Crystallographic and physicochemical studies on metal and anion substituted human lactoferrin

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Abstract

Lactoferrin, isolated from human colostrum, is an 80 kDa glycoprotein capable of binding two ferric ions concomitantly with two carbonate anions. The protein consists of a single polypeptide chain folded into two similar halves, each of which bind one iron and one carbonate. It is a member of the transferrin family of proteins, which includes serum transferrin, an iron transport protein found in the blood, ovotransferrin from avian and reptilian egg-white and melanotransferrin expressed by human melanoma cells. These proteins have important roles in the transport of iron around the body and the iron-binding function of lactoferrin has implications for the health of breast-fed infants.

Two lactoferrin complexes with copper substituted for both iron atoms and the other substituted with copper and an oxalate anion for one of the carbonate anions, have been prepared and crystallised, and the structures of both determined by X-ray crystallography to 2.1 Å resolution. Whereas in diferric lactoferrin, both anions are coordinated in a bidentate fashion to the two iron atoms, in the dicupric complex the carbonate in the N-terminal site is monodentate, resulting in a 5-coordinate copper geometry with an elongated apical ligand (≈2.7 Å). In the second complex, oxalate replaces carbonate in the C-terminal half only. Analysis of the structure indicates that the extent of closure of the lobes has an important role in determining the stereochemistry at the metal binding site.

In addition to the structural studies, the binding of a variety of other metal ions, including other transition metals, aluminium, the lanthanides and thorium have been investigated spectroscopically. All of these metal ions form 2:1 complexes with lactoferrin but with varying degrees of stability. Some binding constants for a number of the lanthanides have been estimated, firstly to provide a comparison with results obtained for serum transferrin and secondly to investigate the relationship between the size of the metal ion and the strength of binding. In the course of the investigation of the binding of the lanthanide ions, it was established that cerium interacts with lactoferrin to give a complex which slowly developes a brown colour after standing for a few days. This can be attributed to the slow oxidation of Ce(III) to Ce(IV).

These results, when compared with earlier structural analyses on lactoferrin, show that the protein is very flexible and can accommodate other metals without altering it's overall structure to any great extent. The predicted binding of other metal ions, including vanadium and the lanthanides, is described based on a combination of the crystallographic and physicochemical studies.

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Abbreviations

Lf	lactoferrin	Tf	serum transferrin
OTf	ovotransferrin	MTf	melanotransferrin
bLf	bovine lactoferrin	apoLf	metal-free human lactoferrin
mLf	mouse lactoferrin	cOTf	chicken ovotransferrin
dOTf	duck ovotransferrin	hTf	human transferrin
bTf	bovine transferrin	rTf	rabbit transferrin
msTf	Tobacco Hornworm transferrin	XTf	Xenopus transferrin
rat Tf	rat serum transferrin	rTf_N	N-lobe of rabbit transferrin
Lf_N	isolated N-lobe of human lactoferrin (from recombinant DNA)		
apoTf	metal-free human serum transferrin	Fe_2Lf	diferric human lactoferrin
apobTf	metal-free bovine transferrin	apoOTf	metal-free hen ovotransferrin
Fe_2Tf	diferric human transferrin	Fe_2OTf	diferric hen ovotransferrin
Fe_2bTf	diferric bovine transferrin	Fe_2rTf	diferric rabbit transferrin
Cu_2Tf	dicupric serum transferrin	Cu_2Lf	dicupric lactoferrin
Cu_2oxLf	dicupric-(oxalato-carbonato) lactoferrin		
Mn_2Lf	dimanganese(III) lactoferrin	Co_2Lf	dicobalt(III) lactoferrin
$Ce(III)_2Lf$	dicerium(III) lactoferrin	$Ce(IV)_2Lf$	dicerium(IV) lactoferrin
EDTA	ethylenediaminetetraacetic acid	EDDA	ethylenediaminediacetic acid
NTA	nitrilotriacetic acid	β-me	β-mercaptoethanol
d.d. water	distilled deionised water	SDS	sodium dodecylsulphate
UV/Vis	electronic absorption spectroscopy	ESR	electron spin resonance
XANES	X-ray-absorption near-edge-structure	CD	circular dichroism
UV	Ultraviolet		
EXAFS	extended-X-ray-absorption-fine-structure		
ESEEM	electron spin echo envelope modulation		