Effect of Alden® on fresh weight, total protein and chlorophyll contents, and soluble acid phosphatase and RNase activity in Spirodela oligorrhiza, as measured after 7 days of cultivation

Treatment (M)	Fresh wt (mg flask^{-1})	Total protein (mg. g^{-1} fr. wt)	Chlorophyll (mg. g^{-1} fr. wt)	Activity of phosphatase (U. g^{-1} fr. wt)	RNase (U. g^{-1} fr. wt)
Control	85[a]	29.4[a]	1.7[a,b]	2.7[a]	3.7[a]
GA_3 (5×10^{-5})	113[b]	28.8[a]	1.7[a]	2.7[a]	5.0[b]
BA (10^{-6})	116[b]	26.5[b]	1.3[c]	2.3[a]	3.1[c]
Alden (10^{-5})	38[c]	37.6[c]	2.2[d]	4.9[b]	5.9[d]
Alden + GA	62[d]	35.3[c,d]	1.9[b]	4.0[b,c]	4.8[b]
Alden + BA	71[e]	32.4[c,d]	1.6[a]	3.8[c]	4.6[b]

Inoculum: 10 fronds per flask. The data followed by unlike postscripts within each group of parameters differ significantly at the 1% probability level.

figure 2). The compound increased by 27% the protein and chlorophyll content per g fr wt (table). The effect on chlorophyll content has also been noted on grapevine, chrysanthemums and other ornamentals[8]. The effects of Alden on protein and chlorophyll content were significantly reduced in the mixtures with BA, which by itself markedly lowered the chlorophyll content per g fr. wt. Of special interest is the fact that GA_3 reduced the stimulatory effect of Alden on chlorophyll content (table).

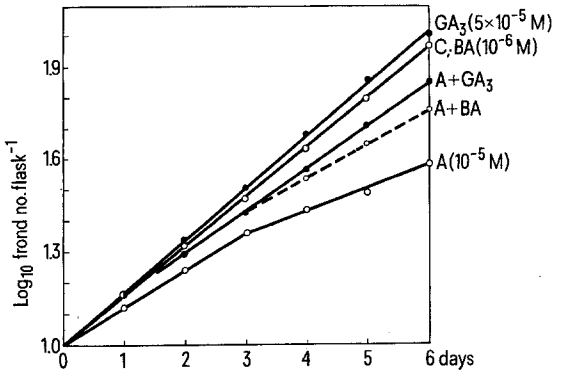

Fig. 2. Kinetics of growth of Spirodela under the influence of Alden, applied alone or in mixtures with GA_3 or BA. C, control.

Alden enhanced acid phosphatase and RNase activity by about 80% and 60%, respectively, in comparison with the control plants. GA_3 also stimulated RNase activity, but in mixtures the effects were not additive (table). In Spirodela there might be an adaptative alkaline phosphatase with pH optimum 7.5, besides a constitutive enzyme showing maximum activity at pH 6.0[9,10]. As phosphatase activity at pH 7.5 was enhanced by about 120% (not shown), against 80% at pH 6.0, Alden may induce the adaptative phosphatase isoenzymes[10].

This study revealed that Alden is about 5 times more active as a growth-retardant for Spirodela than CCC[4]. CCC at 10^{-4} M concentration inhibits chlorophyll synthesis as manifested by yellow colouration of young fronds, whereas Alden does not induce such an effect even at 10^{-3} M concentration. It is possible that Alden interferes with the biosynthesis and/or mode of action of gibberellins, as GA_3 most effectively reduced the symptoms of its action. Nevertheless, a) the increase of protein content in the Alden-treated plants seems to indicate that this compound retards protein breakdown, and b) as it stimulated activity of RNase and phosphatase, it may directly affect the phosphate metabolism in plants[9].

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Regulation of glucose transport in Aspergillus nidulans

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Summary. Pyruvate and acetate inhibited the uptake of glucose by Aspergillus nidulans; although there were significant variations in glucose uptake rate, the intracellular concentration of acetate was almost identical in biotin-supplemented, normal and deficient cells. The in vitro activity of glucose-binding protein was not affected by biotin, avidin, acetate or acetyl-CoA.

In our earlier studies we characterized the glucose transport system in Aspergillus nidulans as energy-dependent, against the concentration gradient, and requiring binding protein for its function[2]. The binding protein for glucose was isolated and purified in a homogenous state, as judged by the gel electrophoresis and its properties were described in detail[3]. In this communication, we wish to report a regulatory aspect of the glucose transport in Aspergillus nidulans.

Materials and methods. The strain, composition of the basal media and the cultural conditions used in the present investigations were the same as reported earlier[2,4]. Biotin

deficiency in this culture was produced as already described[2–5]. Cultures grown in the presence of 5 units of avidin (General Biochemicals, Ohio) were 65% less in fatty acid content as compared with the controls. Biotin was not traceable by the microbiological assay, using Lactobscillus arabinosus as the test organism by the method of Skeggs[6]. These cultures, therefore, were referred to as 'biotin deficient' in this communication,

The established method was used for the uptake studies[2]. Isolation and assay of glucose-binding protein were performed by the 2-stage osmotic shock treatment of Wiley[7] and the equilibrium dialysis technique of Barash and Harlpern[8], with slight modification[2]. Glucose-binding protein was purified as previously described[3]. Glucose and proteins were measured by the methods of Dahlqvist[9] and Lowrey et al.[10], respectively. The method described by Rose[11] was used to determine the acetate content and the intracellular concentration was calculated by using the published value of 4 µl of water/mg of dry cells[12].

Results. The results in the figure show that pyruvate and acetate inhibit the uptake of glucose in A.nidulans. Although there were significant differences in glucose uptake rate, the intracellular concentration of acetate was found to remain identical in biotin-supplemented, normal and deficient cultures of A.nidulans (table 1), indicating that acetate itself is not regulating the glucose uptake in this culture.

Earlier work from this laboratory has indicated that biotin deficiency causes about 20% reduction in glucose uptake[2,13]. Further, it was shown that the lower activity of glucose-binding protein involved in transport of glucose may be responsible for the reduction in glucose uptake by biotin deficient A.nidulans[3]. Considering the well-established role of biotin in conversion of acetyl-CoA to malonyl-CoA[14], and the results discussed above, it is reasonable to believe that not acetate but a metabolite of acetate, more likely acetyl-CoA, may be exerting a regulatory effect on glucose uptake by interacting with glucose binding protein. Thus, the reduction in glucose uptake[2] and the activity of glucose binding protein[3] as a result of biotin deficiency might be due to the higher intracellular concentration of acetyl-CoA, which could be due to the reduction in conversion of acetyl-CoA to malonyl-CoA under this condition. About 65% reduction in the fatty acid content was observed due to biotin deficiency in this culture[4]. However, in vitro studies with purified glucose-binding protein did not show any significant change in the activity by biotin, avidin, acetate or acetyl-CoA (table 2). The observed inhibition of glucose uptake by acetate (figure) is in agreement with the results of Romano and Kornberg[15,16] in A.nidulans. But they failed to observe this effect in a mutant devoid of acetyl-CoA synthetase and suggested that acetyl-CoA as an end-product of glycolysis can regulate the sugar utilization by controlling its uptake. Similar phenomena for the regulation of glucose uptake in A.nidulans and more likely acetyl-CoA might be regulating the in vivo activity of glucose-binding protein, involved in glucose uptake of A.nidulans. However, at present it is difficult to speculate on the nature of the interaction between the glucose-binding protein and acetyl-CoA.

Table 1. Intracellular concentrations of acetate and uptake of glucose in biotin-deficient and biotin-supplemented cultures of A. nidulans

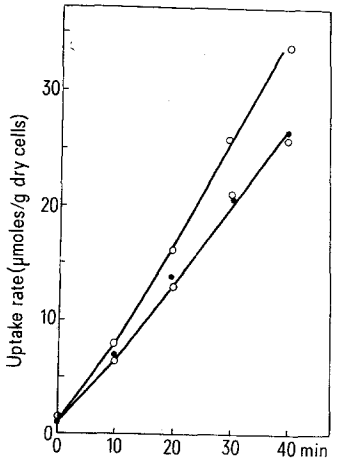
Cultural conditions	Intracellular acetate concentration (mM)	Glucose uptake rate (µmoles/g dry cells/ 30 min)
Normal (control)	5.87	25.00
Control + biotin*	6.12	32.72
Deficient	6.00	20.08
Deficient + biotin*	6.20	31.45

*Biotin was added in 5 µg/ml concentration.

Table 2. Effects of biotin, avidin, acetate and acetyl-CoA on the in vitro activity of glucose-binding protein from A. nidulans

Additions (1 mM)	Specific activity (units*/mg protein)
–	53.5
Biotin	63.0
Avidin	62.6
Acetate	62.8
Acetyl-CoA	61.7

*1unit of glucose-binding protein is the amount of protein, required to bind 1 µmole of D-glucose/24 h at 4 °C.in the given assay conditions.



Effect of pyruvate and acetate on glucose uptake by Aspergillus nidulans. Uptake of glucose was measured in the absence (○) and presence of 250 µg/ml pyruvate (●) and acetate (0).

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