CRYSTALLOGRAPHIC STUDIES OF FOLYL-POLYGLUTAMATE SYNTHETASE AND RECOMBINANT HUMAN LACTOFERRIN

by

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ABSTRACT

This thesis is written in two parts. In the first (chapters 1-4), crystallographic studies on the enzyme folylpolyglutamate synthetase from Lactobacillus casei, both in complex with MgATP\(^3\) and in its apo form, are presented. In the second part (chapters 5-8), a structural analysis of recombinant diferric human lactoferrin is reported.

Folylpolyglutamate synthetase (FPGS) is an ATP-dependent enzyme from eukaryotic and bacterial sources. It catalyzes the addition of glutamate residues to folate to produce folylpolyglutamates which are required for effective intracellular retention of folate and are the preferred substrates for the enzymes of one-carbon metabolism. The crystal structures of L. casei FPGS in both the MgATP\(^3\)-bound and apo forms have been determined by the methods of multiple isomorphous replacement and molecular replacement, and refined by restrained least squares method using data to 2.4 Å resolution. The structural analysis of MgATP-FPGS reveals that folylpolyglutamate synthetase is a modular protein consisting of two domains, one with a typical mononucleotide-binding fold and the other strikingly similar to the folate-binding enzyme, dihydrofolate reductase (DHFR). The ATP-binding site is located in an interdomain cleft and a presumed mode of folate-binding has also been suggested for FPGS by analogy to the structure of DHFR. An unexpected structural similarity has been discovered between FPGS and the UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase (MurD). It is proposed that FPGS and MurD might carry out their biological functions in a very similar way and the structural comparison suggests that a possible domain movement could be involved in the catalytic reaction of FPGS. Two disordered loop regions in the MgATP-FPGS structure are well defined in apo-FPGS, allowing analysis of the interactions between these loops and surrounding structures of the protein.

Human lactoferrin (hLf) has considerable potential as a therapeutic agent. Overexpression of hLf in the fungus Aspergillus awamori has resulted in the availability of large quantities of this protein. Here the crystal structure of the recombinant human
lactoferrin (rhLf) has been determined by X-ray crystallography at 2.2 Å resolution. Superposition of the rhLf structure on to the native milk hLf shows a very high level of correspondence, and their dynamic properties, as indicated by the B factor distribution, also agree closely. This demonstrates that the structure of the protein is not affected by the mode of expression or the use of strain improvement procedures.
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<td>adenylate kinase</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine-5’-diphosphate</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphoblastic leukaemia</td>
</tr>
<tr>
<td>AMPCPP</td>
<td>α,β-methylene-adenosine-5’-triphosphate</td>
</tr>
<tr>
<td>AMPPCP</td>
<td>β,γ-methylene-adenosine-5’-triphosphate</td>
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<td>AMPPNP</td>
<td>β,γ-imido-adenosine-5’-triphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine-5’-triphosphate</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
</tr>
<tr>
<td>CTP</td>
<td>cytidine-5’-triphosphate</td>
</tr>
<tr>
<td>DDATHF</td>
<td>5,10-dideaza-5,6,7,8-tetrahydrofolate</td>
</tr>
<tr>
<td>DHFS</td>
<td>dihydrofolate synthetase</td>
</tr>
<tr>
<td>DHFR</td>
<td>dihydrofolate reductase</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>FeNTA</td>
<td>ferric nitritriacetate</td>
</tr>
<tr>
<td>EF-Tu</td>
<td>elongation factor Tu</td>
</tr>
<tr>
<td>EXAFS</td>
<td>Extended X-ray absorption fine structure</td>
</tr>
<tr>
<td>FOM</td>
<td>figure of merit</td>
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<tr>
<td>FPGS</td>
<td>folylpoly-γ-glutamate synthetase</td>
</tr>
<tr>
<td>FPLC</td>
<td>fast performance liquid chromatography</td>
</tr>
<tr>
<td>GARFT</td>
<td>glycinamide ribonucleotide formyltransferase</td>
</tr>
<tr>
<td>GTP</td>
<td>guanosine-5’-triphosphate</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-[2-Hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>H₄PteGlu</td>
<td>7,8-dihydrofolate</td>
</tr>
<tr>
<td>H₄PteGlu</td>
<td>5,6,7,8-tetrahydrofolate</td>
</tr>
<tr>
<td>hLf</td>
<td>diferric human lactoferrin</td>
</tr>
</tbody>
</table>
rhLf  recombinant diferric human lactoferrin
IgG  Immunoglobulin G
K_m  Michaelis constant
MIR  multiple isomorphous replacement
MurD  UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase
PEG4000  polyethylene glycol 4000
PMN  polymorphonuclear leucocytes
PteGlu  pteroylmonoglutamic acid (folic acid)
PteGlu_a  folylpolyglutamate
rms  root mean square
SDS PAGE  sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TEMED  NNN’N’-Tetramethylethylenediamine
TMV  tobacco mosaic virus
Tris  Tris(hydroxymethyl)aminomethane
TS  thymidylate synthase
UDP  uridine-5’-diphosphate
UK  uridylate kinase
UMA  UDP-N-acetylmuramoyl-L-alanine
UTP  uridine-5’-triphosphate
V_{max}  maximal velocity
RELATED PUBLICATIONS

Some of the material presented in this thesis has already been published, or has been accepted for publication.
