PUROMYCIN DOES NOT INACTIVATE THE GALACTOSYLTRANSFERASE OF GOLGI MEMBRANES

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SUMMARY

The effects of puromycin on galactosyltransferase from four sources, rat liver and lactating sheep mammary Golgi membranes, bovine colostrum and human serum have been investigated. We do not find that the synthesis of N-acetyllactosamine is much inhibited, even in the presence of 3mM puromycin. This is in contrast to previously reported results for rat liver Golgi membranes. We interpret the low level of inhibition observed in terms of pH effects.

The biosynthesis of glycoproteins in mammals utilizes a series of glycosyltransferases which transfer sequentially specific monosaccharides into a specific linkage in the growing oligosaccharide chain. These transferases which are situated in various parts of the endomembrane system, e.g. endoplasmic reticulum, Golgi membranes, have been the subject of considerable recent research.

One of these transferases, a galactosyltransferase which is active in the transfer of galactose from UDP-galactose to free or protein bound N-acetylglucosamine is a specific marker enzyme for Golgi membranes (1). Treloar et al. (2) have recently reported that preincubation of rat liver Golgi membranes with puromycin results in virtually complete inactivation of this enzyme; in contrast various puromycin analogues did not cause a similar loss of activity. It was also reported that during the preincubation, puromycin became bound to components of the Golgi fraction. Since preincubation is required for inhibition of galactosyltransferase, it was suggested that this binding was responsible for the observed enzyme inactivation.

TABLE I

KINETIC PARAMETERS FOR GALACTOSYLTRANSFERASE

Michaelis Constant	Pure Bovine Colostrum Enzyme	Membrane Bound Sheep Mammary Gland Golgi Enzyme
$K_{Mn^{2+}}$	0.705 mM	1.22 mM
$K_{UDP\ gal}$	0.275 mM	0.21 mM
K_{GlcNAc}	24.7 mM	9.76 mM

[Values for sheep mammary gland Golgi enzyme were determined in the presence of 0.5% Triton X-100]

As part of a study of the structure and activity of Golgi membranes and their component proteins, we have investigated the effect of puromycin on the galactosyltransferase from four sources: rat liver and lactating sheep mammary gland Golgi fractions, and soluble galactosyltransferase from bovine colostrum and human serum. We find little inactivation of any of these enzymes by puromycin, even at relatively high concentrations. Further we suggest that the inhibition observed in the previous study (2) may be attributed at least in part to the acidity of puromycin dihydrochloride solutions, as preincubations were performed in unbuffered solutions. Other anomalies of the previous study are also discussed.

MATERIALS AND METHODS

Golgi fractions from rat liver and sheep mammary gland were prepared by the method of Morré (3). Both electron microscopy and marker enzyme assays suggested that the fractions were 70-80% Golgi membranes, uncontaminated by mitochondria or rough endoplasmic reticulum. Bovine colostrum galactosyltransferase was prepared as previously described (4). Serum

galactosyltransferase activity was determined on fresh human preparations.

Galactosyltransferase assays were conducted as previously described using 0.5% Triton X-100, 10mM $MnCl_2$, 0.3mM UDP-galactose and 20mM N-acetylglucosamine (4). Assays were also performed using 2-(N-morpholino)-ethanesulphonate buffer, pH 5.7. Puromycin dihydrochloride was purchased from P L Biochemicals, Inc.

RESULTS

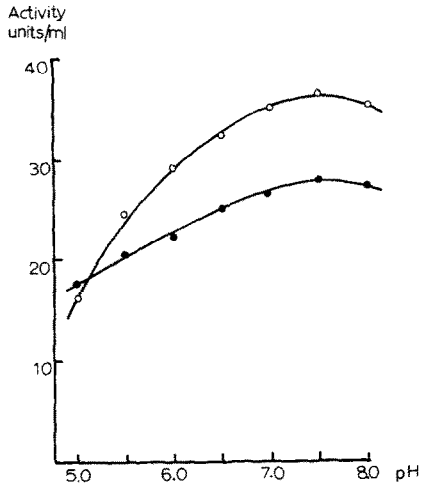
Substrate concentrations of $MnCl_2$, UDP-galactose and N-acetylglucosamine were selected by reference to the K_m values obtained for pure bovine colostrum galactosyltransferase and lactating sheep mammary gland enzyme, Table 1. These values were obtained by initial velocity kinetic studies as previously described (4). Galactosyltransferase activity using 20mM N-acetylglucosamine as acceptor was measured at both pH 5.7 and pH 7.4 for rat liver and lactating sheep mammary gland Golgi fractions, human serum and pure bovine colostrum galactosyltransferase. Assays were also performed in the presence of 1, 2 and 3mM puromycin dihydrochloride, and on samples preincubated at 37° for 5min in the presence of 2, 4 and 6mM puromycin dihydrochloride (giving 1, 2 and 3mM concentration in the assay). The results are shown in Table 2. Both rat liver and sheep mammary gland Golgi fractions show a reduced galactosyltransferase activity (by 20-30%) in the presence of 3mM puromycin dihydrochloride, but this decrease in activity occurs regardless of preincubation. The actual conditions of preincubation without puromycin cause some inactivation of galactosyltransferase in all cases, but is most marked for the sheep mammary gland Golgi enzyme. In all cases enzyme activity is lower at pH 5.7 than at pH 7.4. A much greater difference in activity between these pH values is found for the pure bovine colostrum galactosyltransferase than for the other enzymes, and this is an expression of the difference in pH-activity profiles for pure and membrane bound galactosyltransferase shown in Fig. 1. The effect of puromycin dihydrochloride is mimicked by pH effects, the activity at pH 4.5 being

TABLE 2

SHOWING THE EFFECT OF PUROMYCIN DIHYDROCHLORIDE ON GALACTOSYLTRANSFERASE ACTIVITY

Variable Assay Conditions	Rat Liver Golgi		Sheep Mammary Gland Golgi		Bovine Colostrum Galactosyltransferase		Human Serum	
pH	7.4	5.7	7.4	5.7	7.4	5.7	7.4	5.7
Specific Activity	3.8		113.4		18,500			
no GlcNAc	0.5	0.7	0.4	0.5	0	0	3.5	3.3
Preincubation (5 min 37°)	21.0	14.4	26.9	24.4	37.2	16.3	7.8	6.6
2 mM Puromycin	21.2	14.2	22.5	22.9	37.2			
4 mM Puromycin	18.6	12.3	21.4	21.9	37.3			
6 mM Puromycin	15.4	10.9	19.3	19.7	37.0	7.1	7.1	5.7
With Puromycin in assay (no preincubation)	21.0	15.2	37.8	33.3	37.5	16.4	12.8	10.3
1 mM	21.7	15.0	38.0	23.5	37.5			
2 mM	23.1	13.3	34.3	22.6	37.5			
3 mM	15.1	12.6	32.9	19.8	37.1	6.9	12.3	8.1
Assay at pH 4.5	10.3		19.1		6.6			

Activities are expressed as nmoles [^{14}C]galactose incorporated per min per ml enzyme solution.



1. The effect of pH on the N-acetyllactosamine synthase activity of soluble and Golgi membrane-bound galactosyltransferases. Open circles give data for pure bovine colostrum galactosyltransferase, closed circles show results for lactating sheep mammary gland Golgi enzyme. Activity assayed in the presence of 0.1M cacodylate buffers with 10mM $MnCl_2$, 0.3mM UDP-galactose 20mM N-acetylglucosamine, 0.1% bovine serum albumin; Triton X-100 added to 0.5% for the membrane bound enzyme.

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very similar to that in the presence of 3mM puromycin dihydrochloride. The pH of 3mM puromycin dihydrochloride was measured as 3.5 at 20°.

DISCUSSION

We are unable to find evidence for the total inactivation of N-acetyllactosamine synthesis by puromycin for galactosyltransferase activity from the four sources cited: rat liver and lactating sheep mammary gland Golgi fractions, human serum and pure bovine colostrum galactosyltransferase. Under conditions where we find only 20-30% inhibition of N-acetyllactosamine synthesis, Treloar et al. reported 80-90% inactivation for rat liver Golgi fractions (2). The limited inhibition of galactosyltransferase activity that we observe is consistent with this inhibition resulting from the reduction in pH in the presence of 3mM puromycin dihydrochloride, as in the previous work, preincubations were performed in the absence of buffer. In this respect we note that Treloar et al. (2) found little inhibition of

galactosyltransferase activity in the presence of neutral analogues of puromycin, e.g. 6-dimethyladenosine.

There are several further points of difference between our work and that of Treloar et al. Firstly, Golgi fractions were stored in the presence of 20mM cacodylate buffer pH 7.4. Secondly, substrate concentrations were maintained at levels where Michaelis-Menten kinetics are obeyed. We would suggest that the concentrations of $MnCl_2$ and N-acetylglucosamine (67mM and 110mM, respectively) used by Treloar et al. may be beyond this range, and that the amount of EDTA used to terminate their assays may have been insufficient to complex all the manganese.

Treloar et al. (2) also report the specific binding of [^{3}H]puromycin to Golgi membranes. We have not investigated the binding of puromycin to our Golgi fractions, since we are not confident that preparations of such membranes, obtained by density gradient centrifugation, are completely uncontaminated by other membrane fractions, e.g. smooth endoplasmic reticulum. The reported binding of puromycin to Golgi membranes might be of some interest if it is specific for these membranes, but we would contend that the binding of puromycin, if it does occur, does not modulate the galactosyltransferase activity of Golgi membranes.

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1. B. Fleischer and S. Fleischer (1970) Biochim. Biophys. Acta. 219, 301-319.

2. M. Treloar, J.M. Sturgess and M.A. Moscarello (1974) J. Biol. Chem. 249, 6628-6632.

3. D.J. Morré (1971) Methods in Enzymology 22, 130-144. Academic Press (New York and London).

4. J.T. Powell and K. Brew (1974). Europ. J. Biochem. 48, 217-228.