An Unexpected Symmetry in the LysR-Type Transcriptional Activator, CysB

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CysB is a tetramer of identical $M_r = 36000$ subunits which controls expression of the cysteine regulon (Kredich 1996). This system comprises a number of genes associated with sulphate transport, its reduction to sulphide and the biosynthesis of cysteine from serine and acetyl CoA in Gram negative bacteria. CysB is both an activator and a repressor of transcription whose activity is sensitive to its cofactor N-acetylserine, which is formed intracellularly from the cysteine precursor O-acetylserine. CysB binds upstream of the -35 region of a number of unlinked cys promoters and in the presence of N-acetylserine stimulates transcription. CysB also binds to its own promoter in the -10 region where it acts as a repressor. Repression at this promoter is relieved by N-acetylserine. Thiosulphate and sulphide act as anti-inducers counteracting the effects of N-acetylserine. CysB is a member of the LysR family of prokaryotic transcriptional regulatory proteins which share sequence similarities over $\approx 280$ residues and which have been proposed to contain a helix-turn-helix DNA binding motif at their N-termini (Schell 1993).

The crystal structure of a dimeric chymotryptic fragment of Klebsiella aerogenes CysB comprising residues 88-324, has been solved by multiple isomorphous replacement and multi-crystal averaging and refined against data extending to 1.8 Å resolution (Tyrrell et al 1997). The protein comprises two $\alpha/\beta$ domains each of which contains a central $\beta$-pleated sheet of six and five strands respectively. The two domains are connected by two short segments of polypeptide so as to enclose a cavity lined by polar side chains including those of two residues Thr$^{149}$ and Trp$^{166}$, whose mutation is associated with constitutive expression of the cysteine regulon. A sulphate ion and a number of well-ordered water molecules have been modelled into discrete electron density peaks within this cavity. In the dimer strands $\beta_B$ from domain I and strands $\beta_G$ from domain II come together to form a pair of 0 anti-parallel symmetry-related 11-stranded twisted $\beta$-pleated sheets.

The results provide structural insight into the LysR family of proteins. The overall structure of CysB (88-324) is strikingly similar to those of the periplasmic substrate-binding proteins and in particular to sulphate binding protein. A similar fold has also been observed in the cofactor binding domains of the LacR family of repressor proteins implying a structural relationship between the LacR and the LysR families of proteins. In contrast to Lac repressor and PurR, in CysB the two-fold axis of symmetry that relates the monomers in the dimer is perpendicular rather than parallel to the long axis of the cofactor binding domain (Friedman et al 1995, Schumacher et al 1994, Lewis et al 1996, Tyrrell et al 1997). This seems likely to place the DNA binding domains at opposite extremes of the molecule possibly accounting for CysB's extended DNA footprints.

Possible insights into the mode of DNA binding by CysB from the crystal structure of CysB (88-324) are limited for two reasons. Firstly, the fragment lacks the N-terminal 87 residues which include the putative helix-turn-helix DNA binding domain. Secondly and equally importantly, whereas full-length CysB is a tetramer, CysB (88-324) is a dimer.
identical construction in two crystal forms. Some discussion of DNA binding is warranted, however, in view of the similarities and differences between the structure of CysB (88-324) and those of the cofactor binding domains of the purine repressor PurR, and lactose (Lac) repressor, and in view of the complex half-site arrangements at the various cys promoters (Schumacher et al. 1994; Kredich 1992; Hrymewicz and Kredich 1995).

A comparison of the structure of the CysB (88-324) dimer with that of the cofactor binding domain of Lac repressor (residues 62 to 357) bound to a gratuitous inducer of the lac operon, IPTG, is revealing. It is apparent that the arrangement of the subunits in the two proteins is different. Whereas in Lac repressor the two-fold axis of symmetry which relates the two monomers of the dimer is parallel to the long axis of the cofactor binding domain in CysB (88-324) the dyad axis is perpendicular to this direction. As a result the DNA binding domains, which reside in the N-terminal segments of both proteins, are attached at same end of the dimer in Lac repressor but at opposite ends of the dimer in the case of CysB. For Lac repressor 61 residues are disordered in the crystal and are not visible in the electron density maps. These residues become ordered in the presence of DNA and extend through a linker (residues 46-62) from the cofactor binding domain to the DNA binding head-piece of 45 residues which includes the helix-turn-helix (residues 6 to 25) bound to DNA. The linker segments of each monomer of the dimer lie side-by-side with the head-pieces projecting into adjacent major grooves of a 21 base pair lac operator DNA fragment composed of two 10 base pair inverted repeats (Lewis et al. 1996).

Clearly for CysB the arrangement of the two DNA binding head-pieces in the dimer is different and the most likely possibility is that they are separated by a greater distance than in Lac repressor. One obvious assumption to consider is that the DNA binding head-piece is attached to the cofactor binding domain in a similar manner to that seen in Lac repressor. This would extend the long axis of each monomer by 30 Å and extend the long axis of the dimer from ≈ 65 Å to ≈ 125 Å without taking into account the fact that the length of polypeptide upstream of the cofactor binding domain may be up to 30 residues longer in CysB (87 residues) than in Lac repressor and PurR (≈ 62 residues). There is no direct evidence to support such a structural organisation and other arrangements are possible. However, it has been proposed that interactions between CysB and DNA involve individual subunits of the CysB tetramer binding to 19 base pair half sites. Thus at the activation sites CBS-J1, CBS-K1 and CBS-P1 two subunits each bind to 19 base pair half-sites oriented convergently and separated by either one or two base pairs (Hrymewicz and Kredich 1994). These half sites are twice as long as the half-sites recognised by Lac repressor and PurR. A full binding site therefore comprises ≈ 40 base pairs which would span ≈ 140 Å of B form DNA, a distance similar to the separation of the head-pieces predicted by the simple model described above. The DNA might then follow a path from one 'helix-turn-helix' domain to the other, trailing diagonally across the cofactor binding domains presumably forming interactions significant enough to confer protection against cleavage promoted by hydroxyl radicals. The pseudo two-fold axis relating the two DNA half-sites would be expected to coincide with the two-fold axis of symmetry relating the two monomers in the dimer. There are shallow grooves on both faces of the dimer which may with some structural adjustment accommodate a DNA duplex. Arguing in favour of the front face of the dimer as a DNA binding surface is its wider groove and deeper groove (≈ 17 Å wide and ≈ 12 Å deep) and its more electrostatically positive character. On the other hand, a number of mutations that interfere with cofactor dependent responses, including four mutations in NahR which interfere with DNA binding, map onto the inner faces of the bulges emanating from the rear face of the molecule. This tends to argue that
the rear face of the molecule participates in DNA binding though this would require slight structural adjustments of the loop regions that form the bulges which are ≈ 13 Å apart. Clearly further experiments are needed to test these hypotheses.

The C-terminal helices in Lac repressor are responsible for tetramerisation, coming together to form a four-helix bundle with one helix being contributed by each of the four monomers. In the CysB dimer, the C-terminal residues (292-324) of each monomer form a pair of helices, only loosely associated with the core of the protein, which are followed by a short segment of two to three residues which form the edge strand of the β-sheet. As these segments of polypeptide are situated on opposite sides of the dimer, it seems unlikely that they are involved in constructing a tetramer similar to that seen in Lac repressor PurR, which has a closely similar tertiary structure to Lac repressor, lacks the C-terminal helices of the latter and is active as a dimer (Schumacher et al. 1994). This dimer has the symmetry of the dimer within intact Lac repressor and not that of the CysB (88-324) dimer.

A different model for DNA binding by the LysR family member OxyR has been proposed based on shorter half-site sequences with a different organisation (Toledano et al. 1994). This model has been extended to account for the action of OccR (Wang and Winans 1995). It is proposed that the OxyR tetramer is a dimer of dimers which can bind to DNA such that the four subunits of the tetramer occupy either four adjacent DNA major grooves in the induced state or pairs of adjacent major grooves separated by one turn of the DNA helix in the uninduced state. Such an arrangement requires a side-by-side arrangement of the two DNA binding domains in the dimer, and of all four DNA binding domains in the tetramer in its induced state. This begs the question of whether a single unifying mechanism of action can account for transcriptional activation among all the LysR family proteins, or whether there exist subfamilies with distinct subunit organisations which form different complexes with DNA. There are sufficient differences in the genetics and molecular biology of the LysR proteins to make the latter a possibility. For instance, there is variety in the subunit composition of LysR proteins (dimers and tetramers) and in the genetic organisation of the regulons— for example, expression of some but not all of the LTTRs is negatively autoregulated and the genes encoding some but not all LTTRs are closely linked to, and transcribed divergently from, other genes of the regulon.

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References
The Inhibition of Matrix Metalloproteases by Tissue Inhibitors of Metalloproteases Revealed by X-Ray Crystallography

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Tissue inhibitor of metalloprotease I (TIMP I) is a member of a family of tissue inhibitors of metalloproteases that are important in regulating the activity of a group of zinc dependent enzymes, the matrix metalloproteases (MMP) by binding tightly with 1:1 stoichiometry. The MMPs including collagenase and gelatinase are involved in the degradation and turnover of connective tissue in both normal and pathological conditions; indeed an imbalance in the concentrations of TIMP and MMP has been observed in a number of degradative diseases. In addition, changes in regulation of the 1:1 ratio are seen in neoplastic cells that have become metastatic.

Crystals have been grown and data collected on native (to 1.8 Å resolution) and platinum derivatives (to 2.3 Å). The TIMP I protein used for the crystallisation experiments is a 27kDa protein that has been modified by site directed mutagenesis to yield a 21kDa deglycosylated form of the protein.

The structure of a TIMP MMP complex has been determined (Gomis-Ruth et al 1997) and the coordinates from this structure have been used to build the structure of uncomplexed TIMP I.