Munro, I., Pecht, I., & Stryer, L. (1979) *Proc. Natl. Acad. Sci. U.S.A.* (in press).
Pardee, A. B. (1966) *J. Biol. Chem. 241*, 5886–5892.
Parkinson, J. S. (1974) *Nature (London) 252*, 317–319.
Quiocho, F. A., Gilliland, G. L., & Phillips, G. N., Jr. (1977) *J. Biol. Chem. 252*, 5142–5149.
Schwartz, M., Kellerman, O., Szmelcman, S., & Hazelbauer, G. L. (1976) *Eur. J. Biochem. 71*, 167–170.
Silverman, M., & Simon, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3317–3321.
Springer, M. S., Gay, M. F., & Adler, J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3312–3316.
Strange, P. G., & Koshland, D. E., Jr. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 762–766.
Stryer, L., & Haughland, R. P. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 719–726.
Szmelcman, S., Schwartz, M., Silhavy, T. J., & Boos, W. (1976) *Eur. J. Biochem. 65*, 13–19.
Weber, G. (1952) *Biochem. J. 51*, 145–155.
Weber, G. (1966) in *Fluorescence and Phosphorescence Analysis* (Hercules, D. M., Ed.) pp 217–240, Interscience, New York.
Willis, R. C., Morris, R. G., Curakoglu, C., Schellenberg, G. D., Gerber, N. H., & Furlong, C. E. (1974) *Arch. Biochem. Biophys. 161*, 64–75.
Wu, C. W., & Stryer, L. (1972) *Proc. Natl. Acad. Sci. U.S.A. 64*, 1104–1108.
Yashinsky, G. Y. (1972) *FEBS Lett. 26*, 123–126.
Zukin, R. S., Hartig, P. R., & Koshland, D. E., Jr. (1977a) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1932–1936.
Zukin, R. S., Strange, P. G., Heavey, L. R., & Koshland, D. E., Jr. (1977b) *Biochemistry 16*, 381–386.

Variations in the Sialic Acid Compositions in Glycoproteins of Mouse Ascites Tumor Cell Surfaces†

John F. Codington,* George Klein, Cyla Silber, Keyes B. Linsley, and Roger W. Jeanloz

ABSTRACT: Sialic acid removable by *Vibrio cholerae* neuraminidase from four TA3 mammary carcinoma ascites sublines of the strain A mouse and six TA3-Ha/A.CA hybrid cell lines, in ascites form, resulting from fusion of TA3-Ha cells and normal fibroblasts of the A.CA mouse and adapted for growth in the strain A mouse [Klein, G., et al. (1972) *J. Exp. Med. 135*, 839], consisted of mixtures of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid (NeuNGl). Total cell-surface sialic acid ($\mu g/10^9$ cells) and proportion of NeuNGl (%) averaged, respectively, as follows: TA3-St, 270 μg, 20%; TA3-Ha, 620 μg, 7%; TA3-MM/1, 850 μg, 12%; TA3-MM/2, 1200 μg, 12%; TA3-Ha/A.CA/3B, 700 μg, 13%; TA3-Ha/A.CA/4, 870 μg, 19%; TA3-Ha/A.CA/6, 1180 μg, 17%; TA3-Ha/A.CA/7, 910 μg, 38%; TA3-Ha/A.CA/10, 470 μg, 17%; and TA3-Ha/A.CA/11, 850 μg, 6%. Fractions of effluents obtained by gel filtration chromatography of glycopeptides cleaved from viable cells by proteolysis were analyzed for carbohydrate composition and proportion of NeuNGl. Glycopeptide fractions from the same cells possessed markedly different proportions of NeuNGl. The proportion of NeuNGl increased consistently with the percent of mannose in the carbohydrate moiety and decreased with the percent of *N*-acetylgalactosamine and with the apparent molecular weights (by gel filtration). It was concluded that, in the TA3 tumor system, the proportion of NeuNGl in the sialic acid of cell surface glycoproteins of an ascites cell line correlates directly with the proportion of *N*-glycosyl-linked carbohydrate chains in the glycoproteins.

Many diverse functions have been attributed to sialic acid at the mammalian cell surface. These include the demonstration that masking of penultimate galactose residues in cell-surface glycoproteins may prolong the existence of erythrocytes (Aminoff et al., 1977), platelets (Greenburg et al., 1977), or lymphocytes (Woodruff & Gesner, 1969) in the circulation. Sialic acid may serve as a receptor site for myxo- and paramyxoviruses (Scheid & Choppin, 1974), and it may act as an essential component of both the M and N blood group specific determinants at the surface of erythrocytes (Lisowska & Duk, 1976). It has been shown to bind calcium ions at the cell surface by virtue of its three-carbon hydroxylated segment (Jaques et al., 1977); and it has been suggested that the property of aggregation in mammalian cells is modified by the presence of its negatively charged carboxylic acid group (Deman & Bruyneel, 1975).

Sialic acid does not appear to play a general or universal role in malignancy. Indeed, the concentration of sialic acid may vary widely from one tumor cell type to another, and in the case of certain cells grown in vitro its concentration may be greater in normal than in transformed cells (Jeanloz & Codington, 1976). Yet, the reduced allotransplantability of many tumor cell types at low inocula (10^3 to 10^4 cells per mouse) after sialic acid removal by neuraminidase is well documented (Jeanloz & Codington, 1976; Rios & Simmons, 1973; Bekesi et al., 1976). This effect appears to be primarily due to the exposure of new immunogenic galactose residues, which may also serve as receptors for cytotoxic γM antibodies. However, the removal of sialic acid residues from TA3-Ha mammary carcinoma cell surfaces was not found to exert any detectable effect upon transplantability in foreign mouse strains at inocula of 10^5 to 10^6 cells per mouse (Sanford et al., 1973).

† From the Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114 (J.F.C., C.S., K.B.L., and R.W.J.), and the Department of Tumor Biology, Karolinska Institutet, Stockholm 60, Sweden (G.K.). *Received October 27, 1978.* This is publication No. 769 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School and Massachusetts General Hospital. This investigation was supported by Research Grants CA-08418 (to R.W.J.) and CA-18600 (to J.F.C.) and 5-R01-CA-14054 (to G.K.) from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

0006-2960/79/0418-2145$01.00/0

Consistent with this finding was the observation that sialic acid removal did not alter the capacity of cells in the TA3 mammary carcinoma tumor system to absorb antibody to H-2^a antigens (Sanford et al., 1973). The properties of allotransplantability and reduced antibody absorption in this tumor have been attributed to masking of cell-surface antigens by the large and abundant cell-surface glycoprotein epiglycanin (Sanford et al., 1973; Codington et al., 1973, 1978).

Recent reports have suggested that a correlation exists between cell-surface sialic acid and metastatic properties in a number of PW 20 polyoma-induced renal carcinoma variants (Yogeeswaran & Salk, 1978). Accordingly, it appeared important to reexamine the role of cell-surface sialic acid in the allotransplantability of a number of TA3 tumor sublines and six hybrid lines resulting from fusion of the nonstrain-specific TA3-Ha cell line and normal embryonic fibroblasts of the A.CA mouse. It was also considered expedient to investigate whether any specific function in malignancy could be attributed to either of the two forms of sialic acid present at the surfaces of these cells, *N*-acetylneuraminic acid (NeuNAc) or *N*-glycolylneuraminic acid (NeuNGl).

No unique function has yet been attributed to NeuNGl. It is present in most mammals, often in the O-acylated form, but the proportion of this component is generally minor compared with that of *N*-acetylneuraminic acid (NeuNAc) (Schauer et al., 1974). According to Buscher et al. (1977), *N*-acetylneuraminic acid, either free or glycoprotein bound, may serve as a precursor to its *N*-glycolyl derivatives. To our knowledge, no information is yet available regarding any physicochemical basis in the substrate for the specificity of the hydroxylating enzyme, *N*-acetylneuraminate monooxygenase (Buscher et al., 1977), or for the specificity of the transferase(s) in the reaction of CMP-NeuNAc or CMP-NeuNGl with carbohydrate chains of glycoproteins slated for passage to the cell surface. It was thus of great interest that, during the course of this investigation, information regarding substrate specificity in the biosynthesis of NeuNGl was obtained.

Materials and Methods

Cell Lines and Growth of Cells. All cell lines employed were grown in ascites form in male A/HeJ mice obtained from the Jackson Laboratory, Bar Harbor, ME. Harvests of cells were performed on day 7 following inocula of 10^5 TA3-Ha, TA3-MM/1, or TA3-MM/2 cells or 10^6 cells of each other cell line. The origin and characteristics of the TA3-Ha and TA3-St cell lines have been described (Klein et al., 1972; Friberg, 1972). The six TA3-Ha/A.CA hybrid cell lines utilized in this study resulted from fusion in vitro of TA3-Ha cells and normal fibroblasts of the A.CA mouse (Klein et al., 1973). The origin and development of the ascites forms of these hybrid lines have been described (Codington et al., 1978; Klein et al., 1973; Friberg et al., 1973). Some chemical and immunological characteristics of the hybrid cells at passage numbers equivalent to those employed in this study have been reported (Codington et al., 1978). The nonstrain specific TA3-MM cell line resulted from a change in the strain-specific TA3-St cell in ascites form in A/HeHa mice infected with pneumonia-producing microorganisms. Two sublines, TA3-MM/1 and TA3-MM/2, were established (Cooper et al., 1979; Codington et al., 1979).

Enzyme Reactions. Sialic acid was released from 5–10 × 10^7 intact cells by incubation with 250–500 units of neuraminidase (*Vibrio cholerae*, 500 units/mL, Behring Diagnostics, Somerville, NJ) in 4 mL of phosphate-buffered saline (Dulbecco & Vogt, 1954) at 37 °C for 80 min. Glycoprotein fragments were cleaved from intact cells (2–5 × 10^7/mL) by the action of L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin (Worthington Biochemical Corp., Freehold, NJ) at a concentration of 18 μg/mL during four 30-min incubations at 4 °C, as previously described (Codington et al., 1972). Fractionations of glycopeptides were performed by gel filtration, as previously reported (Codington et al., 1972).

Sialic acid released by neuraminidase was determined by the thiobarbituric acid method (Warren, 1959; Codington et al., 1976). *Vibrio cholerae* neuraminidase has been shown to cleave either NeuNAc[1] or NeuNGl linked by 2→3 or 2→6 bonds (Schauer & Faillard, 1968). Sialic acid with an acetyl substituent in the C-4 position is not a substrate for the enzyme, and an acetyl group at either C-7 or C-8 retards enzyme activity. To determine the proportion of NeuNGl, a solution containing sialic acid was made salt free by ion-exchange chromatography, and the residue was converted into the methyl glycoside–methyl esters by heating with methanol–hydrogen chloride (0.5 M) at 65 °C for 60 min, as previously described (Codington et al., 1976). After pertrimethylsilylating the mixture, it was fractionated by gas–liquid chromatography in a Perkin-Elmer gas chromatograph (Model 900). The composition of glycoprotein-bound sialic acid was determined in a similar manner (Codington et al., 1976). Methanolysis for 60 min released essentially all bound sialic acid, and de-N-acylation occurred in no more than 5–10% of the sialic acid.

In order to release all carbohydrate components from glycopeptides, the material was heated with 1.0 M methanol–hydrogen chloride at 85 °C for 20 h. Under these conditions all sialic acid was de-N-acylated. After conversion to the N-acetylated derivatives, the mixture was analyzed as the pertrimethylsilylated methyl glycoside–methyl esters by gas–liquid chromatography (Reinhold, 1972). To test for the presence of *O*-acyl groups, an aliquot of each sample, containing either free or bound sialic acid, was incubated for 20 min with 0.1 M sodium hydroxide, followed by neutralization with a cation exchanger (AG 50W-X8, hydrogen form, 100–200 mesh, Bio-Rad Laboratories, Richmond, CA). In parallel experiments, bound sialic acid was cleaved by either neuraminidase or 0.5 M sulfuric acid at 80 °C for 60 min prior to treatment with alkali and subsequent analysis. The sialic acid contents of both alkali-treated and untreated samples were determined by the thiobarbituric acid method of Warren (1959). The lower color yields of sialic acids substituted with acetyl groups in various positions have been reviewed (Ledeen & Yu, 1976).

Results and Discussion

Vibrio cholerae neuraminidase removed only two forms of sialic acid, NeuNAc and NeuNGl, from four TA3 mammary carcinoma ascites sublines and six TA3-Ha/A.CA hybrid lines. It appears probable that little or no O-acylated sialic acid derivatives are present at the cell surfaces of these murine tumor cells, since no evidence for these forms of sialic acid could be found in material removed either by neuraminidase or by proteolysis at 4 °C, followed by treatment of the digest with 0.05 M H_2SO_4 (60 min at 80 °C), a procedure which cleaved approximately 60–80% as much sialic acid as was removed from viable cells by neuraminidase. The total amounts of sialic acid removable from ten cell lines by neuraminidase at 37 °C are presented in Table I. These

[1] Abbreviations used: NeuNAc, *N*-acetylneuraminic acid; NeuNGl, *N*-glycolylneuraminic acid; CMP, cytidine monophosphate; GalNAc, *N*-acetylgalactosamine; Man, mannose; Gal, galactose; ManNAc, *N*-acetylmannosamine.

Table I: Amount and Composition of Sialic Acid Removed from Mouse Ascites Cells by *V. cholerae* Neuraminidase

cell line	total sialic acid (μg/10^9 cells)	proportion NeuNGl (%)
mammary carcinomas		
TA3-St	270	20
TA3-Ha	620	7
TA3-MM/1	850	12
TA3-MM/2	1200	12
hybrid cells		
TA3-Ha/A.CA/3B	700	13
TA3-Ha/A.CA/4	870	19
TA3-Ha/A.CA/6	1180	17
TA3-Ha/A.CA/7	910	38
TA3-Ha/A.CA/10	470	17
TA3-Ha/A.CA/11	850	6

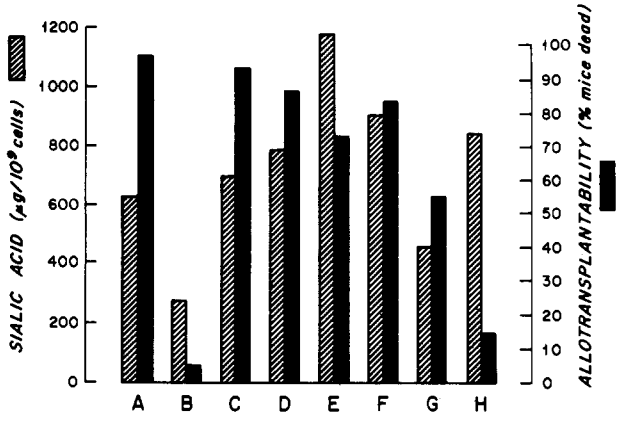

FIGURE 1: Comparison of allotransplantability (% of inoculated mice of foreign strains dead of progressively growing tumors within 30 days) and the total cell-surface sialic acid (μg/10^9 cells). Allotransplantability (solid bars); sialic acid (striped bars). Letters refer to the following cell lines: (A) TA3-Ha; (B) TA3-St; (C) TA3-Ha/A.CA/3B; (D) TA3-Ha/A.Ca/4; (E) TA3-Ha/A.CA/6; (F) TA3-Ha/A.CA/7; (G) TA3-Ha/A.CA/10; (H) TA3-Ha/A.CA/11.

values ranged from approximately 270 μg per 10^9 cells for the strain specific TA3-St subline to about 1200 μg per 10^9 cells for two different cell lines, the TA3-MM/2 and the TA3-Ha/A.CA/6 hybrid cell lines. This large value represents approximately 2.4×10^9 sialic acid residues per cell.

The proportion of NeuNGl present in each sample is also given in the table. These values also varied widely, from 6 to 38%. Values for total sialic acid represent averages of three or more independent determinations. Variations in these values obtained from cells of one passage to those obtained from cells of a later passage were significantly greater for the hybrid lines than for the more stable TA3 sublines. By comparison, the compositions of the sialic acid were far more constant and showed little variation from one cell passage to another.

As shown in Figure 1, no significant correlation could be demonstrated between the total sialic acid concentration, or the concentration of either sialic acid form (Table I), and the capacity of the cells to grow progressively in mice of foreign strains (Codington et al., 1978). Correlation between the latter parameter and the capacity of the cells to absorb anti-H-2^a antibody, in reverse order of magnitude (i.e., the restriction on antibody absorption), has been demonstrated. In that investigation (Codington et al., 1978), it was also shown that a significant correlation did exist between the restriction on antibody absorption, the capacity of the cells to grow in mice of foreign strains, and the concentration of a large endogenous cell-surface glycoprotein, epiglycanin. It was suggested that epiglycanin was involved in masking cell-surface antigens

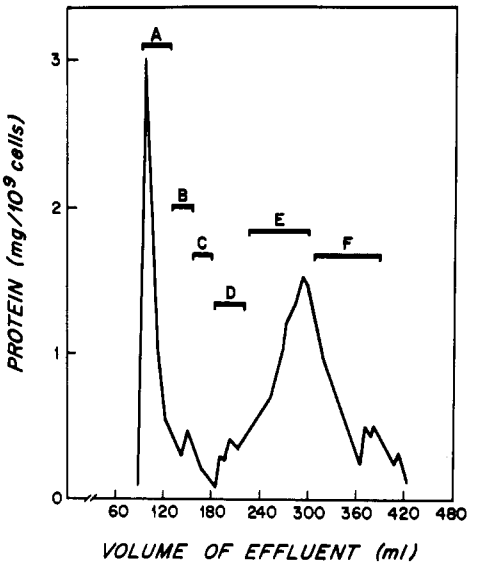

FIGURE 2: Elution profile (protein by Lowry method) of TA3-Ha glycopeptides, removed by proteolysis from 10^9 viable cells from a column of Bio-Gel P-100 (2.5 × 90 cm). Eluent: pyridine acetate, 0.05 M, pH 5.3.

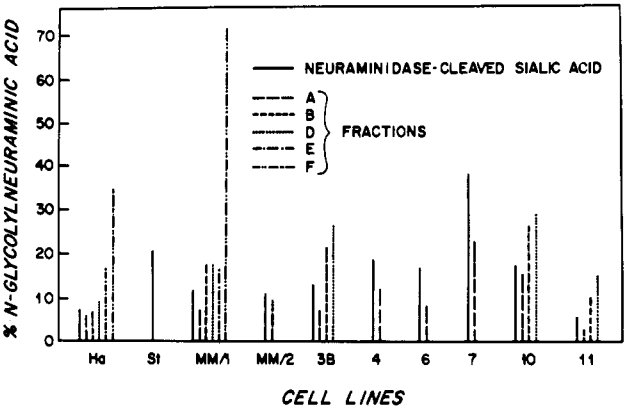

FIGURE 3: Proportion of *N*-glycolylneuraminic acid (%) in total cell-surface sialic acid (—) and in glycopeptide fractions of Bio-Gel P-100 columns (Figure 2) of protease-cleaved material. Fraction A (– – –), B (- - -), D (···), E (-·-·), and F (-···).

(Codington et al., 1973; Sanford et al., 1973) and that allotransplantability in these TA3 sublines and hybrid cell lines was due to this effect (Codington et al., 1978).

Incubation of viable cells in the TA3 ascites cell system with a modified trypsin at 4 °C cleaved a large proportion of the protein and carbohydrate material from the cell surface. Partial fractionation of the glycopeptides could be readily achieved by passage through a gel-filtration column, and this is illustrated in Figure 2 by a fractionation of material cleaved from the TA3-Ha cell surface by this method. The effluent was divided into six fractions, A–F (Figure 2), which were lyophilized. The composition of the bound sialic acid, as well as the total carbohydrate composition, was determined for each fraction. The proportions of NeuNGl in the sialic acid of each fraction are presented in Figure 3. Sufficient material was available for only two cell lines, TA3-Ha and TA3-MM/1, to give significant results for five of the fractions, A, B, D, E, and F. For three additional cell lines values for three fractions, A, B, and D, were obtained. In some of the fractions, the percent of total carbohydrate was too low, less than 5%, for significant sialic acid measurements to be made.

For each cell line, the lowest proportion of NeuNGl was found in fraction A, the fraction with the largest apparent molecular weight (by gel filtration, Figure 2) and the fraction

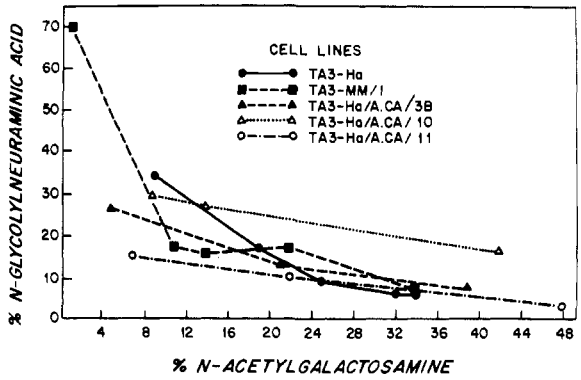

FIGURE 4: Plot of the proportion of *N*-glycolylneuraminic acid (%) in glycopeptide fractions cleaved by proteolysis from viable cells and isolated from a column of Bio-Gel P-100 (Figure 2) vs. the proportion (%) of GalNAc in the carbohydrate moiety, as determined by gas–liquid chromatography. Cell lines: TA3-Ha (●—●); TA3-MM/1 (■--■); TA3-Ha/A.CA/3B (▲--▲); TA3-Ha/A.CA/10 (△···△); TA3-Ha/A.CA/11 (○-·-○).

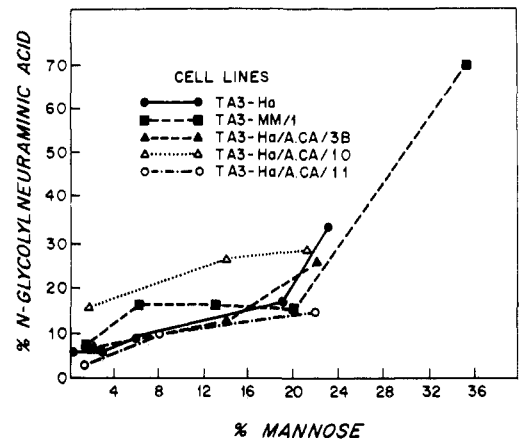

FIGURE 5: Plot of proportion of *N*-glycolylneuraminic acid (%) in fractions, as described in Figure 4, vs. the proportion of mannose in the carbohydrate moiety, as determined by gas–liquid chromatography. Cell lines: TA3-Ha (●—●); TA3-MM/1 (■--■); TA3-Ha/A.CA/3B (▲--▲); TA3-Ha/A.CA/10 (△···△); TA3-Ha/A.CA/11 (○-·-○).

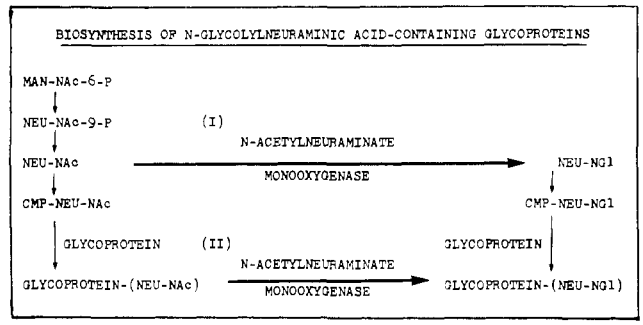

FIGURE 6: Proposed mechanisms for the biosynthesis of *N*-glycolylneuraminic acid, according to Buscher et al. (1977).

which contained epiglycanin material. These values were consistently less than those for samples removed from viable cells by neuraminidase (first bar, Figure 3). The proportions of NeuNGl were consistently greater with each successive fraction eluted from the column, with one exception, fraction E from the TA3-MM/1 cell.

These findings represent the first demonstration that different cell-surface glycoproteins from the same cell line may contain markedly different sialic acid compositions. It has previously been shown that variations in sialic acid composition may occur in different species and in different tissues of the same animal (Schauer et al., 1974; Ng & Dain, 1976).

The correlation of an increasing proportion of NeuNGl with decreasing apparent molecular weight (Figure 3) was observed with protease-digested material, for which no information regarding molecular weights of the native cell-surface macromolecules, with the exception of epiglycanin (500 000), was available. Since for some glycoproteins the rate of proteolysis may be retarded or prevented by the presence of carbohydrate chains (Wang & Hirs, 1977), the possibility was considered that the inverse correlation between the proportion of NeuNGl and the apparent molecular weight was due, at least in part, to the greater frequency of carbohydrate chains, which may prevent proteolysis, in the *O*-glycoproteins, as compared with the *N*-glycoproteins. Accordingly, the proportion of NeuNGl was plotted against the percent of each of two carbohydrate components, *N*-acetylgalactosamine (GalNAc) (Figure 4) and mannose (Man) (Figure 5). The percentage of GalNAc was believed to be approximately proportional to the proportion of *O*-glycosyl-linked carbohydrate chains, and the percentage of Man was considered roughly proportional to the *N*-glycosyl-linked chains. In each figure the slopes of the curves for the five cell lines are in the same direction and indicate a direct correlation in each cell line between the relative number of *N*-glycosyl- (asparagine-) linked chains in the carbohydrate moiety of the glycoprotein fragments A–F and the proportion of NeuNGl. They do not suggest any relationship between the actual percent of *N*-glycoproteins and the proportion of NeuNGl in a particular cell line.

These results do not indicate which of the two pathways proposed for the biosynthesis of NeuNGl in mammalian glycoproteins (Buscher et al., 1977), as illustrated in Figure 6, is employed in the TA3 mammary carcinoma tumor system, but they do suggest how differences in enzyme concentrations and substrate structures may determine the sialic acid compositions. Buscher et al. (1977) have demonstrated that in porcine submandibular gland slices the hydroxylation of both free and glycoprotein-bound NeuNAc required the enzyme *N*-acetylneuraminate monooxygenase. If a pool of CMP-sialic acids exists (mechanism I), the proportion of each form in a particular cell-surface glycoprotein would depend upon the specificity of the sialyl transferase(s) for each glycoprotein substrate. On the other hand, if mechanism II were preferred, each of two major possibilities could explain the specificity of the reaction. This may be related solely to kinetic factors, and since it has been demonstrated that the biosynthesis of epiglycanin (fraction A, Figure 2) is extremely rapid (Miller & Cooper, 1978), it appears plausible that the hydroxylation reaction in glycoproteins which possess mainly *O*-glycosyl linkages is less rapid, and thus less complete, than that in the possibly slower synthesized glycoproteins containing predominantly *N*-glycosyl-linked carbohydrate chains. Another possible explanation is that the high frequency of carbohydrate chains (only 20–30% of which contain sialic acid residues) in the *O*-glycoprotein molecules may result in steric blocking of enzyme–substrate complex formation. On the other hand, the differences in the proportion of NeuNGl may lie in the specificity of the hydroxylating enzyme. In vitro studies with the purified enzyme and the desialylated glycopeptide fractions A–F might help to clarify this question.

Acknowledgments

We thank Mary McDonough, Marianne Jahnke, and Mary D. Maxfield for technical assistance.

References

Aminoff, D., Bruegge, W. F. V., Bell, W. C., Sarpolis, K., & Williams, R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1521.

Bekesi, J. G., Roboz, J. P., & Holland, J. F. (1976) *Ann. N.Y. Acad. Sci. 277*, 313.
Buscher, H. P., Casals-Stenzel, J., Schauer, R., & Mestres-Ventura, P. (1977) *Eur. J. Biochem. 77*, 297.
Codington, J. F., Sanford, B. H., & Jeanloz, R. W. (1972) *Biochemistry 11*, 2559.
Codington, J. F., Sanford, B. H., & Jeanloz, R. W. (1973) *J. Natl. Cancer Inst. 51*, 585.
Codington, J. F., Linsley, K. B., & Silber, C. (1976) *Methods Carbohydr. Chem. 7*, 226.
Codington, J. F., Klein, G., Cooper, A. G., Lee, N., Brown, M. C., & Jeanloz, R. W. (1978) *J. Natl. Cancer Inst. 60*, 811.
Codington, J. F., Cooper, A. G., Miller, D. K., Slayter, H. S., Brown, M. C., Silber, C., & Jeanloz, R. W. (1979) *J. Natl. Cancer Inst.* (in press).
Cooper, A. G., Codington, J. F., Miller, D. K., & Brown, M. C. (1979) *J. Natl. Cancer Inst.* (in press).
Deman, J. J., & Bruyneel, E. A. (1975) *Biochem. Biophys. Res. Commun. 62*, 895.
Dulbecco, R., & Vogt, M. (1954) *J. Exp. Med. 99*, 167.
Friberg, S., Jr. (1972) *J. Natl. Cancer Inst. 48*, 1463.
Friberg, S., Jr., Klein, G., Wiener, F., & Harris, H. (1973) *J. Natl. Cancer Inst. 50*, 1269.
Greenberg, J. P., Rand, M. L., & Packham, M. A. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36*, 453.
Jaques, L. W., Brown, E. B., Barrett, J. M., Brey, W. S., Jr., & Weltner, W., Jr. (1977) *J. Biol. Chem. 252*, 4533.
Jeanloz, R. W., & Codington, J. F. (1976) *Biological Roles of Sialic Acid*, p 201, Plenum Press, New York.
Klein, G., Friberg, S., Jr., & Harris, H. (1972) *J. Exp. Med. 135*, 839.
Klein, G., Friberg, S., Jr., Wiener, F., & Harris, H. (1973) *J. Natl. Cancer Inst. 50*, 1259.
Ledeen, R. W., & Yu, R. K. (1976) *Biological Roles of Sialic Acid*, p 1, Plenum Press, New York.
Lisowska, E., & Duk, M. (1976) *Arch. Immunol. Therap. Exp. 24*, 39.
Miller, D. K., & Cooper, A. G. (1978) *J. Biol. Chem. 253*, 8798.
Ng, S.-S., & Dain, J. A. (1976) *Biological Roles of Sialic Acid*, p 59, Plenum Press, New York.
Reinhold, V. N. (1972) *Methods Enzymol. 25B*, 244.
Rios, A., & Simmons, R. L. (1973) *J. Natl. Cancer Inst. 51*, 637.
Sanford, B. H., Codington, J. F., Jeanloz, R. W., & Palmer, P. D. (1973) *J. Immunol. 110*, 1233.
Schauer, R., & Faillard, H. (1968) *Hoppe-Seylers Z. Physiol. Chem. 349*, 961.
Schauer, R., Buscher, H. P., & Ferreira do Amaral, C. (1974) *Methodology of the Structure and Metabolism of the Glycoconjugates*, p 1075, CNRS, Paris, France.
Scheid, A., & Choppin, P. W. (1974) *Virology 57*, 475.
Wang, F.-F. C., & Hirs, C. H. W. (1977) *J. Biol. Chem. 252*, 8358.
Warren, L. (1959) *J. Biol. Chem. 234*, 1971.
Woodruff, J. J., & Gesner, B. M. (1969) *J. Exp. Med. 129*, 551.
Yogeeswaran, G., & Salk, P. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1299.

Antibiotic Effects on the Photoinduced Affinity Labeling of *Escherichia coli* Ribosomes by Puromycin†

P. G. Grant, W. A. Strycharz, E. N. Jaynes, Jr., and B. S. Cooperman*

ABSTRACT: The effect of ribosomal antibiotics on the photoinduced affinity labeling of *Escherichia coli* ribosomes by puromycin [Cooperman, B. S., Jaynes, E. N., Brunswick, D. J., & Luddy, M. A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2974; Jaynes, E. N., Jr., Grant, P. G., Giangrande, G., Wieder, R., & Cooperman, B. S. (1978) *Biochemistry 17*, 561] has been studied. Although blasticidin S, sparsomycin, lincomycin, and erythromycin are essentially without effect, major changes are seen on addition of either chloramphenicol or tetracycline. The products of photoincorporation have been characterized by one- and two-dimensional gel electrophoresis and by specific immunoprecipitation with antibodies to ribosomal proteins. In the presence of chloramphenicol, protein S14 becomes the major labeled protein. In the presence of tetracycline, L23 remains the major labeled protein, but the yield of labeled ribosomes is enormously increased, and the labeling is more specific for L23. These results are discussed in terms of the known modes of action of these antibiotics and the photoreactivity of tetracycline.

A current goal of research on *Escherichia coli* ribosomes is the development of a structure–function map permitting localization of given ribosomal functions to specific regions of the ribosome. We have been using the technique of photoaffinity labeling as an approach to this problem. In previous work, we showed that when ribosomes are irradiated in the presence of the antibiotic puromycin, the protein L23 is the major site of puromycin incorporation (Cooperman et al., 1975) and that such incorporation proceeds via a true affinity labeling process (Jaynes et al., 1978). Puromycin is unique among naturally occurring ribosomal antibiotics in being a substrate for the ribosome-catalyzed peptidyl transferase reaction. Many other ribosomal antibiotics have been shown to inhibit this reaction, with some acting as apparent competitive inhibitors toward puromycin. In addition, several antibiotics have been shown to induce significant confor-

† From the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104 (P.G.G., E.N.J., and B.S.C), and the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53706 (W.A.S.). *Received December 19, 1978.* This work was supported by research grants from the National Institutes of Health (AM-13212 and AI-14717 to B.S.C.) and the National Science Foundation (PCM-84278 to B.S.C. and PCM-7818490 which was administered by M. Nomura at the University of Wisconsin).

0006-2960/79/0418-2149$01.00/0