Meeting Report

PHOTORESPIRATION

Barry HALLIWELL
Department of Biochemistry, University of London, King's College, Strand, London, WC2R 2LS, England

Received 4 March 1976

A meeting of the British Photobiology Society on the subject of 'Photorespiration' was held at King's College, London, on January 6th, 1976. The following topics were discussed.

Mechanism of CO_2 release during photorespiration

Illuminated green leaves release fixed CO_2 in a light-dependent oxidation process, known as 'photorespiration'. Although most of the CO_2 arises from the carboxyl group of glycollate, some comes from the hydroxymethyl group of this compound [1].

Three reactions have been proposed to account for CO_2 release from the carboxyl group of glycollate. The first, and most widely accepted, is that glycollate is oxidised to glyoxylate by glycollate oxidase in peroxisomes, and the glyoxylate transaminated to glycine, which is then acted upon by an enzyme complex, located in the mitochondria, to give serine and CO_2. Dr A. J. Keys (Rothamsted Experimental Station) reviewed the early ^{14}C-tracer work, which showed that much carbon from glycollate is metabolised in the light to glycine and serine, and then through glycerate into sucrose. Sucrose synthesis from phosphate esters takes place only in the cytoplasm, not in the chloroplast [2–4]. Studies on carbon metabolism during photorespiration using α-hydroxy-sulphonates as inhibitors of glycollate oxidase [5] and isonicotinyl hydrazide as inhibitor of the conversion of glycine to serine [6] strongly suggest that it is the conversion of glycine to serine that is responsible for much of the CO_2 evolved in photorespiration. Studies on wheat leaves photorespiring in air containing 320 or 400 vpm CO_2 show that the amount of carbon metabolised by the glycollate pathway is as much, or more than, is concurrently assimilated ([7,8] and unpublished work) and that the CO_2 evolved can be accounted for almost entirely by the loss of glycine and formation of serine.

Dr Keys also reported that conversion of glycine to serine is coupled to ATP synthesis in leaf mitochondria [9,10], although the glycollate pathway still seems very wasteful of energy (he calculated that, if an equivalent amount of carbon to that assimilated is metabolised by the glycollate pathway, 65 ATP and 52 NAD(P)H molecules are needed per molecule of sucrose formed, whereas direct synthesis from sugar phosphates of the Calvin cycle requires only 37 ATP and 24 NAD(P)H). It was generally agreed by those present that the conversion of glycine to serine is the major reaction releasing CO_2 during photorespiration at temperatures up to 25°C.

A second mechanism which may contribute to CO_2 release from the carboxyl group of glycollate is the direct decarboxylation of glyoxylate to formate in leaf peroxisomes by a non-enzymic reaction with H_2O_2. This can occur because peroxisomal catalase is unable to break down H_2O_2 completely: an observation which has also been made in studies of animal peroxisomes [11]. Studies on leaf peroxisomes showed that this non-enzymic decarboxylation of glyoxylate can still take place when glycine synthesis is proceeding at its maximum rate [12]. However, it is thought unlikely that this non-enzymatic glyoxylate decarboxylation contributes more than 10–20% of the CO_2 released during photorespiration at 25°C [12,13]. Drs B. Grodzinski (University of Cambridge) and V. S. Butt (University of Oxford) reported studies on glycollate decarboxylation by leaf discs from spinach and spinach-beet. Between 25 and 35°C a high Q_{10}

North-Holland Publishing Company – Amsterdam

value (at least 4) is observed for this reaction in both the light and the dark. The Q_{10} value for glycine decarboxylation by leaf mitochondria is approximately 2 [14] and so it appears unlikely that this process alone can account for the increased glycollate decarboxylation. The Q_{10} of purified and peroxisomal glycollate oxidase is found to be 1.73, but that of purified and peroxisomal catalase only 1.26, and they proposed that, as temperature increases, proportionally more of the H_2O_2 produced during glycollate oxidation escapes breakdown by catalase and reacts non-enzymically with glyoxylate. Preliminary experiments supporting these conclusions have been carried out on isolated leaf peroxisomes.

It is known that the formate which might be derived from the 2-carbon of glycollate can be oxidised to CO_2 [15] (perhaps accounting for CO_2 release from C_2 of glycollate [1]) or converted into serine [16]. Also, plant mitochondria can enzymically decarboxylate glyoxylate [17,18], but the contribution of this reaction to photorespiration remains to be assessed.

Dr Grodzinski also referred to work on glycollate metabolism in blue-green algae [19–22] which suggests that glyoxylate is non-enzymically decarboxylated to formate by H_2O_2 produced within illuminated cells of these algae [23,24]. Other work [25], on *Anabaena cylindrica,* has shown that tartronic acid semialdehyde, rather than glycine, is an intermediate in the conversion of glycollate to glycerate.

A third reaction which may convert glycollate to CO_2 is the non-enzymic decarboxylation of glyoxylate by H_2O_2 or $O_2^{\cdot-}$ in illuminated chloroplasts [26,27]. However, chloroplasts contain an NADPH-linked glyoxylate reductase [28] which would help to remove glyoxylate, and it is not clear how much glyoxylate would be available from the peroxisomes, nor how much H_2O_2 in synthesised in illuminated chloroplasts in vivo (but see below and also ref. 11).

Dr J. A. Raven (University of Dundee) discussed recent work on illuminated cells of the alga *Hydrodictyon africanum* [29,30]. O_2 uptake and evolution have been measured by $^{18}O_2$ mass spectrometry. Light-stimulated O_2 uptake is inhibited by DCMU (dichlorphenyl dimethyl urea). Under conditions of light and CO_2 saturation for photosynthesis, light-stimulated O_2 uptake is insensitive to cyanide or CCCP (*m*-chlorocarbonyl-cyanidephenylhydrazone). This suggests the occurrence of pseudocyclic electron flow, generating H_2O_2.

Dr B. Halliwell (King's College, London) mentioned that the action of superoxide dismutase in chloroplasts [31] is bound to lead to generation of H_2O_2.

Dr M. J. Merrett (University of Bradford) discussed recent work on enzymes of photorespiration in *Euglena gracilis* Klebs, which operates a glycine–serine pathway [32]. Although glycollate dehydrogenase and glutamate–glyoxylate aminotransferase are present in peroxisome fractions from this alga, they are also located in mitochondria [33]. Glycollate dehydrogenase is linked to the mitochondrial electron-transport chain and glycollate is oxidised with a P/O ratio of 1.7 [34]. Glycine is oxidised with a P/O ratio of 1, as in higher plants [9,10]. A glycollate dehydrogenase linked to the mitochondrial electron transport chain has also been reported in two marine diatoms [35] and may occur in *Chlorogonium* [36].

Mechanism of glycollate synthesis

Glycollate is derived from one or more intermediates of the Calvin cycle [37]. The most popular of the mechanisms proposed to account for its synthesis are the ribulose diphosphate oxygenase activity of ribulose diphosphate carboxylase and the oxidation of a transketolase-glycolaldehyde complex [37]. The former reaction produces phosphoglycollate, which can be hydrolysed to glycollate by a phosphatase located in the chloroplasts [38].

Dr Raven proposed that the transketolase mechanism might operate under conditions of light and CO_2 saturation in *Hydrodictyon africanum.* However, at the CO_2 compensation point for this alga, the light-dependent O_2 uptake is faster than at CO_2 saturation: that component of O_2 uptake which is stimulated by lowering the CO_2 concentration *is* inhibited by cyanide or CCCP [39]. He proposed that this represents O_2 uptake by RuDP oxygenase, which is known to be competitively inhibited by CO_2 [37]. In contrast, the rate of pseudocyclic electron flow (see above) is insensitive to CO_2 concentration.

Dr G. A. Codd (University of Dundee) discussed the mechanism of glycollate formation by blue-green algae and photosynthetic bacteria. *Rhodospirillum*

rubrum and *Rhodomicrobium vannielli* can tolerate exposure to O_2 and they produce glycollate under these conditions [40,41]. They contain phosphoglycollate phosphatase, RuDP oxygenase and glycollate dehydrogenase [40–43]. *Chromatium* also produces glycollate when exposed to O_2 [44]. All these results support the proposal [45] that synthesis of phosphoglycollate is an inevitable consequence of the active site chemistry of RuDP carboxylase/oxygenase.

Professor D. O. Hall (King's College, London) commented that the ability of such anaerobes to survive transient exposure to O_2 might be related to the presence of superoxide dismutase [46,47].

Dr M. Kerr (Shell Research) reported studies on a phosphoglycollate phosphatase isolated from pea leaves [48]. It has a pH_{opt} of 8.3 and Mg^{2+} is the best metal cofactor. The enzyme shows unusual kinetic properties: the K_M for phosphoglycollate increases 15-fold when the pH is increased from 7.3 to 8.3. There also seem to be 2 types of binding site for Mg^{2+}: a low-affinity site which gives a high V_{max} and predominates at concentrations above 4 mM Mg^{2+}, and a high affinity site which maintains some enzyme activity at very low Mg^{2+} concentrations. Since phosphoglycollate phosphatase is located in the chloroplast stroma, the pH to which it is exposed will increase on illumination (from about 7.2 to 8.2) and the Mg^{2+} concentration also increases from a very low level up to about 15 mM. Dr Kerr proposed that this might represent a regulatory mechanism, the enzyme switching from a form which allows hydrolysis of low concentrations of phosphoglycollate when there is little Mg^{2+} (in the dark) to a form geared to the rapid hydrolysis of high levels of phosphoglycollate in the light. Phosphoglycollate is a powerful inhibitor of triose phosphate isomerase [49] and presumably must be removed rapidly.

Inhibitors of photorespiration

Glycidic acid has been reported to inhibit glycollate synthesis in tobacco [50] and Dr Kerr has confirmed this result using pea leaf discs. He proposed that this could be explained by his observation that glycidic acid is a weak inhibitor of phosphoglycollate phosphatase. However, the Rothamsted group and others [37] have observed that glycidate does not increase net CO_2 assimilation by leaves.

Dr Kerr reported that 2-hydroxy-3-butynoic acid (HBA) is an irreversible inhibitor of glycollate oxidase [51]. Exposure of illuminated leaf discs to this compound causes accumulation of glycollate but there is no observable increase in net CO_2 assimilation. Dr Kerr also reported some recent experiments using a specific inhibitor of phosphoglycollate phosphatase. Partial inhibition of the phosphatase, in leaf discs pretreated with HBA, does cause a decreased accumulation of glycollate, in agreement with the observations with glycidate. However, if the phosphatase is completely inhibited, there is increased (80–100%) build-up of glycollate. This would rule out the oxygenase/phosphatase pathway as the only source of glycollate, although the alternative pathway(s) may not be significant under 'normal' conditions.

Development studies

Professor J. W. Bradbeer (King's College, London) reported studies on the development of enzymes of glycollate metabolism in bean leaves [52]. Dark-grown leaves contain phosphoglycollate phosphatase, catalase, glycollate oxidase and both NADH- and NADPH-linked glyoxylate reductases, together with all the Calvin cycle enzymes. Illumination of the plants increases the activities of all these enzymes. D-*threo*-Chloroamphenicol does not affect light-induced development of the glycollate pathway enzymes, although it inhibits the development of photosynthesis and photorespiration: possibly by inhibiting synthesis of the large subunit of RuDP carboxylase/oxygenase and of a photosystem II component, thus preventing glycollate formation. Hence the glycollate pathway enzymes are not dependent on any intermediate from the Calvin cycle for their synthesis and seem to be made on 80 S ribosomes. Other workers have reached similar conclusions, for wheat [53,54] and *Euglena* [55]. It is not yet clear whether any of the increase in activity of glycollate pathway enzymes in bean leaves is due to activation of pre-existing protein; most of it seems to be due to increased protein synthesis under the control of phytochrome (P_{FR}).

During greening of fat-storing cotyledons, the microbody population present changes from glyoxysomes to peroxisomes. It is not yet clear whether this represents a change in the enzyme composition of

established microbodies, or the destruction of glyoxysomes occurring simultaneously with synthesis of leaf-type peroxisomes [e.g. 56–59]. Dr J. M. Lord (University of Bradford), a proponent of the latter view, presented work showing that the enzymes of phospholipid synthesis in castor-bean endosperm are located in the endoplasmic reticulum [59]. The protein composition of purified glyoxysomal membranes, as determined by SDS-polyacrylamide gel electrophoresis, is very similar to that of the endoplasmic reticulum. Results using [^{35}S]methionine labelling suggest that endoplasmic reticulum is a precursor of the glyoxysomal membrane [60,61], and studies on the metabolism of labelled lecithins strongly suggest that glyoxysomes are not precursors of peroxisomes.

References

[1] Zelitch, I. (1966) Plant Physiol. 41, 1623–1631.
[2] Waidyanatha, U. P. S., Keys, A. J. and Whittingham, C. P. (1975) J. Exp. Bot. 26, 27–32.
[3] Bird, I. F., Cornelius, M. J., Keys, A. J. and Whittingham, C. P. (1974) Phytochemistry 13, 59–64.
[4] Larsson, C. and Albertsson, P. A. (1974) Biochim. Biophys. Acta 357, 412–419.
[5] Zelitch, I. (1975) Annu. Rev. Biochem. 44, 123–145.
[6] Whittingham, C. P., Hiller, R. G. and Bermingham, M. (1963) in: Photosynthetic Mechanisms in Green Plants. pp 675–683, Natl. Res. Council, Washington.
[7] Bird, J. F., Cornelius, M. J., Keys, A. J., Kumarasinghe, S. and Whittingham, C. P. (1974) in: Proc. III. Int. Congress Photosynthesis Res. 2, 1291–1301.
[8] Mahon, J. D., Fock, H. and Canvin, D. T. (1974) Planta 120, 245–254.
[9] Bird, I. F., Cornelius, M. J., Keys, A. J. and Whittingham, C. P. (1972). Biochem. J. 128, 191–192.
[10] Bird, I. F., Cornelius, M. J., Keys, A. J. and Whittingham, C. P. (1972) Phytochemistry 11, 1587–1594.
[11] Halliwell, B. (1974) New Phytol. 73, 1075–1086.
[12] Halliwell, B. and Butt, V. S. (1974) Biochem. J. 138, 217–224.
[13] Grodzinski, B. and Butt, V. S. (1976) Planta, in the press.
[14] Kisaki, T., Yoshida, N. and Imai, I. (1971) Pl. Cell Physiol. 12, 275–288.
[15] Halliwell, B. (1974) Biochem. J. 138, 77–85.
[16] Halliwell, B. (1973) Biochem. Soc. Trans. 1, 1147–1150.
[17] Prather, C. W. and Sisler, E. C. (1972) Phytochemistry 11, 1637–1647.
[18] Davies, D. D. and Corbett, R. J. (1969) Phytochemistry 8, 529–542.
[19] Miller, A. G., Cheng, K. H. and Colman, B. (1971) J. Phycol. 7, 97–100.
[20] Grodzinski, B. and Colman, B. (1975) Planta 124, 125–133.
[21] Colman, B., Miller, A. G. and Grodzinski, B. (1974) Pl. Physiol. 53, 395–397.
[22] Grodzinski, B. and Colman, B. (1976) Pl. Physiol. in the press.
[23] Chua, N. H. (1971) Biochim. Biophys. Acta 245, 277–287.
[24] Patterson, C. and Myers, J. (1973) Pl. Physiol. 57, 104–109.
[25] Codd, G. A. and Stewart, W. D. P. (1973) Arch. Mikrobiol. 94, 11–28.
[26] Zelitch, I. (1972) Arch. Biochem. Biophys. 150, 698–707.
[27] Elstner, E. F. and Heupel, A. (1973) Biochim. Biophys. Acta 325, 182–188.
[28] Tolbert, N. E., Yamazaki, R. K. and Oeser, A. (1970) J. Biol. Chem. 245, 5129–5136.
[29] Raven, J. A. and Glidewell, S. M. (1975) New Phytol. 75, 197–204.
[30] Glidewell, S. M. and Raven, J. A. (1975) J. Exp. Bot. 26, 479–488.
[31] Asada, K., Urano, M. and Takahashi, M. (1973) Eur. J. Biochem. 36, 257–266.
[32] Merrett, M. J. and Lord, J. M. (1973) New Phytol. 72, 751–767.
[33] Collins, N. and Merrett, M. J. (1975) Biochem. J. 148, 321–328.
[34] Collins, N., Brown, R. H. and Merrett, M. J. (1975) Biochem. J. 150, 373–377.
[35] Paul, J. S., Sullivan, C. W. and Volcani, B. E. (1975) Arch. Biochem. Biophys. 169, 152–159.
[36] Stabenau, H. (1974) Plant Physiol. 54, 921–924.
[37] Chollet, R. and Ogren, W. L. (1975) Bot. Rev. 41, 137–179.
[38] Randell, D. D., Tolbert, N. E. and Gremel, D. (1971) Pl. Physiol. 48, 480–487.
[39] Glidewell, S. M. and Raven, J. A. (1976) J. Exp. Bot. 27, 200–204.
[40] Codd, G. A. and Smith, B. M. (1974) FEBS Lett. 48, 106–109.
[41] Codd, G. A. and Turnbull, F. (1975) Arch. Microbiol. 104, 155–158.
[42] Ryan, F. J., Jolly, S. O. and Tolbert, N. E. (1974) Biochem. Biophys. Res. Commun. 59, 1233–1241.
[43] McFadden, B. A. (1974) Biochem. Biophys. Res. Commun. 60, 312–317.
[44] Asami, S. and Akazawa, T. (1974) Pl. Cell Physiol. 15, 571–576.
[45] Lorimer, G. H. and Andrews, T. J. (1973) Nature 243, 359–360.
[46] Hewitt, J. and Morris, J. G. (1975) FEBS Lett. 50, 315–318.
[47] Lumsden, J. and Hall, D. O. (1975) Nature 257, 670–672.
[48] Kerr, M. W. and Gear, C. F. (1974) Biochem. Soc. Trans. 2, 338–340.
[49] Wolfenden, R. (1970) Biochemistry 9, 3404–3407.

[50] Zelitch, I. (1974) Arch. Biochem. Biophys. 163, 367–377.
[51] Jewess, P. J., Kerr, M. W. and Whitaker, D. P. (1975) FEBS Lett. 53, 292–296.
[52] Murray, D. R., Wara-Aswapati, O., Ireland, H. M. M. and Bradbeer, J. W. (1973) J. Exp. Bot. 24, 175–184.
[53] Feierabend, J. and Beevers, H. (1972) Plant Physiol. 49, 28–32.
[54] Feierabend, J. and Beevers, H. (1972) Plant Physiol. 49, 33–39.
[55] Davis, B. and Merrett, M. J. (1975) Plant Physiol. 55, 30–34.
[56] Kagawa, T., Lord, J. M. and Beevers, H. (1975) Arch. Biochem. Biophys. 167, 45–53.
[57] Theimer, R. R., Anding, G. and Schmid-Neuhaus, B. (1975) FEBS Lett. 57, 89–92.
[58] Kagawa, T. and Beevers, H. (1975) Plant Physiol. 55, 258–264.
[59] Bowden, L. and Lord, J. M. (1975) FEBS Lett. 49, 369–371.
[60] Bowden, L. and Lord, J. M. (1976) Biochem. J. 154, 491–499.
[61] Bowden, L. and Lord, J. M. (1976) Biochem. J. 154, 501–506.