Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
REGULATION OF SULFUR ASSIMILATION IN ONION (ALLIUM CEPA L.)

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Physiology and Biochemistry at Massey University, Palmerston North, New Zealand.

Ludivine A. Thomas

2008
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 uptake sulfate (SO\textsubscript{4}\textsuperscript{2-}) to 5'-adenylylsulfate (APS), a reaction catalysed by ATP sulfurylase (ATPS; EC 2.7.7.4). Then, APS is reduced to sulfide (S\textsubscript{2} \textsuperscript{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\textsubscript{2} \textsuperscript{2-} is incorporated into the amino acid skeleton of O-acetylserine (OAS) to form cysteine, and this reaction is catalyzed by OAS (thiol)-lyase (OAS-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 characterisation 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\textsuperscript{+}) media or S-deficient (S\textsuperscript{-}) 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 pl (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.
Acknowledgements

Firstly I would like to send my gratitude to Michael McManus and John McCallum, who trusted me and encouraged me during my PhD, even though my background was not much into biochemistry and protein science.

I would like to acknowledge Crop & Food for providing the PhD scholarship and thus keeping my brain (more or less) functioning. Also I thank IMBS and the NZSPB for their financial support that I used for conferences. It allowed me to learn so much about science and meet very great scientists.

Sarah, thank you so much for being present and, for all your advices and support. You helped me so much, especially at the last minute… as usual! I had so much fun with you. I wish we had more time together, out of the lab. I really do hope you will be successful in the next part of your life!

I would like to thank Susanna, who taught me so much about lab work. I thank also all my labmates, past and present for their support.

Jean-Marc, thank you so much for your everyday support. I could not do it without you. I am so happy that you encourage me to continue and that you trusted me. I am sure also that the next chapter is going to be great.
# Table of Contents

**Abstract** ................................................................. II

**Acknowledgements** .................................................. IV

**Table of Contents** .................................................. V

**List of Figures** ..................................................... IX

**List of Tables** ........................................................ XIII

**List of Abbreviations** ................................................ XIV

**Abbreviations for Amino Acids** ................................... XVIII

1. Introduction ............................................................. 1
   Introduction to the biology of onion .................................. 1
   Sulfur: an essential nutrient in plants .............................. 3

1.2.1. Sulfur assimilation and transport in plants .................. 4
   1.2.1.1. Reductive assimilation pathway ............................... 5
   1.2.1.2. ATP sulfurylase ................................................. 8
   1.2.1.3. APS reductase ..................................................... 11
   1.2.1.4. Sulfite reductase .............................................. 13
   1.2.1.5. Cysteine synthase complex ..................................... 15

1.2.2. Sulfur deprivation in plants .................................... 17

1.3. Light and dark regulation .......................................... 19
   1.3.1. Photoperiodism .................................................... 19
   1.3.2. Circadian rhythms ............................................... 19

1.4. Phosphorylation – dephosphorylation ................................ 21
   1.4.1. Introduction ...................................................... 21
   1.4.2. Protein kinases ..................................................... 23
   1.4.3. Protein phosphatases ............................................. 23
   1.4.4. Regulation by phosphorylation in plants ..................... 24

1.5. 14-3-3 proteins ....................................................... 25

1.6. Studies on S assimilation in onion .................................. 26

1.7. Aims of the project .................................................. 28

2. Material and Methods .................................................. 30
   2.1. Propagation and harvesting methods ............................ 30
      2.1.1. Plant material .................................................. 30
      2.1.2. Plant growth conditions ...................................... 30
      2.1.3. Plant propagation .............................................. 30
      2.1.4. Plant harvest ................................................... 32

2.2. Physiological methods ............................................... 33
   2.2.1. Fresh weight measurement ...................................... 33
2.3. Biochemical methods ............................................................ 33
  2.3.1. Chemicals ........................................................................ 33
  2.3.2. Extraction of proteins ...................................................... 34
    2.3.2.1. Isolation of intact chloroplasts .................................. 34
      2.3.2.1.1. Extraction under light ..................................... 34
      2.3.2.1.2. Extraction in the dark .................................. 35
      2.3.2.1.3. Chloroplast lysis ....................................... 35
    2.3.2.2. Preparation of tissue extracts .................................. 35
    2.3.2.3. Concentration of proteins by acetone precipitation ....... 36
  2.3.3. Protein purification by chromatography ............................ 36
    2.3.3.1. Gel filtration chromatography using Sephadex G-25 ......... 36
    2.3.3.2. Anion exchange chromatography connected to the Fast Protein Liquid Chromatography (FPLC) system .................... 36
    2.3.3.3. Ion metal affinity chromatography (IMAC) using Ni\textsuperscript{2+} -nitritotriacetic acid-agarose connected to FPLC system ........ 37
  2.3.4. Quantification of proteins ................................................ 37
  2.3.5. ATPS analysis ............................................................ 38
    2.3.5.1. Measurement of ATPS activity .................................. 38
    2.3.5.2. ATPS activity determination .................................... 39
  2.3.6. Separation of proteins by one-dimensional polyacrylamide gel electrophoresis (1D-PAGE) ................................................. 40
    2.3.6.1. Sample preparation ................................................ 40
    2.3.6.2. Linear Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) ......................................................... 40
    2.3.6.3. Gradient-PAGE .................................................. 41
  2.3.7. Separation of proteins by two-dimensional electrophoresis (2D-PAGE) ............................................................... 43
    2.3.7.1. Sample preparation ................................................ 43
      2.3.7.1.1. Chloroplast extracts ..................................... 43
      2.3.7.1.2. Leaf extracts ........................................... 43
    2.3.7.2. Isoelectric focusing (IEF) ....................................... 44
    2.3.7.3. Equilibration .................................................... 44
    2.3.7.4. Second dimension by SDS-PAGE .................................. 45
  2.3.8. Detection of proteins by western analysis ........................ 45
    2.3.8.1. Transfer of proteins from polyacrylamide gel to PVDF membranes ................................................................. 45
    2.3.8.2. Probing detection using antibodies on PVDF membranes ..... 46
    2.3.8.3. Immunodetection of proteins immobilised on PVDF membrane ................................................................. 47
      2.3.8.3.1. Chromogenic detection ..................................... 47
      2.3.8.3.2. Chemiluminescence ....................................... 47
  2.3.9. Detection of proteins by gel staining .................................. 48
    2.3.9.1. Coomassie Brilliant blue (CBB) staining ....................... 48
    2.3.9.2. Pro-Q Diamond phosphoprotein staining (Pro-Q DPS) ......... 48
    2.3.9.3. Silver staining ................................................ 49
  2.3.10. Phosphorylation and dephosphorylation treatments ............. 49
    2.3.10.1. Extraction ..................................................... 49
    2.3.10.2. Incubation to preserve or alter the phosphorylation state ...... 49
    2.3.10.3. Affinity chromatography of treated chloroplast extracts through BMH1- and BMH2-Sepharose ........................................ 51
      2.3.10.3.1. Coupling proteins to activated CNBr Sepharose ......... 51

VI
2.3.1.0.3.2. Affinity chromatography of chloroplasts ........................................ 51

2.4. Molecular methods ................................................................. 53
2.4.1. Bacterial culture and recombinant protein production ................................ 53
2.4.1.1. Preparation of Luria-Bertani medium with ampicillin ................................ 53
2.4.1.2. Transformation of E. coli competent cells ........................................... 53
2.4.1.2.1. Preparation of competent cells ...................................................... 53
2.4.1.2.2. Transformation of E. coli/DHSα vector using the heat-shock method ............ 53
2.4.1.2.3. Preparation of glycerol stock .......................................................... 54
2.4.2. Sequencing of cloned DNA in E. coli ................................................. 54
2.4.2.1. Purification of plasmid DNA for DNA sequencing .................................... 54
2.4.2.2. Automated sequencing of DNA ............................................................ 55
2.4.2.3. DNA sequence analysis ........................................................................... 55
2.4.3. Preparation of recombinant proteins ....................................................... 56
2.5. Statistical analyses .............................................................................. 56

3. Characterisation of the S assimilation enzymes and their response to S supply .................. 57
3.1. Influence of S starvation on onion growth and development ................................ 57
3.1.1. Changes in fresh weight of plants and tissues in response to S supply .............. 57
3.1.2. Morphological analysis ............................................................................ 60
3.2. Regulation of the S assimilation enzymes in response to S supply .......................... 63
3.2.1. Variations in ATPS activity ........................................................................ 63
3.2.2. Abundance of the S assimilation enzymes and the influence of S supply .......... 67
3.2.2.1. ATPS abundance .................................................................................. 67
3.2.2.2. APSR abundance .................................................................................. 69
3.3. Light and dark regulation of the S assimilation enzymes ....................................... 73
3.3.1. Changes in ATPS activity over a 24 h period .............................................. 73
3.3.2. Changes in the abundance of the S assimilation enzymes ............................... 75
3.3.2.1. ATPS abundance .................................................................................. 75
3.3.2.2. APSR abundance .................................................................................. 76
3.4. Detection of ATPS isoforms and the influence of S supply .................................... 78
3.4.1. Detection by anion exchange chromatography ............................................. 78
3.4.1.1. Detection of protein in separated chloroplast extracts ................................... 78
3.4.1.2. Detection of ATPS activity in separated chloroplast extracts and the influence of S supply .......................................................................................................................... 81
3.4.1.3. Detection of the abundance of the S assimilation enzymes in separated chloroplast extracts .......................................................................................................................... 81
3.4.1.3.1. ATPS abundance .................................................................................. 81
3.4.1.3.2. APSR abundance .................................................................................. 85
3.4.2. Detection by 2D-PAGE ............................................................................. 89
4. Regulation of ATPS Activity and Accumulation by Phosphorylation ................................................................. 91

4.1. Detection of ATPS phosphorylation .......................................................... 91

4.1.1. Approaches for the detection of ATPS phosphorylation ....................... 91

4.1.2. Bio-informatic analysis of ATPS and OAS-TL sequences reveals putative phosphorylated sites ........................................ 94

4.1.3. Fluorescence based identification of phosphoproteins using 1D-PAGE 95

4.1.4. Detection of phosphatase activity ....................................................... 97

4.1.5. Assessment of phosphorylation of the S assimilation enzymes by mobility shift ......................................................... 99

4.1.6. Identification of phosphorylation by changes in pI ................................ 101

4.1.7. Assessing the influence of phosphorylation and dephosphorylation on ATPS enzyme activity ................................................. 104

4.2. Detection of ATPS binding to 14-3-3 proteins ....................................... 109

4.2.1. Analysis of ATPS and OAS-TL sequences reveal 14-3-3 binding motifs ................................................................. 109

4.2.2. Production of the recombinant 14-3-3 proteins BMH1 and BMH2 from Saccharomyces cerevisiae in E. coli ........................................ 113

4.2.3. Production and purification of recombinant BMH1 and BMH2 ......... 113

4.2.4. Detection of 14-3-3 proteins within onion extracts ............................ 116

4.2.5. Fluorescence-based analysis of phosphoproteins from chloroplast extracts binding to 14-3-3 proteins ........................................ 118

4.2.6. Detection of complex between S assimilation enzymes and 14-3-3 proteins by pull-down ..................................................... 120

5. Discussion .............................................................................................. 128

5.1. Influence of the S supply on onion growth and development ................. 128

5.2. Characterisation of ATPS in the two onion genotypes ......................... 130

5.3. Regulation of APSR abundance by sulfur supply ................................. 139

5.4. Phosphorylation and binding to 14-3-3 proteins ................................ 142

5.5. Future work......................................................................................... 154

Appendices .............................................................................................. 156

References ............................................................................................... 184
LIST OF FIGURES

Figure 1.1. Overview of sulfur uptake, reduction, and transport in higher plants from Crawford et al. (2000) ................................................................. 7

Figure 1.2. Reaction steps in APS reduction by APSR ........................................ 12

Figure 1.3. Phosphorylation/Dephosphorylation of enzymes ................................ 22

Figure 2.1. Arrangement of plants in pots in hydroponic culture ................................ 31

Figure 2.2. Plant position in a pot for hydroponic culture and indication of tissues used for plant growth analysis .......................................................... 32

Figure 2.3. Emission spectrum of the green light used during dark harvest and chloroplast extractions in the dark .......................................................... 33

Figure 2.4. Intact chloroplast extracts visualized under light microscope .................. 35

Figure 2.5. BSA standard curve ........................................................................... 38

Figure 2.6. The coupled reaction for the measurement of the reverse reaction of ATPS ................................................................. 39

Figure 2.7. Formula to calculate the specific activity of ATPS ......................... 40

Figure 2.8. Diagrammetric representation of the pouring system of a linear gradient SDS-PAGE ................................................................. 42

Figure 2.9. Gel and membrane assembly for the electrophoretic transfer ............. 46

Figure 3.1. Influence of the S supply on the total biomass and tissue weights, as indicated, in seedlings of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ................................................. 59

Figure 3.2. Influence of the S supply on the total biomass and tissue weights, as indicated, in mature plants of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ................................................. 59

Figure 3.3. Influence of the S supply on the plant morphology of seedlings and mature plants, as indicated, of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ................................................. 61

Figure 3.4. Influence of the S supply on the root morphology of mature plants of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ................................................. 62
Figure 3.5. Influence of the S supply on the ATPS activity in chloroplasts or the tissues, as indicated, from seedlings of TG and W, as indicated, grown in S-sufficient (S⁺) or S-deficient (S⁻) media, as indicated........................................... 66

Figure 3.6. Influence of the S supply on the ATPS activity in chloroplasts or the tissues, as indicated, from mature plants of TG and W, as indicated, grown in S-sufficient (S⁺) or S-deficient (S⁻) media, as indicated........................................... 66

Figure 3.7. Influence of the S supply on the accumulation of ATPS and APSR in chloroplasts or the tissues, as indicated, from seedlings of TG and W, as indicated, grown in either the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated... 71

Figure 3.8. Influence of S supply on the accumulation of ATPS and APSR in chloroplasts or the tissues, as indicated, from mature plants of TG and W, as indicated, grown in either the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated... 71

Figure 3.9. Influence of the S supply on the accumulation of ATPS and APSR in chloroplasts from seedlings or mature plants, as indicated, of TG and W, as indicated, grown in either the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated... 72

Figure 3.10. Influence of light and dark on the ATPS activity in chloroplasts or the tissues, as indicated, seedlings TG and W, as indicated, grown in the S-sufficient media........................................... 74

Figure 3.11. Influence of light and dark on the accumulation of ATPS isoforms in chloroplast or leaf extracts, as indicated, in seedlings of TG or W, as indicated, grown in the S-sufficient media........................................... 77

Figure 3.12. Comparison of elution patterns after separation of proteins from chloroplast extracts from seedlings of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated........................................... 80

Figure 3.13. Comparison of elution patterns after separation of proteins from chloroplast extracts from mature plants of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated........................................... 80

Figure 3.14. Comparison of ATPS activity and accumulation after the separation by anion exchange chromatography of proteins from chloroplast extracts from seedlings of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated........................................... 83

Figure 3.15. Comparison of ATPS activity and accumulation after the separation by anion exchange chromatography of proteins from chloroplast extracts from mature plants of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated........................................... 84

Figure 3.16. Detection of the accumulation of APSR after the separation by anion exchange chromatography of proteins from chloroplast extracts of seedlings of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated........................................... 87
Figure 3.17. Detection of the accumulation of APSR after the separation by anion exchange chromatography of proteins from chloroplast extracts of mature plants TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ........................................... 87

Figure 3.18. Comparison of the accumulation of ATPS after separation by 2D-PAGE of proteins from chloroplasts of seedlings and mature plants, as indicated, of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ........................................................... ..................... 90

Figure 4.1. Schematic overview of sample preparation steps, analytical techniques and analysis employed for detection of phosphorylation and interactions with 14-3-3 proteins ................................................................................ 93

Figure 4.2. Influence of the phosphorylation (P⁺) and the dephosphorylation (P⁻) treatments, as indicated, on phosphoprotein accumulation in chloroplast and leaf extracts, as indicated, from mature plants of W grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated, separated by 1D-PAGE ........................................ 96

Figure 4.3. Influence of the phosphorylation (P⁺) and the dephosphorylation (P⁻) treatments, as indicated, on albumin, separated by 1D-PAGE........................................ 98

Figure 4.4. Influence of the phosphorylation (P⁺) and the dephosphorylation (P⁻) treatments, as indicated, on the molecular mass of ATPS, APSR, SiR or OAS-TL, as indicated, from chloroplast extracts of mature plants of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated, using PP2A as the phosphatase ........................................................................... 100

Figure 4.5. Influence of the phosphorylation (P⁺) and the dephosphorylation (P⁻) treatments, as indicated, on the isoelectric point of ATPS from chloroplast extracts from mature plants of W grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated .......................... 103

Figure 4.6. Influence of the phosphorylation (P⁺) and the dephosphorylation (P⁻) treatments, as indicated, on the isoelectric point of OAS-TL from leaf extracts from mature plants of W grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ................................. 103

Figure 4.7. Influence of the phosphorylation (P⁺) and the dephosphorylation (P⁻) treatments, as indicated, on ATPS activity in chloroplast extracts from mature plants of TG and W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated .......................................................... 107

Figure 4.8. Influence of the phosphorylation (P⁺) or the dephosphorylation (P⁻) treatments, as indicated, on ATPS activity in chloroplast extracts from seedlings and mature plants, as seedlings, of TG and W, as indicated grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated ......................................................... 108

Figure 4.9. Consensus 14-3-3 binding sites in the translated sequences of the ATPS gene sequences indicated ........................................................................... 110
Figure 4.10. Plot of the predicted surface accessibility at each residue of the translated ATPS sequence of onion.

Figure 4.11. Plot of the known domains, the predicted 14-3-3 site and the predicted surface accessibility at each residue of the translated OAS-TL sequence from onion.

Figure 4.12. Detection of total proteins after IMAC purification of BMH1 and BMH2 recombinant proteins, as indicated, synthesized in E. coli.

Figure 4.13. Detection of 14-3-3 proteins in extracts from seedlings of TG and W, as indicated, grown in S-sufficient media.

Figure 4.14. Detection of 14-3-3 proteins in extracts from mature plants of TG and W, as indicated, grown in S-sufficient (S+) or S-deficient (S-) media, as indicated.

Figure 4.15. Detection of proteins after purification of chloroplast extracts from mature plants of W grown in the S-sufficient (S+) or the S-deficient (S-) media, as indicated, with 14-3-3-Sepharose or BSA-Sepharose, as indicated.

Figure 4.16. Detection of ATPS, APSR, SiR or OAS-TL, as indicated, in chloroplast extracts from seedlings of W grown in the S-sufficient (S+) or the S-deficient (S-) media, as indicated, previously treated to promote phosphorylation (P+) or dephosphorylation (P-) and purified by affinity chromatography with 14-3-3-Sepharose.

Figure 4.17. Detection of ATPS in chloroplast extracts from seedlings or mature plants, as indicated, of TG or W, as indicated, grown in the S-sufficient (S+) or the S-deficient (S-) media, as indicated, previously treated to promote phosphorylation and purified by affinity chromatography with 14-3-3-Sepharose or BSA-Sepharose, as indicated.

Figure 4.18. Detection of APSR in chloroplast extracts from seedlings or mature plants, as indicated, of TG or W, as indicated, grown in the S-sufficient (S+) or the S-deficient (S-) media, as indicated, previously treated to promote phosphorylation and purified by affinity chromatography with 14-3-3-Sepharose or BSA-Sepharose, as indicated.

Figure 4.19. Detection of SiR in chloroplast extracts from seedlings or mature plants, as indicated, of TG or W, as indicated, grown in the S-sufficient (S+) or the S-deficient (S-) media, as indicated, previously treated to promote phosphorylation and purified by affinity chromatography with 14-3-3-Sepharose or BSA-Sepharose, as indicated.

Figure 4.20. Detection of ATPS, OAS-TL, APSR or SiR in leaf extracts from seedlings of W grown in the S-sufficient (S+) or the S-deficient (S-) media, as indicated, previously treated to promote phosphorylation and purified by affinity chromatography with 14-3-3-Sepharose of BSA-Sepharose, as indicated.

Figure 5.1. Regulation of ATPS by phosphorylation and subsequent 14-3-3 protein binding in chloroplasts from onion grown in the sulphur sufficient media.
**LIST OF TABLES**

**Table 2.1.** Composition of the S-sufficient (S⁺) and S-deficient (S⁻) hydroponic media.................................................................................................................. 31

**Table 2.2.** Composition of the heavy and light solutions for the Percoll gradient. 34

**Table 2.3.** Composition of 12% Resolving and 4% Stacking gels for 1D-PAGE. 41

**Table 2.4.** Composition of resolving and stacking gels for gradient-PAGE (8-15%). .................................................................................................................. 42

**Table 2.5.** Running conditions for protein focusing using IPG strips.............. 44

**Table 2.6.** Concentrations of primary and secondary antibodies used for western analyses ........................................................................................................ 47

**Table 2.7.** Full composition of phosphorylation and dephosphorylation treatments.............................................................................................................................. 50

**Table 2.8.** Sequences and melting points of the primers used for DNA sequencing. ................................................................. 55

**Table 3.1.** Summary of results of the detection of ATPS activity, and the accumulation of ATPS and APSR after the separation by anion exchange chromatography of proteins from chloroplast extracts of seedlings or mature plants, as indicated, of TG or W, as indicated, grown in the S-sufficient (S⁺) or the S-deficient (S⁻) media, as indicated.................................................................................................................. 88
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D-PAGE</td>
<td>one dimensional polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>2D-PAGE</td>
<td>two dimensional polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>AAP</td>
<td>antarctic alkaline phosphatase</td>
</tr>
<tr>
<td>Abs</td>
<td>absorbance</td>
</tr>
<tr>
<td>ACSO</td>
<td>alk(en)yl-1-cysteine-sulfoxide</td>
</tr>
<tr>
<td>Amp</td>
<td>ampicillin</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>Arabidopsis thaliana</td>
</tr>
<tr>
<td>APS</td>
<td>5'-adenylylsulfate</td>
</tr>
<tr>
<td>APSR</td>
<td>5'-adenylylsulfate reductase</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5'-triphosphate</td>
</tr>
<tr>
<td>ATPS</td>
<td>ATP sulfurylase</td>
</tr>
<tr>
<td>APS</td>
<td>adenosine 5'phosphosulfate</td>
</tr>
<tr>
<td>BCIP</td>
<td>5-bromo-4-chloro-3-indolyl phosphate</td>
</tr>
<tr>
<td>BMH1</td>
<td>14-3-3 protein from S. cerevisiae, isoform 1</td>
</tr>
<tr>
<td>BMH2</td>
<td>14-3-3 protein from S. cerevisiae, isoform 2</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>C</td>
<td>cross-linker</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>CBB</td>
<td>Coomassie Brilliant blue</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>CHAPS</td>
<td>3-[(3-cholamidopropyl)dimethyl-ammonio]&amp;1-propanesulfonate</td>
</tr>
<tr>
<td>CK2</td>
<td>casein kinase II</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CNBr</td>
<td>cyanogen bromide</td>
</tr>
<tr>
<td>CS</td>
<td>cysteine synthase</td>
</tr>
<tr>
<td>D+x h</td>
<td>dark period after x hours of darkness</td>
</tr>
<tr>
<td>dH2O</td>
<td>distilled water</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
</tbody>
</table>
EC enzyme commission
E. coli Escherichia coli
EDTA ethylenediamine tetraacetic acid
EGTA ethylene glycol-bis(\(\beta\)-aminoethyl ether) \(N,N',N'^{-}\)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-\(\beta\)-\(\text{-D}\)-thiogluco.pyranoside
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
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
$\text{SO}_4^{2-}$ 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
$\mu$A microampere
$\mu$g microgramme
$\mu$L microlitre
$\mu$mol 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

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