Specific binding of non-steroidal anti-inflammatory drugs (NSAIDS) to lactoferrin: Crystal structure of the complex of the C-terminal lobe of bovine lactoferrin with diclofenac at 1.4 A resolution

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Lactoferrin is an 80 kDa bilobal glycoprotein which is folded into two globular N and C lobes. The two lobes are homologous and each contains a binding site for metal ion. The antibacterial activity of lactoferrin is considered to be associated with its capacity to sequester ferric ions. The structural features of iron-bound lactoferrin from a number of species have been studied [1,2]. The structures of apolactoferrins have also been determined from several sources[3,4]. The presence of two homologous N - and C - terminal lobes in lactoferrin gives rise to a number of questions concerning the purpose of their co - existence as well as the sustainability of their functional viability on decoupling, the precise role of interlobe interactions, the functional interdependence of N - and C - terminal lobes, the structural features of monoferric lobes and the roles of other metal ions which can replace the ferric ion and the features of their apoforms.

Lyophilized samples of bovine lactoferrin obtained from Morinaga Co., Japan were saturated with ferric ions and hydrolyzed with proteinase K [5]. The C-lobe so obtained was separated from the N-lobe by on a column (150 15 mm) of CMSephadex C-50 using a salt gradient of 0.0–0.5 M NaCl in 50 mM Tris–HCl pH 8.0. A 50mg/ml protein solution in deionized water was mixed in a 1:1 ratio with the crystallization buffer [0.1 M MES pH 6.5, 25%(v/v) polyethylene glycol monomethyl ether 550 and 0.01 M zinc sulfate heptahydrate] and allowed to equilibrate via vapour diffusion over 1 ml well solution at 298 K. Diclofenac was also added to this solution in excess molar ratio. The irregular-shaped colourless crystals appeared in 2 days and grew to final dimensions of 0.5 x 0.3 x 0.3 mm. X-ray diffraction data were collected at the consortium beamline X-13 at HAYLAB/DESY, Hamburg. The crystals belong to the space group $P2_1$ with a = 61.43Å, b = 49.86 Å, c = 65.16 Å and $\beta = 107.10^{o}$. The structure was solved by molecular replacement using the Zn saturated C-lobe [6] coordinates (PDB code:1SDX).

The first crystal structure of the proteolytically generated monoferric C - terminal lobe of bovine lactoferrin was determined recently [7]. The overall folding of the C-lobe is essentially the same as that of C-terminal half of bovine lactoferrin but differs slightly in conformations of some of the loops and reveals a number of new interactions. There are 20 Cys residues in the C-lobe forming ten disulphide links. Out of these, one involving Cys481-Cys675 provides an inter-domain link at 2.01A while another Cys405-Cys684 is formed between the main C-lobe 342-676 and the hydrolyzed pentapeptide 681-685 fragment. The complex between diclofenac and C-lobe revealed the binding site as well as the mode of binding of C-lobe (Figure 1).

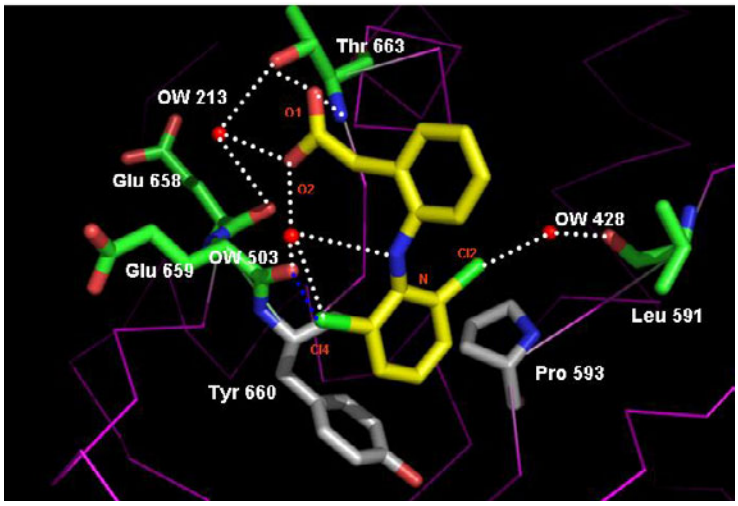

Figure 1: Interactions between diclofenac and C-lobe of lactoferrin

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