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Differential Effects of EDTA, Metal Ions, and Nucleotides on Glycoprotein Sialyltransferase Activity of Serum and Liver (39841)

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The properties of serum CMP-NANA:glycoprotein sialyltransferase have been studied in detail (1, 2). Hudgin and Schachter (2) compared the properties of membrane-bound and soluble porcine liver sialyltransferase with those of serum enzyme and showed that the serum enzyme resembled the liver enzyme in several respects. They concluded that in both liver and serum a single sialyltransferase is responsible for the transfer of sialic acid to protein. The present study, however, illustrates several important differences in the properties of the soluble, serum CMP-NANA:glycoprotein sialyltransferase and the liver enzyme. It is shown that EDTA increases the transfer of NANA from CMP-NANA to protein and that divalent cations decrease the transfer by serum enzyme, while CMP-NANA transfer to protein by liver microsomes is enhanced by divalent ions. It is also shown that nucleotides have strong inhibitory effects on the serum enzyme and that the magnitude of the inhibitory effect increases with the number of phosphate groups in the molecule, while the enzyme in liver is either stimulated or not affected by nucleotides.

Materials and methods. Human blood obtained from local hospitals, and rat blood, were allowed to clot at room temperature and centrifuged for 15 sec to obtain serum. The serum was stored at −20° until use. Liver microsomes were prepared exactly as described by Bernacki (3). Fetuin was desialyzed as described elsewhere (4). [^{14}C]-Labeled CMP-NANA (200 μCi/μmole) was obtained from New England Nuclear Corp., Boston, Massachusetts. Incubation mixtures for serum enzyme assays contained 50 μl of serum, 1 mg of desialyzed fetuin, 2.5 μmoles of EDTA, 50 m*M* HEPES buffer, pH 6.8, and 0.05 μCi of CMP-[^{14}C]NANA, in a final volume of 0.3 ml. Incubation mixtures for liver microsomal enzyme contained 0.3 mg of microsomal protein, 1 mg of desialyzed fetuin, 10 m*M* Mg^{2+} and Mn^{2+}, 10 m*M* Triton X-100, 50 m*M* HEPES buffer, pH 6.8, and 0.05 μCi of CMP-[^{14}C]NANA in a final volume of 0.3 ml. Incubations for serum enzyme were carried out for 120 min and for liver enzyme for 30 min, at 37° and reactions were terminated by the addition of 2 ml of 10% trichloroacetic acid (TCA). The mixtures were centrifuged, and the precipitates were washed successively twice with 2 ml of 5% TCA, twice with 2 ml of acetone, and twice with 2 ml of chloroform:methanol (2:1) mixture. The washed pellet was dissolved in 0.5 ml of formic acid, and the [^{14}C] was assayed by liquid scintillation spectroscopy.

Results. Preliminary experiments with human serum indicated that the transfer of [^{14}C]NANA from CMP-[^{14}C]NANA to desialyzed fetuin was linear up to a serum concentration of 60 μl per incubation and over a time interval of 180 min. The transfer was unaffected by the addition of detergents. These properties of the enzyme were essentially similar to those reported by Kim *et al.* (1). However, the addition of EDTA to the incubation medium resulted in an increase in the transfer of [^{14}C]NANA from CMP-[^{14}C]NANA to desialyzed fetuin (Table I). The increase in the transfer was concentration-dependent and reached a maximum at an 8 m*M* EDTA concentration. The results suggested that EDTA was interacting with endogenous divalent cations, and hence the effects of divalent cations on serum sialyl transferase were studied. As is shown in Table II the addition of Mg^{2+} or Mn^{2+} to the incubation medium resulted in a decrease in the transfer of NANA to the protein. In general, Mn^{2+} was more inhibitory than Mg^{2+}, but with either ion approximately 90% inhibition was obtained at a 10

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TABLE I. EFFECT OF EDTA ON [^{14}C]NANA TRANSFER TO PROTEIN.[a]

EDTA concentration (mM)	[^{14}C] Transferred to protein (cpm)	Increase (%)
0	1960	–
2	2461	25.6
4	3286	67.7
8	3708	89.2
16	2905	48.2

[a] Human serum (50 μl) was incubated with CMP-[^{14}C]NANA under conditions described in the text. Varying concentrations of EDTA were added as indicated.

TABLE II. EFFECTS OF DIVALENT CATIONS ON [^{14}C]NANA TRANSFER TO PROTEIN.[a]

Addition	Concentration (mM)	[^{14}C] Transferred to protein (cpm)	Decrease (%)
None	0	4018	–
Mg^{2+}	2.5	3924	2.3
	5.0	1912	52.4
	10.0	558	86.7
	20.0	374	90.7
Mn^{2+}	2.5	3336	17.0
	5.0	676	83.2
	10.0	324	92.0
	20.0	270	93.3

[a] Human serum (50 μl) was incubated with CMP-[^{14}C]NANA under conditions described in the text.

mM concentration. Table III summarizes the effects of several nucleotides, at 1 mM concentrations, on the transfer reaction. As can be seen, AMP had no inhibitory effect, but with increasing number of phosphate groups in the molecule the inhibitory effect increased. Thus ADP caused about 70% inhibition, while ATP caused near total inhibition of the activity. Similarly, UMP and GMP had little or no effect, while the corresponding di- and triphosphates had progressively greater inhibitory activities. Both CMP and CTP caused significant inhibition, but this inhibition is probably related to the substrate used, viz., CMP-NANA.

The response of rat serum enzyme to EDTA, metal ions, and nucleotides was essentially similar to that of the human serum enzyme. Thus, EDTA stimulated the transfer, while divalent metal ions and nucleotide di- and triphosphates inhibited the transfer reaction (Table IV). The results of rat liver enzyme shown in Table V are essentially similar to those reported by Bernacki (3). The presence of divalent metal ions is necessary for the optimal activity, and UDP and UTP both stimulate the reaction by approximately 50%.

Discussion. Kim *et al.* (1) have shown that addition of 25 mM EDTA inhibited the serum sialyltransferase activity. The present study shows that EDTA, at lower concentrations, has a stimulatory effect on the

TABLE III. EFFECTS OF VARIOUS NUCLEOTIDES ON [^{14}C]NANA TRANSFER TO PROTEIN.[a]

Addition	[^{14}C] Transferred to protein (cpm)	Decrease (%)
None	2419	–
AMP	2656	–
ADP	1004	58.5
ATP	315	87.0
UMP	1979	18.2
UDP	905	62.6
UTP	366	84.9
GMP	1863	23.0
GDP	556	77.0
GTP	218	91.0
CMP	365	85.0
CTP	218	89.1

[a] Human serum (50 μl) was incubated with CMP-[^{14}C]NANA under incubation conditions described in the text. Nucleotides were included, at 1 mM concentrations, as indicated.

TABLE IV. EFFECTS OF EDTA, METAL IONS, AND NUCLEOTIDES ON SIALYLTRANSFERASE ACTIVITY OF RAT SERUM.[a]

Addition/omission	[^{14}C] Transferred to protein (cpm)	Decrease (%)
None	3362	–
Experiment A		
minus EDTA	471	86.0
+ 10 mM Mg^{2+}	212	93.7
+ 10 mM Mn^{2+}	103	96.9
+ AMP	3028	9.9
+ ADP	1329	60.5
+ ATP	366	89.1
+ UMP	2619	22.1
+ UDP	1105	67.1
+ GMP	2258	33.0
+ GDP	790	76.5
+ GTP	413	85.3
Experiment B[b]		
None	1579	53.0
minus EDTA	328	90.2

[a] Rat serum (50 μl) was incubated with CMP-[^{14}C]NANA under conditions described in the text. The concentrations of nucleotides were 1 mM.

[b] Dialysed serum (50 μl) was used as the enzyme source. Protein concentration of dialyzed serum was reduced by approximately 60% because of increase in volume during dialysis.

TABLE V. EFFECTS OF METAL IONS AND NUCLEOTIDES ON SIALYLTRANSFERASE ACTIVITY OF RAT LIVER MICROSOMES.[a]

Addition/ omission	[^{14}C] Transferred to protein (cpm)	Percentage of control
Experiment A		
None	3205	100
Minus Mg^{2+} and Mn^{2+}	203	6
+ UDP	5256	163
+ UTP	5537	172
+ GDP	2939	92
+ GTP	3352	104
+ ATP	2877	90
Experiment B[b]		
None	1179	100
+ UDP	2245	190
+ UTP	1506	128

[a] Microsomal protein (0.3 mg) was incubated with CMP-[^{14}C]NANA under conditions described in the text. Concentrations of nucleotides were 1.15 m*M*.

[b] Microsomes from liver were treated with 10 m*M* Triton X-100 and recentrifuged. Solubilized protein (120 μg) was then used for enzyme assay.

transfer reaction; the transfer was nearly doubled at an 8 m*M* EDTA concentration. Consistent with this observation is the result that addition of divalent metal ions significantly inhibited the transfer reaction. Similar results were obtained with dialysed serum as well. These results thus indicate that EDTA perhaps interacts with endogenous metal ions present in serum in a bound manner. The results thus contrast with the divalent cation requirement of the rat liver enzyme, as reported by Bernacki (3) and also observed by us in the present study (Table V).

The effects of nucleotides on the serum enzyme are different from those for rat liver enzyme. Results of our present study and those of Bernacki (3) show that addition of uridine nucleotides increases the activity of rat liver sialyltransferase almost twofold. GDP, GTP, and ATP were without significant effect. In the case of the soluble serum enzyme (human and rat), however, all of the nucleotides tested had inhibitory activity. The extent of inhibition was progressively less with decreasing number of phosphate groups in the nucleotide molecule. Thus, ATP, UTP, and GTP had the greatest inhibitory effects, while AMP, UMP, and GMP were without significant inhbitory effects. The inhibitory effect of CMP is perhaps related to the substrate used, vis., CMP-NANA.

Hudgin and Schachter (2) have compared the properties of pork liver and blood serum sialyltransferases and have shown that the serum enzyme resembles the liver enzyme. They have further concluded that in both liver and serum a single sialyltransferase is responsible for the incorporation of sialic acid into protein. Kuhlenschmidt *et al.* (5), from kinetic studies on human liver and serum sialyltransferases, have suggested that under their experimental conditions, the sialyltransferases of serum and liver may be different. The contrasting effects of divalent cations and of nucleotides on the serum and liver enzymes support the latter view.

Summary. The effects of EDTA, divalent metal ions, and nucleotides on serum and liver glycoprotein sialyltransferase were studied. EDTA, at concentrations up to 16 m*M*, increased the transfer of [^{14}C]NANA from CMP-[^{14}C]NANA to desialyzed fetuin, by serum enzyme, while Mg^{2+} and Mn^{2+} at concentrations of 5–20 m*M* strongly inhibited the transfer. Nucleotides showed marked inhibitory effects on the transfer reaction. The extent of inhibition increased with the number of phosphate groups in the molecule. The enzyme in liver, on the other hand, required divalent metal ions for optimal activity. Also, UDP and UTP stimulated the enzyme in the liver.

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1. Kim, Y. S., Perdomo, J., Bella, A., Jr., and Nordberg, J., Biochim. Biophys. Acta **244,** 505 (1971).
2. Hudgin, R. L., and Schachter, H., Canad. J. Biochem. **49,** 829 (1971).
3. Bernacki, R. J., Eur. J. Biochem. **58,** 477 (1975).
4. Raghupathy, E., Ko, G. K. W., and Peterson, N. A., Biochim. Biophys. Acta **286,** 339 (1972).
5. Kuhlenschmidt, M. K., Peters, S. P., Pinkard, O. D., Glew, R. H., and Sharp, H., Biochim. Biophys. Acta **359** (1976).

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