Lectin-Resistant CHO Cells: Selection of Seven New Mutants Resistant to Ricin

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Abstract—In attempts to isolate new CHO glycosylation mutants, selection protocols using plant lectins that bind galactose residues of cell surface carbohydrates were applied to mutagenized CHO populations. The lectins were used alone or in combination to obtain seven ricin-resistant phenotypes. Each mutant had distinctive properties compared with previously described ricin-resistant CHO cells. One of the new phenotypes was dominant in somatic cell hybrids, and the others were recessive. Complementation analyses between related lectin-resistant (LecR) phenotypes indicated that each new isolate represented a novel genotype. Five of the mutants had properties typical of new CHO glycosylation mutants. The remaining two mutants were not readily categorized. Although they did not appear to be ricin-internalization or protein-synthesis mutants, they also did not display the marked alterations in sensitivity to several lectins of different sugar specificity expected for glycosylation mutants. The seven new LecR mutants described in these studies brings the total number of different LecR CHO mutants isolated by this and other laboratories to about 40. Criteria for identifying new LecR mutations in CHO cells are discussed.

INTRODUCTION

Lectin-resistant mutants that are affected in glycosylation reactions have been extremely useful in defining the pathways of carbohydrate biosynthesis in mammalian cells and in providing cell lines with which to investigate the functions of carbohydrates (1-4). Of equal importance is the potential that these mutants offer for cloning glycosylation enzymes that are difficult to purify, by transfection and gene rescue (5, 6). Therefore, in order to isolate a complete range of glycosylation mutants that will identify the genes that code for glycosylation enzymes or regulatory molecules involved in mammalian carbohydrate biosynthesis, we have continued to use cytotoxic plant lectins as selective agents. Because of their exquisite specificities for mammalian carbohydrates and also their broad range of affinities for related structures (7), lectins offer great potential for isolating many different glycosylation mutants that express altered cell surface carbohydrates.

Previous studies from several laboratories have identified more than 20 different CHO glycosylation mutants that were selected with lectins (1-4). A common characteristic of these mutants is their cross-resistance to lectins not used in their selection and hypersensitivity to lectins that recognize the altered carbohydrates synthesized as a result of their glycosylation defect. This lectin-resistance (LecR) phenotype is distinct both
qualitatively and/or quantitatively for each different glycosylation mutation. Therefore, new glycosylation mutants can be rapidly identified by testing their resistance to a panel of lectins and determining their ability to complement related LecR phenotypes in somatic cell hybrids (8, 9).

Since a large number of LecR CHO mutants, including those affected in glycosylation (1-4), lectin internalization (10), endosome acidification (11-14), or protein synthesis (15, 16) have been isolated, a major concern in designing selection protocols for new mutants is to avoid the repeated isolation of known phenotypes. This can be achieved by using new lectins (14) and/or different lectins in combination as selective agents (17, 18). In this paper we have used combined lectin selections to isolate seven new LecR CHO mutants. Phenotypic and genetic studies provide evidence that the mutants are novel.

MATERIALS AND METHODS

Cell Lines. The CHO auxotrophs Pro-5 and Gat-2 were used in the selection of all mutants (8). Previously isolated mutants that were used for lectin-resistance comparisons and complementation analyses included the single mutants (8, 9) Pro-Lec1.3C (Lec1), Pro-Lec1A.2C (Lec1A), Pro-Lec5.B211 (Lec5), Pro-Lec9.12A (Lec9), Pro-Lec10.3C (Lec10), Pro-Lec15.B4 (Lec15), and the double mutants Pro+Lec9. Lec2.1A (19) and Pro+Lec15.TG (a thio- guanine-resistant, Pro+ revertant of Pro-Lec15.B4). Cells were routinely cultured in suspension in alpha medium (Gibco Laboratories, Grand Island, New York) containing 10% horse serum (Hazeltine Biologies, Lenexa, Kansas) and 1% fetal calf serum (Flow Laboratories, McLean, Virginia). The absence of Mycoplasma contamination was confirmed by cocultivation of CHO cells with a 3T6 indicator strain and subsequent staining with Hoechst 33258 (20).

Mutagenesis. Suspension cultures of Pro-5 or Gat-2 CHO cells at ~2 x 10^6 cells per milliliter were incubated in 100-200 μg/ml ethyl methane sulfonate (EMS; Eastman Kodak, Rochester, New York) or ethyl nitrosourea (ENU; Sigma Chemical Co., St. Louis, Missouri) for 18 h or in 40 ng/ml N-methyl-N-nitrosoguanidine (MNNG; ICN Biomedicals, Costa Mesa, California) for 2 h at 34°C or 37°C. After mutagen treatment, cells were pelleted, washed, and resuspended in culture medium and an aliquot was plated to determine relative plating efficiencies. The latter were routinely ≥50% following mutagen treatment.

Mutant Selections. Cell populations were subjected to selection with the cytotoxic lectins ricin (RIC; EY Laboratories, San Mateo, California); concanavalin A (Con A; Pharmacia, Uppsala, Sweden); the erythroagglutinin from P. vulgaris (E-PHA), the lymphoagglutinin from P. vulgaris (L-PHA), and abrin (ABR) all from Vector Laboratories (Burlingame, California); and modececin (MOD), that was from Pierce Laboratories (Rockford, Illinois) or a gift of Dr. April Robbins (National Institutes of Health). The lectins were used alone, sequentially, or in combination as described in Table 2 below, and selections were always performed in alpha medium containing 10% fetal calf serum at 34°C or 37°C in a humidified atmosphere of 5% CO_2 for 8-12 days.

Lectin-Resistance Phenotype. Colonies arising on selection plates were picked into sterile plastic tubes and tested as soon as possible for their resistance to a panel of lectins by titration in 96-well microtiter dishes as described previously (8). Isolates with apparently novel LecR phenotypes were cloned by limiting dilution and subjected to lectin-resistance tests with eight different lectins, including RIC, ABR, MOD, E-PHA, L-PHA, Con A, wheat-germ agglutinin (WGA; Sigma Chemical Co.) and Lens culinaris agglutinin (LCA; Vector Laboratories). The concentration of lectin giving approximately