Stimulation of Glycollate Excretion of Algae by Disalicylidenepropanediamine and Hydroxypyridinemethanesulfonate

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In a previous paper we have shown that disalicylidenepropanediamine (DSPD), an inhibitor of photosynthetic reduction of ferredoxin [1], stimulates ^{14}C-labelling of glycollic acid in algal cells [2]. Further experiments led to the observation that also the excretion of ^{14}C-labelled glycollate is enhanced by DSPD [3]. We supposed that the increase of glycollate formation and excretion by DSPD is due either to an inhibition of photosynthesis by preventing the reduction of ferredoxin or to an inhibition of glycollate oxidase [2]. In consequence we tested a) the influence of inhibitors of photosynthesis, which, like DSPD, preferentially affect the cyclic photophosphorylation in vivo, probably catalyzed by ferredoxin [2, 4] and b) the influence of hydroxypyridinemethanesulfonate (HPMS), an inhibitor of glycollate oxidase [5], on the excretion of glycollate in *Ankistrodesmus*.

Like DSPD, antimycin and salicylaldoxime produce an increased glycollate excretion [3], whereas neither inhibition of noncyclic electron transport by 3-(3,4-dichlorophenyl)-1,1-dimethylurea nor uncoupling or inhibition of ATP-formation by carbonyl cyanide *m*-chlorophenyl hydrazone, atebrin and phloridzin have a stimulating effect on glycollate excretion [3, 6]. On the other hand, as shown in table 1 a, also HPMS stimulates the excretion of ^{14}C-labelled glycollate in a similar way like DSPD. Up to 9×10^{-3} M HPMS no significant inhibition of total ^{14}C-fixation was observed but a continuous increase of glycollate excretion to about five times the control. The excreted glycollate represents about 1,5% of the total ^{14}C fixed in the control and 6,3% in the presence of 9×10^{-3} M HPMS, i. e. about 30% of the ^{14}C-fixation in the soluble fraction. This is inconsistent with Tolbert and Hess, who neither found a stimulation of glycollate excretion by HPMS nor any glycollate oxidase activity in several strains of algae including *Ankistrodesmus* [7, 8]. They suggested that the glycollate pathway in algae is different from that in higher plants and that algae excrete glycollate into the culture medium, because they are lacking in glycollate oxidase. Our results can be explained by the recent findings of Zelitsch and Day [9], who succeeded to de-

	Additions	mmole/*l*	^{14}C-fixation in % of control	^{14}C-glycollate excretion in % of control	^{14}C-glycollate excretion in % of total ^{14}C-fixation
a) *Young cells*	—	—	100	100	1,5
6 hours in the light	HPMS	1	107	174	2,2
period (in normal cul-	HPMS	3	108	215	2,7
ture medium, but 5 hours	HPMS	6	99	378	4,7
without phosphate)	HPMS	9	98	476	6,3
	DSPD	0,5	64	343	8,2
b) *Autospores*					
2 hours in the light	—	—	100	100	0,5
period (normal culture	HPMS	6	111	1132	3,2
medium)					
Young cells					
6 hours in the light	—	—	100	100	6,8
period (normal culture	HPMS	6	105	224	15,1
medium)					

Table 1. Influence of HPMS and DSPD on ^{14}C-fixation and excretion of ^{14}C-labelled glycollate in synchronized cultures of *Ankistrodesmus braunii*. Incorporation of ^{14}C was carried out in Warburg vessels at 25 °C and 17 000 lux. The reaction mixture contained in a final volume of 5 ml: algae containing about 60 µg chlorophyll; culture medium 2 ml; Tris puffer (pH 8,0) 250 µmole and inhibitors as indicated. Incorporation was started after 10 min of preillumination by adding 5 µmole $NaHCO_3$ (containing 0,02 mCi ^{14}C) and stopped after 15 min by injecting 2 ml of the sample into trichloroacetic acid to measure total ^{14}C fixed and by filtering the rest of the sample instantly to estimate the ^{14}C-labelled glycollate excreted into the culture medium. Details of the procedure will be published elsewhere [3].

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tect glycollate oxidase activity in algae. A reason for these divergent results may be the dependency of the glycollate oxidase activity on the physiological state of algae as already described [6]. We have good evidence for a variation of glycollate excretion during synchronous life cycle of *Ankistrodesmus*. Autospores, harvested 2 hours after the beginning of the light period, show a small excretion of glycollate, which is, however, strongly enhanced by HPMS (table 1 b). Young cells, harvested after 6 hours in the light, excrete more glycollate into the culture medium, but the stimulation of this process by HPMS is less (table 1 b). From this we suggest that the activity of glycollate oxidase changes during the life cycle of synchronized algae.

The inhibition of cyclic photophosphorylation in vivo as well as the stimulation of glycollate excretion by DSPD, antimycin and salicylaldoxime suggest a connection between these two processes. The similar response of these processes to the various inhibitors could be explained by the possibility that ferredoxin is catalysing both, cyclic photophosphorylation in vivo [2, 4, 10], and glycollate oxidation by a ferredoxin type of glycollate oxidase in *Ankistrodesmus*, as has been found in wheat [11]. A more detailed publication will be presented elsewhere.

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