type A that has a molecular weight of 150KDa with three approx. 50KDa functional domains was discussed. Human placental ribonuclease inhibitor (hRI) that binds to human Angiogenin (Ang), a blood vessel inducing protein, with high affinity. The hRI-Ang complex structure determined at 2.0• resolution was discussed.

ÒProblematics in macromolecular structures (I): PhasingÓ was the topic of discussion of one of the microsymposia, in which a talk on detecting and exploiting non-crystallographic symmetry in MR concentrated on making the distinction between 'frustrated' crystallographic symmetries i.e. those that are almost crystallographic ones and 'standard' non-crystallographic symmetries. These have been successfully applied to many body macromolecular crystal structures with save in time and computational effort. Another talk focussed on the use of maximum likelihood for MR. The theory has been developed and the initial tests seem to be promising. Likelihood based MR seems to have much better signal to noise ratio and significantly greater accuracy. Also structure determination of *E.coli*. CLPP using 14-fold non-crystallographic symmetry averaging was also discussed.

Under the topic Protein-Nuclei acid interaction, the structure, action, inhibition of Human Topoisomerase I was discussed. The crystal structure of several complexes with DNA was elucidated after inspecting over thousand crystals. The insight into the functioning of the enzyme was discussed. Another talk was on the mismatch recognition and mismatch repair by the *E.coli*. G:U/T mismatch glycosylase. The structure of the free enzyme and the complexes with abasic products have been studied. Another talk was on the crystal structure of a hexameric B-DNA type duplex in complex with a metalloporphyrin solved by MAD experiments at 0.9• resolution.

Under the title ÒLarge unit cells: sources, detectors and dataÓ the topics discussed were, design of beamlines for macromolecular crystallography at III generation sources, data collection from large unit cell crystals using CCD and large IP detectors on ID14 at the ESRF. The CCD used on the ID14/EH4 can record 500-550 diffraction orders with a duty cycle of 10 frames/minute. Weissenberg camera with an active area of 800mm by 800mm consisting of two image plates 40cm by 80cm placed on a flat wall by a robot from an IP storage/erasure device has been developed. Results of data collection were presented. A Data Processing Suite (DPS) developed by the Rossmann group for data collected on oscillating single crystals was presented. Also, crystallographic study of a T=7, dsDNA virus capsid with a diameter of 650• was presented. The structure was solved by phase extension with a cryoEM reconstruction serving as an initial phase model at 30• resolution.

ÒNew frontiers in macromolecular crystallizationÓ was a topic of discussion in which the use of Dynamic light scattering (DLS) was discussed. 81% of the proteins crystallized had DLS profile with a homogeneous size distribution, while only 61% of the proteins that stained as single band on native PAGE, produced crystals. The micro-gravity protein crystal growth at the center for macromolecular crystallography (CMC) performing growth experiments on 37 U.S. space shuttle missions was discussed. Another talk was on optimizing the crystallization condition using the analytical centrifuge. One other talk was on the post-crystallization soaking improving the diffraction quality of MTCP-1 crystal. The MTCP-1 crystal grown form 1.5M ammonium sulfate in Tris buffer pH7.8 diffracted to 3.0• with streaky spots and diffuse scattering. This was soaked in an artificial solution with 2.0M ammonium sulfate, the crystals had much stronger diffraction up to 1.9• and no streaking was observed. Also, NASA's biological crystal growth program on the international space station was discussed.

Under the topic Metalloproteins, Electron transport and EXAFS, the crystal structure of reaction intermediate of cytochrome P450cam was discussed in which the biochemically important oxygen complex of P450cam obtained by reducing ferric P450.camphor.O2 complex was generated by fusion of oxygen, the method used and the structure obtained was discussed. A hydroxylating dioxygenase-Naphthalene dioxygenase which catalyzes the dihydroxylation of naphthalene to cis-naphthalene dihydrodiol has been studied with structure of enzyme complexed with substrate/inhibitors with the Fe in both the oxidized and reduced states, was discussed. Polarized EXAFS measurements on oriented single crystals of carbonmonoxy-myoglobin (MbCO) was used to deduce the geometry of the heme-Fe-C-O site in MbCO. The polarized Fe K-edge X-ray absorption spectra of single crystals of MbCO in six orientations were recorded and fitted a 3-dimensional model of the heme-Fe-C-O site simultaneously to the six sets of EXAFS data. The results were discussed.

Macromolecules at high resolution: Refinement and Validation was a topic of discussion in which, the relationship between R_{free} and R in a correctly refined protein structure was discussed. The presenter had derived a statistically expected value of the ratio of the free R-factor to the R-factor where weights correctly account for experimental and model errors. The estimates compared with the observed ratios from nearly 725 macromolecular structures with relationship to resolution and number of reflections/atom was discussed. Another talk focussed on the structure of subtilisin at atomic resolution, in which the structure was solved at 0.85• for the enzyme in a fully active state and compared with the structure obtained at 10% optimal activity. Also, Ultra high-resolution structure of Crambin at 0.54• resolution and the first ever charge density analysis of protein was discussed.

Viruses and viral proteins was another topic of discussion of the microsymposia. Crystal structure of human hepatitis B capsid with T=4 icosahedral symmetry determined at 3.3• was discussed. The structure was solved using phases derived from a 7.4• resolution map determined by electron cryo microscopy. Also, the effective combination of cryo-electron microscopy (cryo-EM) technique with data obtained from X-ray diffraction providing models at near-atomic resolution as applied to Human Rhinovirus (HRV) was the subject of another talk. One other talk was on probing the interior of the Bluetogue virus core particle, while another talk concentrated on understanding the mechanism of adenovirus entry onto the host cells by studying the crystal structure of Adenovirus knob bound to its cellular receptor cap.

The MAD method was another topic of discussion. Solve - an automated procedure for determination of heavy atom sites and calculation of native phase in a MAD experiment or in all derivatives in a MIR experiment was a topic of discussion. Another talk was on the crystal structure of the synaptic fusion machinery solved by MAD phasing in which the multi-MAD technique was used for the structure solution since the crystals were highly mosaic, weakly diffracting and highly variable. Another topic of presentation was structure of the *E.coli*. FIMC-FIMH chaperone-adhesin complex solved at 2.5• resolution. The MAD structure determination at the CORNELL high energy synchrotron source and the structural biology center commissioning of 19ID undulator beamline at APS useful for the MAD experiments was discussed.

Poster presentation

The poster presentations were broadly classified in several categories. The most number of posters were on the section ÒCrystallography of Biological macromoleculesÓ under various sub-titles. The use of synchrotron radiation combined with CCD cameras has become the common mode of data collection. Some of the structures had been solved at ultra high-resolution and a large number of charge density analyses has been done on small molecules and certain macromolecules due to availability of high-

resolution data. A few posters were on the structural elucidation of enzymes that are involved in the degradation pathway of aromatic compounds. There were also posters that described the structure of the ribosomal proteins and of other macromolecular machine and organelles. There were also posters that described proteins of the immune system, receptor and signal transduction, multi-domain proteins, structural motifs, etc. NMR applications to macromolecules, time resolved studies, enzymatic catalysis, protein-DNA, protein-RNA interactions and protein engineering were some of the topics which were interesting. Some significant development were seen in the methods of structure determination, computational methods in structure prediction, anomalous dispersion, MAD, MIR phasing, direct methods of phase determination, etc.

Some of the other major topics of poster presentation were crystallography of biological small molecules, crystallography of organic compounds, Inorganic crystallography, crystallography in material science, fiber diffraction, crystal growth, electron microscopy, electron diffraction, databases and industrial crystallography. A large number of posters were presented by a large contingent of Japanese crystallographers.

A large number of stalls with exhibits of various companies were seen at the congress, these companies also happen to be the sponsors of the IUCr congress. Several dinners and banquets were hosted during the conference and there were organized tours on the 10^{th} of August (the rest day). The sessions were stopped for half an hour between 11:00 am and 11:30 am for visualizing the rare event of a full-solar eclipse.