Characterization of the Carbonic Anhydrase Isozymes of *Zea mays*

Thesis submitted by

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Abstract

In maize, CA catalyzes the first reaction in the C_4 photosynthetic pathway, hydrating carbon dioxide that has diffused into the mesophyll cell cytoplasm to bicarbonate, providing an inorganic carbon source for the C_4 pathway. The beta-CA isozymes from maize, as well as other agronomically important C_4 crops such as sorghum and sugarcane, differ significantly from other reported forms of the enzyme and have remained relatively uncharacterized.

The mRNA transcripts encoding the CA isozymes contain repeating sequences of approximately 600 bp that encode multiple protein domains (Repeat A, Repeat B and Repeat C). In maize, three cDNA sequences had been determined and designated CA1, CA2 and CA3. There are at least three genes in the maize genome, and one of these encodes two identical protein domains, with distinct groups of exons corresponding to the repeating regions of the transcript. The first exon of the CA2 gene encodes a putative chloroplast transit peptide, indicating an additional non-photosynthetic role for CA in maize, such as in lipid biosynthesis pathways and/or replenishing the Krebs cycle intermediates together with PEP carboxylase. This is supported by the identification of CA transcripts in root tissue and analysis of the gene sequence, which identified promoter elements that direct constitutive expression.

The expression of a single repeat region of the transcript produced active enzyme, able to catalyze the reversible hydration of carbon dioxide to bicarbonate producing hydrogen ions. The carbon dioxide hydration activity of Repeat B was relatively high compared to the activity of either Repeat A or C. Repeat B was also found to be a dimer and is composed primarily of alpha-helices, in agreement with that observed for other plant CAs. The active site of the individual protein domains, Repeat A, Repeat B and Repeat C was identified and found to contain the conserved amino acids proposed to coordinate the catalytic zinc ion and act as a proton acceptor during regeneration of the active enzyme complex.

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Abbreviations

× *g*	times gravity
°C	degrees Celsius
A_{260}	absorbance at 260 nm
aa	amino acid
ABA	abscisic acid
ABRE	ABA-responsive element
ARE	anaerobic responsive element
ATP	adenosine-5′-triphosphate
BLAST	basic local alignment search tool
bp	base pair
BSA	bovine serum albumin
CA	carbonic anhydrase
CA-RP	carbonic anhydrase-related protein
CCM	carbon concentrating mechanism
CD	circular dichroism
cDNA	complementary deoxyribonucleic acid
cm	centimetre
dCTP	deoxycytidine triphosphate
DEAE	diethylaminoethyl
dicot	dicotyledon
DRE	dehydration-responsive element
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
Dof	DNA-binding with one finger
dpm	disintegrations per minute
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immuno-sorbent assay
EREBP	ethylene-responsive element binding protein
EXAFS	extended X-ray absorption fine structure
Fig.	figure
g	gram
GST	glutathione *S*-transferase
h	hour
HRP	horse radish peroxidase
IgG	Immunoglobulin G
IPTG	isopropyl-β-D-thiogalactopyranoside
IMAC	immobilized metal affinity chromatography
kb	kilo base pair
k_{cat}	catalytic rate of an enzyme
K_d	dissociation constant
K_{dist}	distribution coefficient
kDa	kilodaltons
K_m	Michaelis-Menten constant
L	litre
LB	Luria Broth

LDH	lactate dehydrogenase
M	molar
MDH	malate dehydrogenase
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
monocot	monocotyledon
mRNA	messenger ribonucleic acid
Mw	molecular weight
n	number
NAD	nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
NIP	nearly identical paralog
ng	nanogram
nm	nanometre
ocs	octopine synthase
OD	optical density
ORF	open reading frame
p	probability
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEP	phospho*enol*pyruvate
PEP-CK	PEP carboxykinase
pfu	plaque forming unit
pH	$-\log_{10}[H^+]$
pI	isoelectric point
pmol	picomole
PPDK	pyruvate orthophosphate dikinase
PR gene	pathogenesis-related gene
PS II	Photosystem II
PVDF	polyvinylidene fluoride
RA	Repeat A
RACE	rapid amplification of cDNA ends
RB	Repeat B
RC	Repeat C
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal RNA
RT-PCR	reverse-transcriptase PCR
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	ribulose-1,5-bisphosphate
s	second
SA	salicylic acid
SABP3	salicylic acid-binding protein 3
SAP	shrimp alkaline phosphatase
SDS	sodium dodecyl sulphate

snRNPs	small nuclear ribonucleoproteins
TCA	trichloroacetic acid
TE	10 mM Tris-HCl pH 7.5, 1 mM EDTA
Tris	tris (hydroxymethyl) aminomethane
µg	microgram
µl	microliter
µm	micrometer
µM	micromolar
µmol	micromole
UTR	untranslated region
UV	ultra violet light
V	volts
v_e	volume of the elution peak height
v_o	void volume
V_{max}	maximum reaction rate
vol (or) v	volume
WOC	water-oxidizing complex
w	weight
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

Publications

Tems, U. and Burnell, J. "Carbonic Anhydrase Isozymes from *Zea mays*." Poster. Combio 2006 Combined Conference (ASBMB, ASPS, AuPS, ANZSCDB, NZSBMB and NZSPP), Brisbane, Australia.

Tems, U. and Burnell, J. (2009) "The structure of the maize β-carbonic anhydrase gene contains two repeat regions with expression of a single repeat region producing an active enzyme." *(Manuscript in preparation)*.