Occurrence of Isoenzymes of Glutamine Synthetase in the Alga *Chlorella kessleri*

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**ABSTRACT**

Two forms (GS1 and GS2) of glutamine synthetase have been isolated, separated by ion exchange chromatography, and partly characterized from cells of the green alga *Chlorella kessleri*. Both forms are present in cells grown autotrophically or heterotrophically on various nitrogen sources, but under all nutritional conditions GS1 was found to be the major isoenzyme present (60–80%). The activity of both isoenzymes was greatest in cells grown under nitrogen-limiting conditions. Both isoenzymes have molecular weights in the range 340 to 350,000 daltons. GS1 was found to have a greater thermostability than GS2; GS2 was stable at 30°C while GS1 lost 95% of its activity in 30 minutes. GS2 was much less sensitive to thiol reactive reagents than GS1.

Recent work has shown that glutamine synthetase (EC 6.3.1.2), the key enzyme of ammonia metabolism, occurs in multiple molecular forms in many higher plants (1, 7, 12, 15, 16, 18–20, 23, 27). Ion exchange chromatography and subcellular localization studies have shown the presence of two isoenzymes in photosynthetically active tissue: GS1 is localized in the cytoplasm whereas GS2 is found in chloroplasts (10, 11, 17). These isoenzymes differ in several respects, including their pH optima, heat stability, and Km for glutamate (1, 9, 16, 18, 19).

Although glutamine synthetase has been much studied in microorganisms, there is little evidence for the occurrence of isoenzymic forms; in only two species, *Bacillus caldolyticus* (26) and *Rhizobium japonicum* (6), have distinct isoenzymes been demonstrated. There appear to be no reports of glutamine synthetase isoenzymes in algae, although the enzyme has been purified from a thermophilic strain of *Chlorella pyrenoidosa* and characterized in some detail (21). In the present paper, we report the presence of two distinct forms of glutamine synthetase in *Chlorella kessleri* and that their properties resemble those of GS1 and GS2 isoenzymes present in some higher plants.

**MATERIALS AND METHODS**

Organism and Growth Conditions. *Chlorella kessleri* was obtained from the culture center of Algae and Protozoa at Cambridge (No. 211/11g) and maintained on agar slopes. Cells were grown as batch cultures for 3 to 4 d either autotrophically in aerated Dretschel bottles at 25°C in the light (4) or heterotrophically in penicillin flasks on a rotary shaker at 25°C in the dark.

![Image](https://example.com/image.jpg)

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In general, the specific activities of GS₁ and GS₂ in dark-grown cells on a particular nitrogen regime are greater than those in corresponding cells grown in the light.

Enzyme Stability. A major difference observed between the GS₁ and GS₂ isoenzymes of higher plants is their heat stability: GS₂ always appears to be less heat stable than GS₁ (1, 9, 16, 18, 19). This difference in heat stability is also shown by the Chlorella isoenzymes (Fig. 2). GS₂ was found to inactivate more rapidly than GS₁ at all temperatures examined; at 30°C GS₁ was quite stable but GS₂ lost 95% of its activity in 30 min.

Influence of Thiol-Reactive Reagents. A further characteristic which distinguishes the GS₁ and GS₂ isoenzymes from higher plants is their sensitivity towards thiol-reactive reagents such as N-ethylmaleimide and 5,5-dithiobis(2-nitrobenzoic acid). It is evident from the results shown in Figure 3 that GS₂ of Chlorella was more sensitive to both NEM³ and DTNB. A concentration of 0.5 mM NEM gave complete inactivation of GS₂ but only 60% inactivation of GS₁. Similarly, 0.1 mM DTNB brought about 80% inactivation of GS₂, but less than 10% inactivation of GS₁. GS₂ in higher plants is also more susceptible to inactivation by DTNB and NEM (1, 16, 18, 19).

Table 1. Comparison of Specific Activities of Glutamine Synthetases from Chlorella kessleri Grown under Different Conditions

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Specific Activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GS₁</td>
<td>GS₂</td>
</tr>
<tr>
<td>Ammonium, light</td>
<td>5.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Nitrate, light</td>
<td>8.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Nitrogen starved, light</td>
<td>31</td>
<td>8.3</td>
</tr>
<tr>
<td>Ammonium, dark</td>
<td>13.3</td>
<td>8.4</td>
</tr>
<tr>
<td>Nitrate, dark</td>
<td>28.3</td>
<td>11.9</td>
</tr>
<tr>
<td>Nitrogen starved, dark</td>
<td>42.8</td>
<td>11.9</td>
</tr>
</tbody>
</table>

In general, the specific activities of GS₁ and GS₂ in dark-grown cells on a particular nitrogen regime are greater than those in corresponding cells grown in the light.

Abbreviations: NEM, N-ethylmaleimide; DTNB, 5,5-dithiobis(2-nitrobenzoic acid).

1 Abbreviations: NEM, N-ethylmaleimide; DTNB, 5,5-dithiobis(2-nitrobenzoic acid).
DISCUSSION

Cells of *C. kessleri*, like photosynthetic tissue of many higher plants, have two isoenzymes of glutamine synthetase. These exhibit differences in their heat stability and sensitivity toward thiol-reactive reagents. *Chlorella* GS₂ was found to be less heat stable and more readily inactivated by NEM and DTNB than GS₁. It is tempting to assume, given the similarity in chromatographic behavior, heat stability, and sensitivity to NEM and DTNB between the *Chlorella* isoenzymes and those of higher plants that GS₁ and GS₂ of *Chlorella* correspond to cytoplasmic and chloroplastic forms of glutamine synthetase.

Both *Chlorella* isoenzymes are influenced by light and nitrogen availability, although both these factors appear to exert a greater influence on GS₁. The activity of GS₂ in cells subject to different nitrogen regimes was always greater when the cells were grown in the dark. This contrasts with results obtained on higher plants; in both barley (16) and rice (9), GS₂ was found to be present at low levels in etiolated seedlings and to increase rapidly following exposure to light. Tischner and Hutterman (25) working with *Chlorella sorokiniana* cells grown in synchronous cultures reported a light activation of total glutamine synthetase activity in response to the light-dark transitions. In the present study, comparisons were made between cells grown nonsynchronously in continuous illumination or continuous darkness with a carbon source supplied (as glucose).

Another difference in regulatory behavior between the *Chlorella* and higher plant isoenzymes is the effect of nitrogen availability on their activity. Although the isoenzymes of barley show little change in activity in response to nitrogen source or concentration (17), the specific activity of *Chlorella* GS₁ varies 7-fold and that of GS₂ 4-fold. Maximum activities of GS₁ and GS₂ were found in nitrogen-starved cells grown in the dark whereas the lowest activities were present in light-grown cells assimilating ammonia. Hipkin and Syrett (8) similarly demonstrated increased glutamine synthetase activity in *Ankistrodesmus braunii* during nitrogen starvation.

Under all growth conditions employed, GS₁ was found to be the major component in *C. kessleri*. In light-grown cells supplied with nitrate or ammonia, GS₁ accounted for 50 to 60% of the total activity while under conditions of nitrogen starvation, GS₁ comprised 70 to 80% of the total activity. Keys et al. (14) have suggested that the cytoplasmic isoenzyme (GS₁) in higher plants functions in the re-assimilation of ammonia released in the photorespiratory nitrogen cycle while the chloroplastic isoenzyme (GS₂) functions in primary ammonia assimilation. The depression of *Chlorella* GS₁ in dark grown cells would appear to rule out an exclusive role in the photorespiratory nitrogen cycle.

![Graph 2](image2.png)

**Fig. 2.** Thermal inactivation of GS₁ and GS₂. Partially purified GS₁ and GS₂ were incubated at different temperatures and aliquots taken at various time intervals to determine the residual activity. Decay constants \((K_d)\) were calculated from semilog plots of residual activity against time.

![Graph 3](image3.png)

**Fig. 3.** Effect of thiol-reactive reagents on GS₁ and GS₂. (a), NEM; (b), DTNB. Partially purified GS₁ and GS₂ were incubated with varying concentrations of NEM and DTNB at 30°C for 30 min and aliquots were removed to determine residual activity.
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LITERATURE CITED