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CRYSTALLOGRAPHIC STUDIES OF FOLYLPOLYGLUTAMATE 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 MgATP²⁻ 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 MgATP²⁻-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. Over-expression 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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ABBREVIATIONS

ADK	adenylate kinase
ADP	adenosine-5'-diphosphate
ALL	acute lymphoblastic leukaemia
AMPCPP	α,β -methylene-adenosine-5'-triphosphate
AMPPCP	β,γ -methylene-adenosine-5'-triphosphate
AMPPNP	β,γ -imido-adenosine-5'-triphosphate
ATP	adenosine-5'-triphosphate
CHO	Chinese hamster ovary
CTP	cytidine-5'-triphosphate
DDATHF	5,10-dideaza-5,6,7,8-tetrahydrofolate
DHFS	dihydrofolate synthetase
DHFR	dihydrofolate reductase
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
cDNA	complementary DNA
FeNTA	ferric nitrilotriacetate
EF-Tu	elongation factor Tu
EXAFS	Extended X-ray absorption fine structure
FOM	figure of merit
FPGS	folylpoly- γ -glutamate synthetase
FPLC	fast performance liquid chromatography
GARFT	glycinamide ribonucleotide formyltransferase
GTP	guanosine-5'-triphosphate
HEPES	N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
HPLC	high pressure liquid chromatography
H₂PteGlu	7,8-dihydrofolate
H₄PteGlu	5,6,7,8-tetrahydrofolate
hLf	diferric human lactoferrin

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_n	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.

Sun, X., Bognar, A. L., Baker, E. N., and Smith, C. A. (1998). Structural homologies with ATP- and folate-binding enzymes in the crystal structure of folypolyglutamate synthetase. *Proc. Natl. Acad. Sci. USA* **95**, 6647-6652.

Sun, X., Baker, H. M., Shewry, S. C., Jameson, G. B., and Baker, E. N. Crystal structure of recombinant human lactoferrin, expressed in *Aspergillus awamori*. *Acta Cryst.* in press.