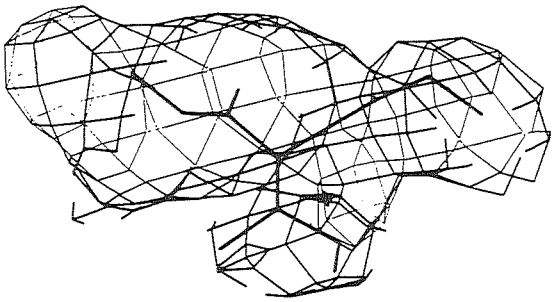
01.4–4 CONSISTENT ELECTRON DENSITY METHOD: A POSSIBLE SOLUTION TO THE PHASE PROBLEM. By T.N. Bhat, LMB, NIADDK, NIH, Bldg. 2, Rm. 312 Bethesda, MD. 20205, U.S.A.

The phase problem is the basic stumbling block in structure solution by diffraction methods. For small molecules direct methods provide a generally acceptable approach to the problem. However the structure determination of macromolecules is a time consuming and difficult task. I shall present a method which is very promising and may be used for ab initio phasing for molecules of all sizes. This method is not limited to any particular resolution of diffraction data. Several model calculations have been done using the method. These calculations cover low, medium and high resolution data and include small medium and large molecules. In all these calculations I will demonstrate that starting from random phases and correct or error introduced amplitudes electron density maps that are similar to the true electron density maps have been obtained.

The method, called the consistent electron density (CED) method, attempts to produce a set of mutually unbiased electron density values which are consistent with certain restraints and the observed amplitudes. The set of electron density values which are either inconsistent with the restraints or which result from undesired bias are altered in an iterative process. The method makes use of restraints both in real space and in reciprocal space and thus permits the best visualisation, incorporation and alteration of the desired restraints. The steps in the CED procedure may be summarized as follows: the starting set of phases is altered on the basis of restraints in reciprocal space (e.g; symmetry effects) and an electron density map is calculated. This electron density map is altered according to restraints in real space (e.g; positivity, solvent flattening). From the restrained electron density map, an unbiased electron density map which is consistent with the restraints is calculated. Effects of undesired bias or incompatible restraints are reduced by the choice of suitable weights for each Fourier coefficient. A fresh set of starting phases is calculated from this new electron density map and the procedure is iterated. The result converged in all the model calculations that have been performed.

In one of the test calculations an atomic model which has 16 atoms and space group P1 was chosen. Structure factor amplitudes, FCAL extending up to 3 Å were calculated from the model. FCAL were randomly altered to obtain FOB such that the R-value between FOB and FCAL is 0.1. These FOB and random phases were subjected to several cycles of the CED procedure.



The atomic model and the electron density map calculated using FOB and the phases derived by the CED method are shown above.

I am gratefull to Drs. D.R. Davies and G.H. Cohen for useful discussion.

01.5–1 CRYSTALLOGRAPHIC REFINEMENT OF 2ZN INSULIN STRUCTURE. By K.Sakabe[1], N.Sakabe[1] and K.Sasaki[2]. 1, Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan. 2, College of Medical Technology, Nagoya University, Higashi, Nagoya 461, Japan.

Stereochemically restrained refinement is useful for protein structures at medium and high resolution, and stereochemically unrestrained refinement followed by a regularization of the structure is powerful at very high resolution. The crystal structure of 2Zn insulin was refined using the latter method with 1.1Å resolution data, and these results were presented at the last conference (Acta Cryst.(1981) A37 C50).

Since that time, the location and refinement of disordered insulin and water structures were investigated. We concluded that the most reliable indicator for such structures was to compare the Fourier map, removed D-Fourier map and the refined positions. The convergence of the refinement and the evaluation of the refined structure will be discussed at the meeting.

In the crystals, two zinc ions are crystallographically independent but in almost equivalent positions. The zinc ion is on a three fold axis and binds six ligands in a distorted octahedral coordination, namely three crystallographically equivalent imidazole rings and also three water molecules. Dr. E.N. Maslen in University of Western Australia suggested to us the possibility of observing the redistribution of electron density due to bonding near the zinc atom in our D-Fourier map.

The $zinc^{2+}$ ion has a $d_{10}$ configuration which in the absence of a perturbing field will be spherically symmetric. However, there will be a perturbation due to the three ligating nitrogen atoms of imidazole and three water molecules. The geometrical arrangement of these atoms around the zinc is approximately octahedral. The perturbation of the zinc atom by this field redistributes density towards the corners of a cube. This is slightly distorted because the coordination is not exactly octahedral. The difference Fourier maps show two planes containing the corners of the cubes. The direction of the peaks nicely coincides with that of the [111] direction for the cube.

The peaks are approximately 1.3Å from the zinc atom. This large distance is expected because of the limited resolution of the maps.

We are very grateful to Dr. E.N. Maslen for his suggestions and discussions.

Section of the difference electron density around Zn