The specific cellular and biochemical changes that occur in the mammary gland during the non-lactating period have not been fully defined. The 39-kilodalton glycoprotein was isolated from goat dry secretions. The protein is glycosylated and immunologically distinct from other milk-associated or serum proteins and from cytoskeletal proteins. The N-terminal sequence and complete cDNA sequence have been determined. It shows 45-50 percent sequence identity with members of the chitinase protein family but lacks chitinase-like binding site. The crystal structure has been determined by molecular replacement method and refined to an R-factor of 0.185 for all the data to 2.9 Å resolution. The structure shows a fold similar to that of the prototype chitinases but lacks chitinase-like binding site. The shortening of loops makes the structure very compact. The loop 203-213 is particularly noteworthy as it adopts a conformation that is completely different from those observed in the proteins of chitinase family.

Keywords: CRYSTAL STRUCTURE, BP-39, GOAT DRY SECRETIONS


THE FIRST CRystal STRUCTURE OF A HALOTOLERANT PROTEIN: CARBONIC ANHYDRASE FROM D. SALINA AT 1.86 Å RESOLUTION

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Although proteins exist which can function optimally under extreme conditions, very few, if any, exhibit activity both in mild and extreme conditions. In studies of the halotolerant unicellular alga Dunaliella salina, we have identified a new protein class - halotolerant proteins -, which retain their function in the entire salinity range (0 to 4M NaCl). Herein we present the crystal structure of a prototypic halotolerant protein – a 30 kDa carbonic anhydrase (p30) from D. salina. p30 is a salt-inducible, extracellular, α-type carbonic anhydrase, catalyzing reversible hydration of CO₂. It was cloned by the replacement method and refined to an R-factor of 0.185 for all the data to 2.9 Å resolution. The structure shows a fold similar to that of the prototype chitinases but lacks chitinase-like binding site. The shortening of loops makes the structure very compact. The loop 203-213 is particularly noteworthy as it adopts a conformation that is completely different from those observed in the proteins of chitinase family.

Keywords: DUNALIELLA SALINA HALOTOLERANT PROTEINS, CARBONIC ANHYDRASE


CRYSTAL STRUCTURES OF THE STIMULATORY AND INHIBITORY COMPLEXES OF RAT GTPCHI AND GFRP

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GTP cyclohydrolase I (GTPCHI, EC 3.5.4.15) catalyses the initial step in the de novo synthesis of tetrahydrobiopterin (BH₄) from GTP. BH₄ is an essential cofactor for key enzymes producing nitric oxide and neurotransmitters such as catecholamines and serotonin and thus is involved in diverse body functions including neurotransmission, blood pressure regulation, immune function, and the conversion of phenylalanine to tyrosine. Genetic defects affecting GTPCHI activity cause hyperphenylalaninemia and severe neurological disorders such as 3,4-dihydroxyphenylalanine-responsive dystonia. Recent findings that guanine and 8-hydroxyguanine inhibit GTPCHI activity in a GTPCHI feedback regulatory protein (GFRP)-dependent manner raise the possibility that a BH₄ deficiency occurs in Lesch-Nyhan syndrome and Parkinson's disease. The identification of GFRP, which functions as both a positive and negative regulator of GTPCHI, has revealed the tight regulation of GTPCHI activity that maintains intracellular BH₄ levels at and below those needed by BH₄-requiring enzymes. GFRP mediates feed-forward activation of GTPCHI activity by enhancing GTP binding in the presence of phenylalanine while it induces feedback inhibition of enzyme activity in the presence of BH₄. GTPCHI is a decamer of 260 kDa with a subunit consisting of 230 amino acid residues, and GFRP is a pentamer of 50 kDa with a subunit of 83 amino acid residues. Both stimulatory and inhibitory complexes consist of three layers, (GFRP)₅(GTPCHI)₁₀(GFRP)₅, with overall dimensions of 130 Å height and 93 Å diameter. The structural comparison between the stimulatory and inhibitory complexes provides a deeper understanding of the allosteric regulation of GTPCHI by GFRP.

Keywords: CYCLOHYDROLASE TETRAHYDROBIOPTERIN ALLOSTERIC REGULATION


STRUCTURAL STUDIES OF BACTERIAL TRANSCRIPTION INITIATION

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In bacteria, the binding of a single protein, the initiation factor, to a multi-subunit RNA polymerase core enzyme results in the formation of a holoenzyme, the active form of RNA polymerase essential for transcription initiation. We have determined the crystal structure of the RNA polymerase (RNAP) holoenzyme from Thermus thermophilus at 2.6 Å resolution (R-free = 27.4%; R-factor = 22.8%). In the structure, two N-terminal domains of the subunit (conserved regions 1.2 and 3.1) form a V-shaped structure near the opening of the upstream DNA binding channel of the active site cleft. The C-terminal domain of (region 4) is located in the vicinity of the RNA exit channel outlet, at a distance of ~57 Å from the N-terminal domains. The extended linker domain (region 3.2) forms a hairpin protruding into the active site cleft, and then stretches through the RNA exit channel to connect the N- and C-terminal domains. The holoenzyme structure allows us to identify the structural changes in the core induced by binding, and to correlate them with the known functional properties of the holoenzyme that are biologically relevant. The holoenzyme structure provides insight into all the steps of transcription initiation: formation of the closed promoter complex; DNA melting and formation of the open complex; RNAP translocation along the DNA; promoter escape and dissociation from the core.

Keywords: RNA POLYMERASE CRYSTAL STRUCTURE