PROTEIN MIR PHASES IMPROVED BY DIRECT METHODS

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Direct methods have been applied to multiple-isomorphous replacement (MIR) data of a known protein containing 682 amino-acid residuals in the asymmetric unit. The data set consists of 14,500 unique reflections at 3.0 Åresolution with F(obs) greater than 2σ. Test calculation showed that the phases from conventional MIR phasing could be considerably improved by direct methods. Main points of the new phasing procedure are as follows. (i) Conventional MIR phasing is first performed. Phases with a figure of merit greater than a certain limit, say 0.99, are accepted as the input to the following step. (ii) The MIR data are divided into n-sets (n equals the number of heavy-atom derivatives) of single isomorphous replacement (SIR) data. Each SIR set is treated by direct methods separately to break the phase ambiguity with starting phases from step (i). (iii) Direct-method phases from different sets of SIR data are combined to give a unique set of phases. (iv) Resultant phases from step (iii) are further combined with that of step (i) to give the final phases. The result in comparison with that from conventional MIR phasing is shown below. Averaged figure of merit: increased from 0.71 to 0.80. Correlation coefficient: increased from 0.51 to 0.55. Fobs weighted averaged phase error: decreased from 58.74° to 56.10°.

This project was initiated according to a very helpful discussion with Professor Liang Dong-cai.

Keywords: DIRECT METHODS MIR PROTEIN PHASING

COMBINING SELENOMETHIONINE AND SELENOCYSTEINE PROTEIN LABELLING FOR MAD EXPERIMENT : A PROMISING METHOD FOR THE STRUCTURAL GENOMICS

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Seleno-methionine incorporation for MAD phasing (1) is routinely used and has become the norm in protein crystallography (2).In order to improve the phasing power of seleno-proteins, we propose here to substitute both Met and Cys residues for SeMet and SeCys. A protocol for the incorporation of these two seleno-residues into recombinant proteins overexpressed in E. coli is described. It allows the isomorphous replacement with a high yield of both cysteine and methionine and moreover, it is fully compatible with the formation of diselenide bridges.We demonstrate that the double labelling will significantly enhance the phasing power,therefore improving and extending the applications of the MAD technique to solve new structures. This new strategy is particularly promising in high throughput structural genomics projects.
Here we report the X-ray structure of the cathelicidin motif of protegrin-3,the first structure solved by MAD using a seleno-cysteine labelled protein. Cathelicidins are a family of antimicrobial proteins isolated from leucocytes that contributes to the innate host defense mechanisms in mammalians (3,4). The overall structure of this protein represents a fold homologous to the cystatin family and adopts two native states, a monomer and a domain-swapped dimer. This is the first structural characterization of the highly conserved cathelicidin motif and thus provides insights into the possible mechanisms of activation and release of the antibiotic peptide from the cathelicidin platform.

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RECOGNITION OF STRUCTURAL FRAGMENTS IN CRYSTALLOGRAPHIC ELECTRON DENSITY MAPS

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In macromolecular crystallography the process of interpretation of an electron density map is not always straightforward and unambiguous. In particular, interpretation of density maps computed with poor phases or limited resolution of the X-ray data requires sufficient expertise and is often subjective. Initial identification of known structural motifs can be of great aid. Several successful approaches have been reported that implement pattern recognition methods for elucidation of structural motifs during the process of structure solution or refinement. These methods split the problem into a selection of a structural template followed by an attempt to find its orientation and location in the unit cell. The conformational freedom of a structure in the search problem is addressed by a search for overlapping small rigid fragments. We have developed a new automated technique based on pattern recognition methods for an identification of structural elements. An electron density map is first parameterised by a set of N free atoms. Each structural element is represented as a set of smaller fragments consisting of a subset of M free atoms. Each possible subset is considered. Fragments are parameterised as a set of interatomic distances, valence and dihedral angles and are classified using linear discriminative analysis. The top hits are selected and overlapped thus facilitating reconstruction of larger structural elements. The method has shown promising results in fragment classification with models that have average displacement of their Cα positions up to 1.5 Å which makes it applicable to a MR solution and the majority of MAD or SAD cases.

Keywords: **STRUCTURE SOLUTION, MODELLING, PATTERN RECOGNITION**

PHASE DETERMINATION BY WAVELENGTH-MODULATED DIFFRACTION. 2.NONCENTROSYMMETRIC CASE

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Wavelength-modulated diffraction was developed by the present authors as a method for phase determination, in which the intensity of Bragg reflections is recorded using radiation whose wavelength is changing continually over a range in the vicinity of the absorption edge of an atom in the crystal. Using a ferrocene derivative crystal (chemical formula $C_{37}H_{34}O_7Fe$) with the Fe atoms chosen as anomalous scatterers, measurements were made of the intensity gradient of the Bijvoet pair of reflections with an imaging plate as a detector on a synchrotron radiation source at Ritsumeikan University. In the case of a non-centrosymmetric crystal, the phase of the structure factor could be derived by solving a set of simultaneous linear equations with the measured intensity gradient as constant term (Iwasaki et al, 1999). Comparison was made between the phase thus determined and the phase calculated from the structure model of that crystal and reasonable agreement was obtained.

Reference
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