CARBOHYDRATE RECOGNITION BY α-AMYLASE ISOZYMES: THEIR CRYSTAL STRUCTURES REVEAL NEW BINDING SITES AND REMARKABLE FUNCTIONAL DIFFERENCES

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KEYWORDS: GLYCOSIDE-HYDROLASE, PROTEIN/SUGAR INTERACTIONS, CRYSTAL STRUCTURE

THE BARLEY α-AMYLASE ISOZYMES, AMY1 AND AMY2, SHARE NEARLY 80% SEQUENCE IDENTITY BUT DISPLAY QUITE DIFFERENT PHYSICO-CHEMICAL PROPERTIES. THE CRYSTAL STRUCTURES OF BOTH AMY2 AND AMY1 WERE ESTABLISHED IN THE NATIVE STATE, BUT ALSO IN COMPLEX WITH VARIOUS INHIBITORS (LIKE THE DIABETIC DRUG ACARBOSE) AND SUBSTRATE ANALOGUES, AS FOR AMY2 WITH THE ENDOGENOUS BIFUNCTIONAL BARLEY α-AMYLASE/SUBTILISIN PROTEIN INHIBITOR BASI WHICH ONLY INHIBITS AMY2. REMARKABLY, THESE ISOZYMES HAVE VERY SIMILAR 3D STRUCTURES BUT BEHAVE DIFFERENTLY WHEN INTERACTING WITH SUBSTRATES AND INHIBITORS. A SUBTLE, BUT VERY SIGNIFICANT CONFORMATIONAL CHANGE OCCURS WHEN SUGARS BIND, LEADING TO THE IDENTIFICATION OF AN ADDITIONAL SUGAR RECOGNITION SITE IN THE C-TERMINAL DOMAIN OF AMY1 NAMED "THE PAIR OF SUGAR TONGS", NOT PRESENT IN AMY2 (ROBERT *ET AL.*(2003) *STRUCTURE*). A CRUCIAL ROLE IS OBSERVED FOR A TYROSINE CONTRIBUTING TO CAPTURE THE POLYSACCHARIDE. CHANGES IN THE IMMEDIATE VICINITY OF THIS KEY TYROSINE TOGETHER WITH DIFFERENCES IN THE ELECTROSTATIC SURFACE POTENTIAL CONFER TO THE C-TERMINAL DOMAIN A NEW FUNCTION AS A CARBOHYDRATE-BINDING MODULE. INTERESTINGLY, THIS EXTRA CARBOHYDRATE SITE IN AMY1 IS CONSISTENT WITH AN INCREASED AFFINITY (COMPARED TO AMY2) WHEN ACTING ON STARCH GRANULES. THIS SITE IS ALSO CONFIRMED BY THE CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN THE INACTIVE MUTATED ENZYME AND A TRUE SUBSTRATE, MALTOHEPTAOSE. IN CONTRAST TO THE ACTIVE SITE, THE "SUGAR TONGS" REGION REVEALS THE CIRCULARIZATION OF THE SUGAR SUBSTRATE, PROBABLY CONTROLLED BY THE KEY TYROSINE SIDE-CHAIN. MOREOVER, WE VERY RECENTLY HAVE ESTABLISHED THE FIRST 3D-STRUCTURES OF AN α–AMYLASE (AMY1) IN COMPLEX WITH A CYCLODEXTRIN, THIS LIGAND BINDING ONLY AT THE NEW "SUGAR TONGS" SITE. THEREFORE WE PROPOSE THAT THE C-TERMINAL DOMAIN, UNTIL NOW FUNCTION-LESS APPEARS, WITH THE NEW CARBOHYDRATE SITE, TO CONTRIBUTE TO THE FINE-TUNING OF THE ENZYME/POLYSACCHARIDE INTERACTIONS AND TO BE A MAJOR DETERMINANT FOR THE BARLEY α–AMYLASE ISOZYME SPECIFICITY, AND EVENTUALLY FOR OTHER ENZYMES THAT WORK ON α-LINKED GLUCOPYRANOSE-DERIVED SUBSTRATES.