METABOLISM OF SULPHATE IN WHEAT

by

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TABLE OF CONTENTS

Page

Page

SUMMARY

1. ATP sulphurylase has been purified from wheat leaves and some of its properties determined. APS was the sulphonucleotide utilized by the wheat plant for subsequent reduction to the thiol level. $PAP^{35}S$ was not detected in extracts of wheat leaves even in the presence of 5'-AMP which is known to protect the sulphur nucleotide from the action of degradative enzymes.

The stimulatory effect of light on enzyme activity is in keeping with the location of the sulphate activating enzyme as well as the reducing enzymes in the chloroplasts.

The properties of ATP sulphurylase from wheat leaves are similar to those for the enzyme from other plant sources. The purified enzyme was inhibited by PCMB but not by the end products of sulphate reduction, namely L-cysteine and methionine.

2. APS sulphotransferase has been purified from wheat leaves and some of its properties determined. APS but not PAPS was the substrate utilized for the reduction of activated sulphate to $^{35}SO_3^{2-}$. The purified enzyme utilized dithioerythritol and GSH as acceptors for the transfer of SO_4^{2-} from APS.

Bound SO_3^{2-} was shown to be produced in the reductive pathway because it readily exchanged its radioactivity when carrier SO_3^{2-} was added.

The properties of the enzyme from wheat leaves are similar to those of *Chlorella*, *Rhodospirillum rubrum* and leaves of *Spinacea oleracea*. The end product of APS sulphotransferase reaction, 5'-AMP, inhibited the purified enzyme; however cysteine, the end product of sulphate reduction, did not inhibit the enzyme.

3. APS thiosulphonate reductase was purified from wheat leaves and some of its properties determined. The reduction of APS to S^{2-} which was ferredoxin-dependent in extracts of wheat leaves was detected only when GSH or dithiothreitol was included in the reaction mixture. The addition of APS-sulphotransferase and thiosulphonate reductase to the reaction mixture containing APS was essential for its reduction to S^{2-}. The protein carrier associated with thiosulphonate reductase bound the sulphonyl group of APS to it only when APS sulphotransferase was present to mediate the transfer reaction.

Bound $^{35}S^{2-}$ was the product of the reductive pathway and it readily exchanged its radioactivity with carrier sulphide. The purified enzyme reduced bound GS-SO_3H to bound GS-SH. Free sulphide was released by an exchange reaction between the bound GS-^{35}SH and the carrier sulphide.

When sulphite reductase was coupled to the APS sulphotransferase reaction, S^{2-} was not detected, proving that sulphite reductase was not the functional reductase system in wheat leaves. Sulphite reductase activity was demonstrated only when exogenous sulphite was added as a substrate to the reaction mixture.

The end product of sulphate reduction, cysteine, inhibited the enzyme. The reduction of APS to cysteine had a strict requirement for O-acetyl,L-serine as well as for the three coupled enzymes involved in

the reductive pathway, namely:- APS sulphotransferase, APS thiosulphonate reductase, and O-acetyl,L-serine sulphydrylase.

4. O-acetyl,L-serine sulphydrylase was isolated and purified from wheat leaves. The substrate for the enzyme was O-acetyl,L-serine. Methionine inhibited the enzyme competitively with respect to S^{2-} while a non-competitive inhibition was observed for L-cysteine, homoserine, PCMB, N-ethylmaleimide and 5,5'-dinitrobis-2-nitrobenzoic acid respectively.

5. An integrated scheme is proposed for the activation and reduction of sulphate in wheat leaves involving bound intermediates. Feedback inhibition by cysteine, the end product of sulphate reduction, occurred with thiosulphonate reductase and O-acetyl,L-serine sulphydrylase.