REGULATION OF SULFUR ASSIMILATION IN ONION (*ALLIUM CEPA* L.)

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ABSTRACT

Onion (Allium cepa L.) is an example of a species that accumulates very high levels of reduced sulfur (S)-containing compounds, particularly in the bulb as alk(en)yl-L-cysteine-sulfoxides (ACSOs) and it is these compounds, or their derivatives, that confers the distinct odour and pungent flavour.

In common with higher plants, the S assimilation pathway in onion begins with the activation of uptaken sulfate (SO_4^{2-}) to 5'-adenylylsulfate (APS), a reaction catalysed by ATP sulfurylase (ATPS; EC 2.7.7.4). Then, APS is reduced to sulfide (S^{2-}) in a two-step process catalysed by the enzymes APS reductase (APSR; EC 1.8.4.9) and sulfite reductase (SiR; EC 1.8.7.1). To complete the reductive assimilation pathway, S^{2-} is incorporated into the amino acid skeleton of *O*-acetylserine (*O*AS) to form cysteine, and this reaction is catalyzed by *O*AS (thiol)-lyase (*O*AS-TL; EC 4.2.99.8). While the regulation of the pathway is quite well defined in the plant model *Arabidopsis*, much less is known about its regulation in S accumulating species such as onion.
The primary aim of this thesis, therefore, was to characterise the enzymes of the S assimilation pathway in onion, with a particular emphasis on ATPS. As part of this charaterisation two genotypes of onion were compared. These comprised a mild genotype, 'Texas Grano 438' (TG) with a lower level of S-containing compounds in the bulb tissues, and 'W202A' (W), a cultivar with a higher level of S containing compounds in the bulb tissues. As well, comparisons were made between seedlings (typically harvested at 7 weeks) and plants at a designated mature stage (at bulbing; typically after 4 months growth), and for plants grown in S-sufficient (S^+) media or S-deficient (S^-) media, as appropriate.

In terms of plant growth, S-deprivation generally had a negative influence for both genotypes, with significant reductions in total biomass (measured as fresh weight) for TG at both the seedlings and mature stages. ATPS activity and accumulation were shown to be present in all tissues examined (leaf, root, bulb) as well as the chloroplasts, with highest activity measured in the roots, particularly in seedlings. ATPS activity and accumulation were also compared between the two genotypes (TG and W) with ATPS activity and accumulation higher in W, particularly at the seedling stage.

In terms of the influence of S supply, in general higher ATPS activity was measured in chloroplast, leaf and root extracts from plants of both genotypes grown in the S- media, at the seedling stage. In roots of mature plants of both genotypes, a significant increase in activity was measured in response to S-deprivation, while in chloroplasts isolated from mature plants of both genotypes, highest activity was measure in those grown in the S+ media. Finally diurnal variations were observed in chloroplast, leaf and root extracts of both genotypes with a general trend of an increase in ATPS activity and accumulation a few hours after illumination and upon the onset of the dark period.

Although a single gene coding for ATPS is presumed to be present in onion, the enzyme was characterized as two electrophoretic forms using 1D-PAGE during western analyses following fractionation of chloroplasts by anion exchange chromatography and also as an alignment of spots using 2D-PAGE. As protease inhibitors were routinely included in the extraction buffers, these forms suggest the occurrence of ATPS isoforms that may arise as a consequence of post-translational modifications.

The regulation of ATPS by one mechanism of post-translational modification, phosphorylation, was therefore investigated using several techniques including the detection of a shift in molecular mass, a change in enzyme activity or pI (as determined by 2D-PAGE) and the capability to bind to 14-3-3 proteins using affinity chromatography. Following treatments of chloroplast extracts to promote either the phosphorylation (P^+) or the dephosphorylation (P^-) of proteins, no molecular mass change or change in activity was observed. However, after fractionation by 2D-PAGE, differences in the spot alignment of ATPS were visualized, suggesting that ATPS is a phosphoprotein. The enzyme was detected in pull-downs after affinity chromatography, suggesting that ATPS may also interact with 14-3-3 proteins (although this needs to be confirmed unequivocally). A model is advanced, therefore, in which upon phosphorylation, no variation in ATPS activity occurs but a change in the surface charged and possibly a change in conformation of the protein does occur to make the enzyme competent to interact with 14-3-3 proteins.

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1D-PAGE	one dimensional polyacrylamide gel electrophoresis
2D-PAGE	two dimensional polyacrylamide gel electrophoresis
AAP	antartic alkaline phosphatase
Abs	absorbance
ACSO	alk(en)yl-$_L$-cysteine-sulfoxide
Amp	ampicillin
ANOVA	analysis of variance
Arabidopsis	*Arabidopsis thaliana*
APS	5'-adenylylsulfate
APSR	5'-adenylylsulfate reductase
ATP	adenosine 5'-triphosphate
ATPS	ATP sulfurylase
APS	adosine 5'phosphosulfate
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BMH1	14-3-3 protein from *S. cerevisiae*, isoform 1
BMH2	14-3-3 protein from *S. cerevisiae*, isoform 2
bp	base pair
BSA	bovine serum albumin
C	cross-linker
°C	degree Celsius
CBB	Coomassie Brilliant blue
cDNA	complementary DNA
CHAPS	3-[(3-cholamidopropyl)dimethyl-ammonio]&1-propanesulfonate
CK2	casein kinase II
cm	centimetre
CNBr	cyanogen bromide
CS	cysteine synthase
D+x h	dark period after x hours of darkness
dH_2O	distilled water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol

EC	enzyme commission
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetraacetic acid
EGTA	ethylene glycol-bis(*β*-aminoethyl ether) *N,N,N',N'*-tetraacetic acid
EtOH	ethanol
F2KP	fructose-2,6-biphosphatase
FAOSTAT	Food and Agriculture Organization of the United Nations – statistic data
FPLC	fast protein liquid chromatography
g	g-force
g	gram
GS	glutamine synthase
GSH	glutathione
h	hours
IEF	isoelectric focusing
IMAC	ion metal affinity chromatography
IPG	immobilized pH gradient
IPTG	isopropyl-β-$_D$-thioglucopyranoside
kDa	kiloDaltons
kPa	kilo Pascal
L	litre
L+ x h	light after x hours of illumination
LB	Luria-Bertani
m	meter
M	mole L^{-1}
meOH	methanol
meq	milliequivalent
mg	milligram
MgATP	magnesium ATP
min	minute
mL	millilitre
mm	millimeter
mM	millimolar
MW	molecular weight
Na	sodium

NAD^+	β-nicotidamide adenine dinucleotide
NADH	nicotidamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
$Na_4P_2O_7$	sodium pyrophosphate
NaCl	sodium chloride
NaF	sodium fluoride
NBT	nitrotetrazolium blue chloride
NCBI	National Center for Biotechnology Information
nm	nanometer
NOA	National Onion Association of the United States
NR	nitrate reductase
*O*AS	*O*-acetylserine
*O*AS-TL	*O*-acetylserine thiol lyase
OKA	okadaic acid
p	probability
P^+	treatment for protein phosphorylation
P^-	treatment for protein dephosphorylation
PAGE	polyacrylamide gel electrophoresis
PALP	pyridoxal phosphate dependent enzyme
PAPS	3'-phospho-5'-adenylylsulfate
PBS	phosphate buffered saline
PBST	PBS-Tween 20
PEG_{8000}	polyethylene glycol, MW 8000
pI	isoelectric point
pH	potential of hydrogen
PMSF	phenylmethyl sulphonyl fluoride
PP1	protein phosphatase, type 1
PP2A	protein phosphatase, type 2A
PP2B	protein phosphatase, type 2B
PP2C	protein phosphatase, type C
PPi	inorganic phosphate
Pro-Q DPS	ProQ Diamond phosphoprotein staining
PTPase	protein tyrosine phosphatase
PVDF	polyvinylidene difluoride

RO	reverse osmosis
rpm	rotation per minute
RuBisCO	ribulose-1,5-biphosphate carboxylase oxygenase
S	sulfur
S^+	high level of sulfur
S^-	low level of sulfur
SAT	serine acetyltransferase
S. cerevisiae	*Saccharomyces cerevisiae*
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	second
SiR	sulfite reductase
SO_4^{2-}	sulfate
SPS	sucrose phosphate synthase
T	total monomer
TCA	trichloroacetic acid
TEMED	*N,N,N',N'*-tetramethylethylenediamine
TG	'Texas Grano 438'
Tris	tris(hydroxymethyl)aminomethane
Tween-20	poly(oxyethylene) sorbitane-monolaurate
U	unit
µA	microampere
µg	microgramme
µL	microlitre
µmol	micromole
v	volume
V	volt
v/v	volume to volume ratio
Vhrs	volt hours
w	weight
W	'W202A'
w/v	weight by volume ratio
WHO	world health organization

ABBREVIATIONS FOR AMINO ACIDS

Amino acid	Three-letter abbreviation	One letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V