

Deoxyglucose-Resistant Mutants of *Neurospora crassa*: Isolation, Mapping, and Biochemical Characterization

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Neurospora crassa mutants resistant to 2-deoxyglucose have been isolated, and their mutations have been mapped to four genetic loci. The mutants have the following characteristics: (i) they are resistant to sorbose as well as to 2-deoxyglucose; (ii) they are partially or completely constitutive for glucose transport system II, glucamylase, and invertase, which are usually repressed during growth on glucose; and (iii) they synthesize an invertase with abnormal thermostability and immunological properties, suggesting altered posttranslational modification. All of these characteristics could arise from defects in the regulation of carbon metabolism. In addition, mutants with mutations at three of the loci lack glucose transport system I, which is normally synthesized constitutively by wild-type *N. crassa*. Although the basis for this change is not yet clear, the mutants provide a way of studying the high-affinity system II uncomplicated by the presence of the low-affinity system I.

In studies of fungal carbon metabolism, much useful information has come from the isolation of mutants resistant to toxic sugar analogs. One such analog is 2-deoxyglucose, which is readily phosphorylated by fungal hexokinases (16). The resulting compound, 2-deoxyglucose-6-phosphate, accumulates and inhibits early glycolytic enzymes as well as the incorporation of glucose and mannose into cell wall polysaccharides. The depletion of ATP during phosphorylation of 2-deoxyglucose may also contribute to the toxicity of this sugar analog.

A variety of 2-deoxyglucose-resistant mutants have been found in *Saccharomyces cerevisiae*. Some carry mutations in the structural gene for hexose kinase PI, hexose kinase PII, or glucokinase, leading to reduced phosphorylation of 2-deoxyglucose (11-13, 28). Others have increased amounts of a phosphatase which dephosphorylates 2-deoxyglucose-6-phosphate (8, 15). Many deoxyglucose-resistant mutants appear to have pleiotropic regulatory alterations in the activity of enzymes such as invertase, α -glucosidases, and malate dehydrogenase (3-7, 28). Less is known about 2-deoxyglucose resistance in filamentous fungi, but *dgr* mutants have been isolated in *Neurospora crassa* and appear, from preliminary data, to map at several distinct genetic loci (L. T. Dunn, M.A. dissertation, University of North Carolina, Greensboro, 1980; B. Eberhardt, *Neurospora* Newsl. 27:19-20, 1980).

A second glucose analog, L-sorbose, alters the growth characteristics of filamentous fungi dramatically and has also been used to select resistant mutants. Perhaps the simplest results are those for *Coprinus cinereus*, when Moore and Stewart (18) isolated 450 sorbose-resistant mutants mapping at a single locus (*ptr*) involved in fructose and glucose uptake (17). By contrast, Elorza and Arst (2) found that sorbose-resistant mutants of *Aspergillus nidulans* map to two loci: *sorA* mutants appear defective in sugar uptake, whereas *sorB* mutants display abnormally low phosphoglucomutase activity and an altered cell composition. Even more complex results have been reported for *N. crassa*, for which Klingmueller (9) has determined that there are at least six unlinked

sor loci. The biochemical basis for resistance in these strains is not yet clear, but some of the mutants appear to take up sorbose at a reduced rate.

In this report, we describe the isolation of eight 2-deoxyglucose-resistant mutants of *N. crassa*. We have mapped the mutations to four genetic loci, established their relationship to previously described *sor* and *dgr* *Neurospora* mutants, and found some of the mutants to be altered in the synthesis of a family of glucose-repressible enzymes.

MATERIALS AND METHODS

Strains, growth, and genetic techniques. In addition to the strains listed in Table 1, the following strains of *N. crassa* were used in the present study: RL21a, the wild-type strain from which the KHY mutants were derived (see below); 74-OR23-1A, a different wild-type strain which was the progenitor of the *dgr* mutants isolated by Eberhart (*Neurospora* Newsl. 27:19-20, 1980) and Dunn (M.A. dissertation); and two sorbose-resistant mutants, DS (*sor-4*; FGSC 1741) and T9 [*sor(T9)*; FGSC 3429], isolated by Murayama and Ishikawa (19) and obtained from the Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center, Kansas City. Several marker strains, also from the Fungal Genetics Stock Center, were used in mapping the mutations: *caf-1*, FGSC 993; *lys-1*, FGSC 1535; *leu-3;arg-1*, FGSC 1210; *al-2;arg-13*, FGSC 1723. All stocks were maintained on agar slants on Vogel medium N (27) supplemented appropriately for their nutritional requirements.

To isolate 2-deoxyglucose-resistant mutants, conidia from wild-type strain RL21a were mutagenized by exposure to UV light and plated on agar medium containing Vogel N salts, 0.3% 2-deoxyglucose, and 1% fructose. Eight mutants were selected on this medium and, after being back-crossed to the wild-type strain RL3-8A, were designated KHY2, KHY3, KHY5, KHY6, KHY7, KHY11, KHY15, and KHY19.

Mutations in the newly isolated *dgr* mutants were mapped by using randomly collected ascospores as described by Davis and DeSerres (1). Individual progeny were scored in either of two ways: for deoxyglucose resistance, they were

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TABLE 1. Strains used in this study

| Locus | Map location | Alleles | FGSC no. | Derivation ^a |
|--------------|----------------------|---------|----------|-------------------------|
| <i>dgr-1</i> | Chromosome V (left) | KHY3 | 4352 | a |
| | | KHY6 | | a |
| | | KHY15 | | a |
| | | BE52 | | b |
| <i>dgr-2</i> | Chromosome I (left) | KHY5 | 4327 | a |
| | | L-1 | | b |
| <i>dgr-3</i> | Chromosome I (left) | KHY19 | 4334 | a |
| | | BEX6 | | b |
| <i>dgr-4</i> | Chromosome I (right) | KHY2 | | a |
| | | KHY7 | | a |
| | | KHY11 | | a |

^a a, Mutants isolated in this study from the wild-type strain RL21a; b, Mutants isolated by Eberhart (*Neurospora Newsl.* 27:19-20, 1980) and Dunn (M.A. dissertation) from wild-type strain 74-OR23-1A.

scored by growth on agar medium containing Vogel N salts, 0.3% 2-deoxyglucose, and 1% fructose; or for sorbose resistance, they were scored by the ability to grow as mycelia (instead of as colonies) on agar medium containing Vogel N salts, 2% xylose, and 2% sorbose.

Quantitative measurements of resistance were made by inoculating 20-ml standing cultures with 10^4 conidia per ml, incubating the cultures for 3 days at 25°C, harvesting the mycelial mat, and determining the dry weight. For these measurements, the medium contained Vogel N salts, 1% fructose, and various concentrations of 2-deoxyglucose or sorbose.

Transport assay. The ability to transport the nonmetabolizable glucose analog 3-O-methylglucose was assayed by using shaking cultures grown for 15 h in Vogel N medium with 2% sucrose or fructose as the carbon source. The cells were harvested by filtration, washed thoroughly with water, and suspended at a density of 1 to 3 mg (dry weight) per ml in buffer containing $0.29 \times$ Vogel salts, 2.3 mM CaCl_2 , and 20 mM sodium 3,3-dimethylglutaric acid (pH 5.8). At time zero, 3-O-[¹⁴C]methylglucose (specific activity, 10 to 100 $\mu\text{Ci}/\text{mmol}$) was added. Cells were collected at intervals on membrane filters (Type RA; Millipore Corp., Bedford, Mass.), washed well with water, dried overnight, weighed, and counted with Aquasol (Du Pont, NEN Research Products, Boston, Mass.) in a liquid scintillation counter (no. 3150T; Beckman Instruments, Inc., Fullerton, Calif.). The initial rates of uptake were determined and computer fitted by means of the Marquardt algorithm (14) to the Michaelis-Menten equation to determine the K_m and V_{\max} for the high-affinity transport system.

Enzyme assays. Invertase and glucamylase were assayed as previously described (10, 25). The rate at which wild-type and mutant cells synthesize glucamylase relative to total protein was measured by using polyclonal rabbit antibody directed against the polypeptide as previously reported (25). The thermostability of invertase was determined by heating cellular extracts to 60°C for various times and then assaying the remaining activity. Titers of wild-type and mutant invertases were found with two antisera: one prepared at State University of New York at Buffalo by Lee and Free (10) and the other a gift from H. D. Braymer. In these experiments, an indirect immunoprecipitation procedure was used because the antisera precipitated the enzyme poorly.

Materials. Sorbose and 2-deoxyglucose were purchased from Sigma Chemical Co., St. Louis, Mo.; [³H]leucine,

3-O-[¹⁴C]methylglucose, and 2-[¹⁴C]deoxyglucose were purchased from Du Pont, NEN Research Products.

RESULTS

Isolation and genetic analysis of the mutants. The starting point for this study was the isolation of a group of eight *N. crassa* mutants that could form colonies on fructose in the presence of 0.3% (1.83 mM) 2-deoxyglucose. By using standard mapping procedures (1), the mutations were mapped to four loci (Table 2): *dgr-1*, located on the left arm of chromosome V and represented by mutant alleles KHY3, KHY6, and KHY15; *dgr-2*, located on the left arm of chromosome I and represented by KHY5; *dgr-3*, also located on the left arm of chromosome I, and represented by KHY19; and *dgr-4*, located on the right arm of chromosome I and represented by KHY2, KHY7, and KHY11. These map positions suggested that some of the mutants might be allelic to 2-deoxyglucose-resistant mutants previously isolated by Eberhart (*Neurospora Newsl.* 27:19-20, 1980) and Dunn (M.A. dissertation). Indeed, subsequent crosses revealed no recombinants (in 100 to 200 progeny tested) between the Eberhart mutant BE52 and KHY15, placing BE52 at the *dgr-1* locus; between the Dunn mutant L-1 and KHY5, placing L-1 at *dgr-2*; and between the Eberhart mutant BEX6 and KHY19, placing BEX6 at *dgr-3*.

In additional growth tests, the newly isolated *dgr* mutants proved to be resistant to sorbose as well, growing with filamentous morphology instead of the usual colony morphology on agar medium containing 2% xylose and 2% sorbose. Therefore, it was important to examine them for possible allelism with previously described sorbose-resistant (*sor*) mutants of *N. crassa*. The *sor-4* and *sor(T9)* mutations of Murayama and Ishikawa (19) have been reported to map on the left arm of chromosome I, where both *dgr-2* and *dgr-3* are located. Accordingly, crosses were carried out between *sor-4*, *sor(T9)*, and representatives of both *dgr-2* and *dgr-3*. No recombinants were found between *sor-4* and KHY19 (200 spores isolated), between *sor(T9)* and KHY19 (200 spores isolated), or between *sor-4* and *sor(T9)* (400 spores isolated), suggesting that both *sor-4* and *sor(T9)* are alleles of *dgr-3*. Other sorbose-resistant mutations of *N. crassa* map on the right arm of chromosome III, chromosome V, and the left arm of chromosome VI and therefore are clearly unlinked to any of the *dgr* strains.

The map locations of known alleles of *dgr-1*, *dgr-2*, *dgr-3*, and *dgr-4* are summarized in Table 1.

Growth studies. To characterize the phenotypes of the mutants in a more quantitative way, we grew the various *dgr* and *sor* strains in standing cultures for 3 days at 25°C on liquid medium containing Vogel N salts, 1% fructose, and increasing concentrations of 2-deoxyglucose or L-sorbose. In general, similar data were obtained for all of the isolates at any given locus; the results for four representative mutants are illustrated in Fig. 1. Three of the mutants (KHY15 [*dgr-1*], KHY5 [*dgr-2*], and KHY19 [*dgr-3*]) showed a relatively small but reproducible shift in the concentration of 2-deoxyglucose required to give 50% inhibition of growth (IC_{50}): from ca. 0.1 mM in the wild-type strain to ca. 0.3 to 0.6 mM in the mutants. The remaining strain (KHY7, *dgr-4*) was much more resistant, with a 2-deoxyglucose IC_{50} of ca. 9 mM.

A similar test showed that the IC_{50} of sorbose was significantly increased for only two of the mutants: ca. 3 mM for KHY15 (*dgr-1*) and 8 mM for KHY5 (*dgr-2*), compared with ca. 1 mM for the wild-type strain. The other two

TABLE 2. Two- and three-point crosses

| Zygote genotype and recombination % | | | | Parental combinations | No. of recombinants | | | Total germination (%) | Allele no. |
|-------------------------------------|------|--------------|---------------|-----------------------|---------------------|------------------|---------|-----------------------|----------------------------|
| | | | | | Singles region 1 | Singles region 2 | Doubles | | |
| <i>dgr-1</i> | + | + | | 73, 87 | 13 | 31 | 2 | 206 (96%) | KHY15 KH101(r) 33933 |
| + | 7.3 | <i>caf-1</i> | 16.0 | | | | | | |
| + | + | | | 30, 30 | 19 | 2 | 0 | 81 (81%) | KHY5 R156 B369 |
| <i>leu-3</i> | 23.0 | <i>arg-1</i> | 2.5 | | | | | | |
| + | | <i>dgr-3</i> | + | 27, 38 | 11 | 5 | 0 | 82 (82%) | KHY19 R156 B369 |
| <i>leu-3</i> | 13.5 | + | 6.0 | | | | | | |
| + | | <i>dgr-2</i> | | 342 | 16 | | | 358 (89%) | KHY5 KHY19 |
| <i>dgr-3</i> | 8.9 | + | | | | | | | |
| <i>dgr-4</i> | + | + | | 45, 62 | 1 | 54 | 0 | 162 (81%) | KHY2 15300 RU12 |
| + | 0.6 | <i>al-2</i> | 34.0 | | | | | | |
| | | | <i>arg-13</i> | | | | | | |

mutants (KHY19 [*dgr-3*] and KHY7 [*dgr-4*]) were as sensitive as the wild type in liquid medium, even though they were morphologically resistant to sorbose in the standard growth test on agar plates.

High-affinity glucose transport. One obvious mechanism by which cells might become resistant to toxic sugar analogs is by a reduction in sugar transport. *Neurospora* spp. are known to possess two kinetically distinct transport systems for glucose (20, 21, 23): system I is expressed constitutively during growth on glucose or sucrose and has a relatively low affinity for substrates, whereas system II is derepressed during carbon starvation or growth on fructose and has a high affinity. Both systems can transport glucose analogs such as 2-deoxyglucose (20; K. E. Allen, H. S. Lowendorf, and C. W. Slayman, unpublished results) and sorbose (22). To test whether any of the *dgr* mutants might be altered in either of the two glucose transport systems, we measured the uptake of 3-*O*-[¹⁴C]methylglucose (a nonmetabolizable

analog known to be a substrate for both system I and system II [20, 22, 24]). The measurements were made under repressing conditions (glucose- or sucrose-grown cells) and derepressing conditions (fructose-grown cells) over a range of substrate concentrations that allowed the activity of both transport systems to be assessed. The key results are summarized in Fig. 2 and Table 3.

As expected, glucose- and sucrose-grown wild-type cells took up very little substrate at concentrations below 150 mM (Fig. 2), consistent with the virtual absence of system II under these conditions. System I was present, however, as evidenced by the substantial uptake seen at concentrations between 1 and 30 mM (Fig. 2A). In fructose-grown wild-type cells, system I remained and, in addition, system II became derepressed, with a characteristic K_m of 87 μ M and a V_{max} of 6.96 mmol/liter of cell water per min (Table 3). Very similar kinetic parameters were obtained after 2 h of carbon starvation, an alternative route for the derepression of system II (data not shown).

Strikingly, changes in both transport systems were seen in all mutants tested. System I, assayed in either fructose-grown cells (Fig. 2A) or glucose-grown cells (data not shown), was virtually undetectable in KHY15, KHY5, and KHY19 (*dgr-1*, *dgr-2*, and *dgr-3*, respectively) but was significantly enhanced in KHY7 (*dgr-4*). Under the same conditions, system II was expressed constitutively in all the mutants: at intermediate levels in the *dgr-1* and *dgr-3* mutants and at elevated levels in the *dgr-2* and *dgr-4* mutants. Very similar results were obtained when other alleles were tested at each locus or when glucose or 2-deoxyglucose was the transport substrate. Taken together, the results show that none of the *dgr* mutants lacks the ability to transport glucose or its analogs; indeed, when uptake rates via systems I and II are summed for fructose-grown cells, the strain with the highest rate (*dgr-4*) is the one most resistant to 2-deoxyglucose.

Glucose-repressible enzymes. The results in the previous section demonstrate that the resistance of the *dgr* mutants to 2-deoxyglucose cannot be explained simply by a reduction in

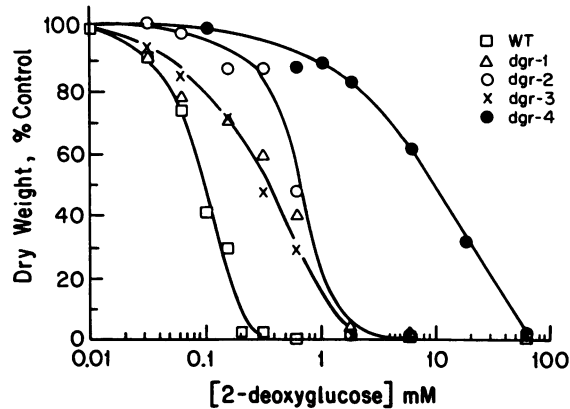


FIG. 1. Inhibition of growth by 2-deoxyglucose. Standing cultures were incubated for 3 days at 25°C, as described in Materials and Methods, and mycelial mats were harvested and weighed. Each point is the mean of triplicate determinations. WT, Wild type.

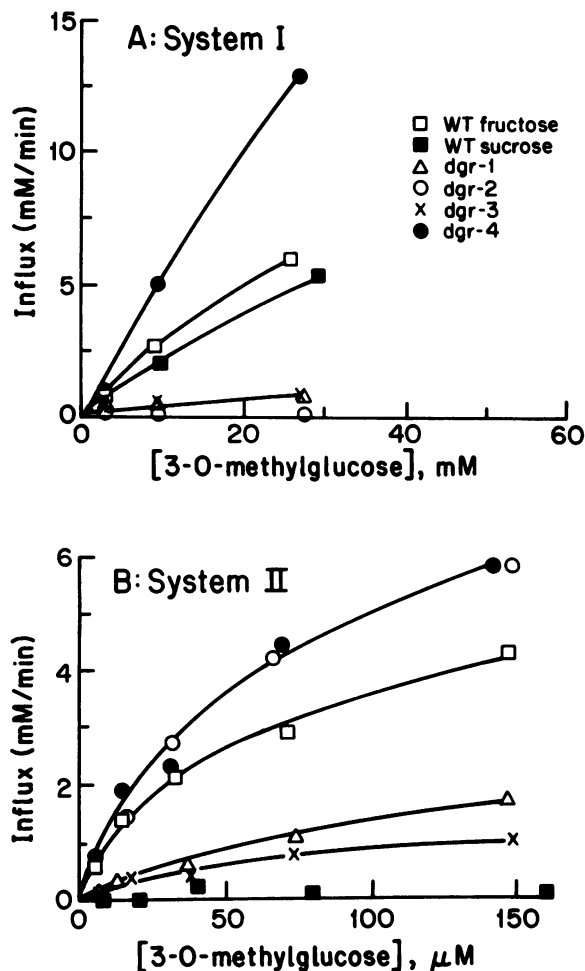


FIG. 2. Concentration dependence of 3-*O*-methylglucose transport. (A) System I; (B) System II. Cells were grown in Vogel medium containing 2% sucrose (RL21a, *dgr-1*, *dgr-2*, *dgr-3*, and *dgr-4*) or 2% fructose (RL21a only), harvested, suspended in flux medium, and assayed for the uptake of 3-*O*-[¹⁴C]methylglucose over a wide range of concentrations (0 to 30 mM) as described in Materials and Methods. Initial rates of uptake were calculated from time points with the linear range (2 min). All values for system I have been corrected for the contribution of system II. At the low concentrations used to assay system II, however, the contribution of system I is negligible and required no correction.

sugar transport. Rather, the pattern of transport changes in systems I and II suggests that the mutants might be altered in some aspect of carbon regulation. To test this hypothesis, we assayed glucose-grown cells for two enzymes known to be glucose repressible in wild-type *Neurospora* spp.: glucamylase (25) and invertase (10). The results are summarized in Table 4. Three of the mutants (KHY15, KHY19, and KHY4 [*dgr-1*, *dgr-3*, and *dgr-4*, respectively]) showed significant increases in the activities of both enzymes, whereas one mutant (KHY5 [*dgr-2*]) showed elevated invertase activity but normal activity for glucamylase.

To determine whether the elevated glucamylase activity arose as a result of an increased rate of synthesis, cells from one of the mutants (KHY7 [*dgr-4*]) and from the wild-type strain were labeled with [³H]leucine for 10 min during growth under repressing conditions (2% glucose). Cellular extracts were prepared, and glucamylase was immunoprecipitated with specific antiserum (25). Less than 0.1% of the

TABLE 3. Kinetic parameters for the high-affinity glucose transport system in wild-type and *dgr* mutants^a

| Strain | Sucrose-grown cells | | Fructose-grown cells | |
|----------------------|---------------------|--------------------|----------------------|--------------------|
| | K_m (mM) | V_{max} (mM/min) | K_m (mM) | V_{max} (mM/min) |
| Wild type (RL21a) | 0.042 | 0.08 | 0.087 | 6.96 |
| <i>dgr-1</i> (KHY15) | 0.171 | 3.47 | 0.080 | 4.44 |
| <i>dgr-2</i> (KHY5) | 0.094 | 8.86 | 0.125 | 6.21 |
| <i>dgr-3</i> (KHY19) | 0.080 | 1.79 | 0.080 | 3.45 |
| <i>dgr-4</i> (KHY7) | 0.063 | 9.09 | ND ^b | ND |

^a Cells were grown in Vogel medium containing 2% sucrose or fructose as the carbon source, suspended, and assayed for 3-*O*-[¹⁴C]methylglucose uptake over a range of concentrations as described in Materials and Methods. The data were computer fitted to the Michaelis-Menten equation to obtain values for K_m and V_{max} ; standard errors in replicate experiments averaged $\pm 15\%$.

^b ND, Not determined.

leucine incorporated by the wild type was precipitated in this experiment, whereas 1.8% of the leucine incorporated by the mutant was precipitated (a value similar to that previously reported for wild-type cells under derepressing conditions [25]). Thus, the rate of glucamylase synthesis is clearly increased in the *dgr-4* mutant. Similar results were obtained for *dgr-1* and *dgr-3* mutants (data not shown).

Finally, two kinds of experiments were carried out to examine the qualitative properties of the invertase produced by the *dgr* mutants. In the first, the thermostability of the enzyme was determined by incubating cell extracts at 60°C. The half time for inactivation was ca. 7 min in the wild-type strain, 4.5 min in KHY15 (*dgr-1*), 3.5 min for KHY5 (*dgr-2*) and KHY19 (*dgr-3*), and 2 min for KHY 7 (*dgr-4*) (Fig. 3); five other mutants [BE52, BEX6, L-1, *sor-4*, and *sor(T9)*] also produced an invertase that was relatively temperature sensitive (data not shown). In the second set of experiments, immunoprecipitation of invertase by two independently prepared polyclonal antisera was measured. More antiserum was required to precipitate the enzyme from mutant extracts than from wild-type extracts (representative results from KHY19 [*dgr-3*] are given in Fig. 4). Because the structural gene for invertase has been mapped to linkage group VR, right of *pab-2* (3%) (10, 21), the amino acid sequence of invertase in the *dgr* mutants should be normal, and the changes seen in thermostability and antigenicity are likely to reflect changes in posttranslational modification.

TABLE 4. Glucamylase and invertase activities in wild-type and *dgr* mutants^a

| Strain | Growth conditions | Activity (μ mol glucose released/min per mg of protein) of: | |
|----------------------|-------------------|--|-----------|
| | | Glucamylase | Invertase |
| Wild-type (RL21a) | Glucose | 0.003 | 0.03 |
| | Carbon starved | 0.021 | 6.5 |
| <i>dgr-1</i> (KHY15) | Glucose | 0.009 | 3.4 |
| <i>dgr-2</i> (KHY5) | Glucose | 0.004 | 2.7 |
| <i>dgr-3</i> (KHY19) | Glucose | 0.028 | 5.6 |
| <i>dgr-4</i> (KHY7) | Glucose | 0.019 | 5.7 |

^a Cells were grown in Vogel medium containing 2% glucose (repressing conditions). Carbon-starved wild-type cells were obtained by incubation of glucose-grown cells in carbon-free medium for 60 min (derepressing conditions). Glucamylase and invertase activities were assayed in cell extracts as described in Materials and Methods. The results are the mean of three determinations; standard errors averaged $\pm 10\%$.

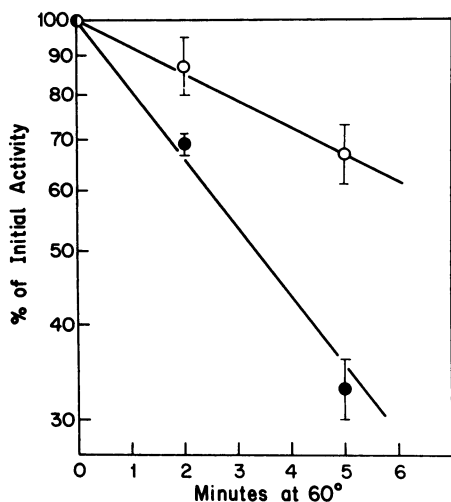


FIG. 3. Thermolability of invertase from *dgr-3*. Cellular extracts from KHY19 and the wild-type strain RL21a were heated to 60°C for various times and then assayed for invertase activity. The remaining activity is plotted as the percentage of initial activity. Symbols: ○, RL21a; ●, KHY19.

DISCUSSION

In this paper, we report the genetic and physiological characterization of *N. crassa* mutants that are resistant to toxic glucose analogs. Thirteen independently isolated mutants have been studied in all: eight KHY strains, newly isolated on the basis of 2-deoxyglucose resistance; three strains (BE52, BEX6, and L-1) previously isolated by Dunn (M.A. dissertation) and Eberhart (*Neurospora* Newsl. 27: 19–20, 1980) in a similar manner; and two strains [*sor-4* and *sor(T9)*] selected by Murayama and Ishikawa (19) for resistance to sorbose. The mutations have been mapped to four distinct genetic loci, which have been named *dgr-1*, *dgr-2*, *dgr-3*, and *dgr-4*.

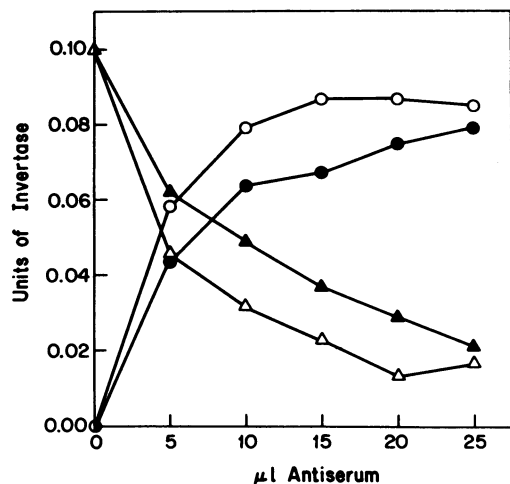


FIG. 4. Determination of titer of invertase from *dgr-3*. Invertase (0.1 U) from the *dgr-3* strain, KHY19, and the wild-type strain, RL21a, was subjected to indirect immunoprecipitation by using increasing amounts of antiserum. The amounts of enzymatic activity present in the immunoprecipitate and the supernatant were determined by assay. Symbols: ○, RL21a immunoprecipitate; ●, KHY19 immunoprecipitate; △, RL21a supernatant; ▲, KHY19 supernatant.

Although the actual *dgr* gene products have not yet been identified, it seems likely that they play either a direct or an indirect role in the regulation of carbon metabolism in *Neurospora* spp. All of the mutants display pleiotropic alterations in enzymes that are normally glucose repressible. Glucamylase, invertase, and the high-affinity glucose transport system are synthesized constitutively in the *dgr* strains, and at least for invertase, there are qualitative changes in thermostability and immunological properties that may reflect abnormalities in glycosylation or other posttranslational processing.

The finding that the mutants also show changes in the low-affinity glucose transport system, which has generally been thought to be constitutive (20, 22, 23), is of particular interest. Three of the *dgr* mutants appear to lack system I, whereas one mutant displays elevated activity for this system. These results may point to an unexpected role of carbon-regulatory genes in transcriptional, translational, or posttranslational regulation of system I or, alternatively, may arise from some physiological cross-regulation between systems I and II. Tests of these and other hypotheses will be possible once the structural genes for the two transport systems have been cloned and the corresponding proteins identified.

In the meantime, the *dgr* mutants should prove useful in dissecting the physiological roles of systems I and II. For example, because system II is known to be driven by the proton electrochemical gradient across the plasma membrane (26), its thermodynamic characterization requires measurement of the steady-state gradient of substrate that can be accumulated across the membrane as a function of the pH gradient and the membrane potential. In wild-type *Neurospora* spp., such measurements are complicated by the presence of the low-affinity system I, which can be expected to act as a shunt for glucose and prevent the build-up of high intracellular sugar concentrations by system II. Indeed, preliminary experiments (Allen et al., unpublished) have shown that the maximal ratio of [3-*O*-methylglucose]_{in} to [3-*O*-methylglucose]_{out} rises from ca. 6,000 in the wild-type strain RL21a to ca. 12,000 in KHY5 (*dgr-2*), which lacks system I but possesses a high level of system II. Thus, the way may now be clear to undertake quantitative studies of the bioenergetics of the H⁺-linked sugar transport in *Neurospora* spp.

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