Ionic Remediability of a Mutational Transport Defect in *Chlamydomonas*

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A modifying gene (*mod-1*) influences the utilization of exogenously supplied quinolinic and nicotinic acids by strains of *Chlamydomonas eugametos* containing either of two different nicotinamide-auxotrophic genes (*nic-5* and *nic-6*). Although strains containing the *nic-5* gene can grow on minimal medium supplemented with nicotinamide, and strains containing the *nic-6* gene can grow on minimal media supplemented with either nicotinic or quinolinic acid, double mutants containing one of the auxotrophic genes and the modifier (*nic-5 mod-1* or *nic-6 mod-1*) do not grow on media supplemented with nicotinic or quinolinic acid (or both). The modifying gene has no demonstrable effect on the utilization of exogenously supplied nicotinamide (K. Nakamura and C. S. Gowans, Genetics 51: 931, 1965).

Two observations strongly indicate that the modifying gene does not operate through a modification of the pathway of synthesis of niacin. First, the nicotinamide auxotrophs carrying the modifying gene grow normally on media supplemented with nicotinamide, even though it appears, in a variety of organisms, that nicotinamide is first deamidated to nicotinic acid before its utilization in the synthesis of nicotinamide adenine dinucleotide (J. Preiss and P. Handler, J. Biol. Chem. 233: 493, 1958). Second, strains carrying *mod-1* alone (no nicotinamide-auxotrophic genes) grow normally on minimal medium, and therefore are utilizing endogenously synthesized quinolinic and nicotinic acids. It is thus most likely that the modifying gene exerts its effect on a transport mechanism involving nicotinic and quinolinic acids.

Two mutant strains were utilized in these experiments: (i) Mo 11, which carries the *nic-6* nicotinamide-auxotrophic gene and the *mod-1* modifying gene, and which therefore grows on minimal medium only when it is supplemented with nicotinamide (cannot grow on nicotinic or quinolinic acid); and (ii) N554-3, which also carries the *nic-6* gene but has the wild-type allele at the *mod-1* locus, and which therefore grows on standard minimal medium when supplemented with nicotinamide, nicotinic acid, or quinolinic acid. The media used were standard minimal medium (C. S. Gowans, Z. Vererbungslehre 91: 63, 1960) and tris(hydroxymethyl)aminomethane (Tris) minimal medium. Tris minimal medium is standard minimal medium with the phosphate buffer replaced by 0.025 M Tris chloride (adjusted to pH 7.2). Tris medium prevents the precipitation of added calcium, but reduces growth to one-third to one-half the growth in standard medium. Media were supplemented with 5 mM quinolinic acid. The stock solution of this supplement was previously adjusted to pH 7.0 with NH₄OH. Cultures were grown in 5 ml of the various testing media in test tubes (18 by 150 mm) for 6 days at 25°C under fluorescent lights (ca. 350 ft-candles). Growth was measured as turbidity as previously described (K. Nakamura and C. S. Gowans, Genetics 51: 931, 1965).

The first indication of the ionic remediability of the *mod-1* phenotype was the observation that, although Mo 11 (*nic-6 mod-1*) would not grow on minimal medium supplemented with quinolinic acid, the extra addition of 50 mM KCl would permit growth in this medium. Remediability is not due to increased osmotic pressure of the medium, since glycerol, sucrose, and glucose (5, 10, 50, 100, and 150 mM) failed to produce the effect. Salts of potassium, ammonium, calcium, and magnesium also proved effective in permitting growth of Mo 11 in media supplemented with quinolinic acid (Fig. 1). The results with sodium salts were inconclusive, possibly owing to the less rapid permeability of *Chlamydomonas* to sodium (R. R. Ronkin and K. M. Buretz, J. Protozool. 7: 109, 1960). In tests with nicotinic acid (20 mM) instead of quinolinic acid, the results were also inconclusive, probably partially owing to the lower efficiency of the vitamin in supporting the growth of even strain N554-3 under the conditions employed.

Optimal remedial concentrations of added salts were constant for a given cation, and not for anion (Fig. 1). Total ionic strength [ionic strength

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FIG. 1. Effects of various salts on the growth of (solid lines) nic-6-mod-1 (Mo 11) and of (broken lines) nic-6 mod-1+ (N554-3) in Tris minimal medium supplemented with quinolinic acid.
(μ) = \frac{1}{2} \left( \frac{\text{gram-ion}}{1,000 \ g \ \text{of solvent}} \times \text{valence}^2 \right) \] (F. J. Ryan et al., J. Bacteriol. 65:434, 1953) does not explain the results.

It is not known whether or not mod-1 influences the actual uptake of cations, but a large cation uptake difference between mod-1 stocks and wild type is made unlikely by the normal growth of mod-1 stocks (without auxotrophic genes) on standard minimal medium. It seems likely that the cations are involved in a carrier mechanism or energy supply mechanism in the transport of quinolinic acid, either at the membrane or intracellularly.

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