PLATELET ADHESIVENESS AND AGGREGATION: THE COLLAGEN:GLYCOSYL, POLYPEPTIDE:N-ACETYLGALACTOSAMINYL AND GLYCOPROTEIN:GALACTOSYL TRANSFERASES OF HUMAN PLATELETS[1]

H. Bruce Bosmann[2]

Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620

Received April 21, 1971

Summary Four glycoprotein:glycosyl transferases were identified, purified, and characterized from human platelets. The enzymes present were collagen:glc, collagen:gal, polypeptide:galNAc, and glycoprotein:gal; the fetuin:glcNAc transferase was absent. Each of the 4 transferases was found to be almost exclusively bound to the platelet plasma membrane. Incubation of platelet homogenates without exogenous acceptors yielded no transfer of monosaccharide, indicating the complete lack of endogenous acceptors. These results lead to the interesting speculation that the transferases may not be responsible in the mature platelet for glycoprotein synthesis at all, but rather that they may function for intercellular adhesion and the primary step in hemostasis, the adhesion of collagen to platelets.

Introduction Glycoprotein:glycosyl transferases have been isolated from a variety of tissues and cell types (1-12). Except for an abstract (13) little information has been published on glycoprotein:glycosyl transferases in human platelets. The present communication describes two secreted glycoprotein:glycosyl transferases, the collagen:glucosyl (coll:glc) and collagen:galactosyl (coll:gal) transferases, and two membrane glycoprotein: glycosyl transferases, the polypeptidyl:N-acetylgalactosaminyl (polyp:galNAc) and glycoprotein:galactosyl (glyc:gal) transferases in human platelets. The results presented herein are of importance since it has been suggested that glycoprotein:glycosyl transferases may function as bridge or receptor molecules for cell:cell adhesiveness and contact inhibition phenomena (14) and since it has been suggested that the collagen:glucosyl transferase of platelets may function in the primary step in hemostasis, the adhesion of collagen to platelets (13). Each of the human platelet glycosyl transferases was found to be located on the platelet plasma membrane.

[1]Supported in part by Grant P529 from the American Cancer Society and GM-15190 from NIGMS.

[2]Research Career Development Awardee of NIGMS.

Materials and Methods

Platelet isolation. Platelets were isolated from the blood of healthy young male donors immediately after drawing by the method of Zucher and Lundberg (15). One to two units of blood were used for each preparation.

Platelet plasma membrane isolation. Platelet plasma membranes were isolated by the method of Barber and Jamieson (16).

Purification of platelet glycosyl transferases. The platelets from 1 liter of blood were homogenized with 5 volumes of 0.1% Triton X-100 for 30 strokes in a Ten Broeck homogenizer. This crude homogenate served as the starting material for enzyme purification. The homogenate was centrifuged at 10,000 x g for 1 hour and the supernatant fluid and pellet were analyzed for activity. 10 ml of the supernatant fluid were applied to a Sephadex G-150 column which was packed in and eluted with 0.1% Triton X-100. A flow rate of 2 ml per minute was used. Fractions of the G-150 column were tested for activity. Also tested for activity were preparations of platelet plasma membranes extracted with 5 volumes of 0.1% Triton X-100.

Radioactively labeled compounds. UDP-[^{14}C]glc (sp. ac. 240 Ci/mole), UDP-[^{14}C]gal (240 Ci/mole), GDP-[^{14}C]man (241 Ci/mole), UDP-[^{14}C]galNAc (40 Ci/mole), UDP-[^{14}C]xyl (100 Ci/mole), UDP-[^{14}C]ara (100 Ci/mole), and UDP-N-[^{14}C]glcNAc (40 Ci/mole) were purchased from New England Nuclear Corp.

Protein. Protein determinations were made by the procedure of Lowry et al. (17).

Acceptor preparation. Fetuin (18, 19), porcine submaxillary mucin (PSM) (20, 21), guinea pig skin collagen (22, 23), bovine submaxillary mucin (BSM) (9) and their degraded products were prepared as previously described (5-9, 24).

Enzyme assays. The complete system for each of the glycoprotein: glycosyl transferases is given below. In each instance endogenous activity was determined by replacing the acceptor in the system with an equal volume of 0.1% Triton X-100; this activity was subtracted from the total activity yielding exogenous activity in each instance. Transferred radioactivity and activity with small molecules was determined as given previously (5, 22, 23).

Collagen:glucosyl transferase (22, 25, 26). The complete system for the collagen:glucosyl transferase, which was incubated for 60 min at 37°C, contained 50 µl of the enzyme extract (approx. 10 µg protein), 50 µl of the acceptor (guinea pig skin collagen minus glucose, approx. 800 µg protein), 10 µl of 0.25 M $MnCl_2$, 10 µl of UDP-[^{14}C]glucose (approx. 40,000 cpm; 1.4×10^{-10} mole) and 10 µl of 0.1% Triton X-100, to a final volume of 0.130 ml. After 60 min incubation, the protein of the reaction mixture was precipitated

with 0.1% phosphotungstic acid in 0.5 N HCl, and the radioactivity was determined as given elsewhere (22, 25, 26).

Collagen:galactosyl transferase (23, 26, 27). The complete system for the collagen:galactosyl transferase contained 50 µl of the enzyme extract (approx. 10 µg protein, 20 µl of receptor preparation (guinea pig skin collagen minus glc, gal, approx. 900 µg protein), 10 µl of 0.1% Triton X-100, 10 µl of 0.1 M $MnCl_2$, 10 µl of UDP-[^{14}C]galactose (approx. 40,000 cpm; 1.4 x 10^{-10}mole), and 10 µl of 0.1 M Tris buffer, pH 7.2 to a final volume of 110 µl. Radioactivity was determined as above.

Fetuin:N-acetylglucosaminyl transferase (5). The complete system for the fetuin:glcNAc transferase contained in a final volume of 250 µl:UDP-[^{14}C]glcNAc, 0.98 nmole; $MgCl_2$, 0.25 µmole; enzyme extract (10 µg as protein) and 1 mg of fetuin minus NANA, gal, glcNAc. Final pH was 7.5 and 0.01 M in Tris-HCl. Radioactivity was determined as above.

Polypeptide:N-acetylgalactosaminyl transferase (9). The complete system (final volume 150 µl) contained UDP-[^{14}C]galNAc, 1.1 nmole, 10 µl of BSM minus NANA, galNAc (0.8 mg), 20 µl of 0.25 M $MnCl_2$, 50 µl of 0.1 M Tris buffer, pH 7.2, 10 µl of 0.1% Triton X-100 and 50 µl of enzyme extract (10 µg protein).

Glycoprotein:galactosyl transferase (9). The complete incubation mixture (final volume 155 µl) contained UDP-[^{14}C]gal, 1.0 nmole, 20 µl of 0.25 M $MnCl_2$, 15 µl of fetuin minus NANA, gal (0.9 mg protein), 50 µl of enzyme extract (10 µg protein), 50 µl of 0.1 M Tris buffer, pH 7.0, and 10 µl of 0.1% Triton X-100. Radioactivity was determined as given above.

Results

Endogenous transferase activity. Initially 50 µl of the crude 0.1% Triton X-100 homogenate of human platelets in 0.1 M Tris, pH 7.0, was incubated with 10 µl of 0.1 M $MnCl_2$ and 0.1 µCi of UDP-[^{14}C]glc, UDP-[^{14}C]gal, GDP-[^{14}C]man, UDP-[^{14}C]glcNAc, UDP[^{14}C]galNAc, UDP-[^{14}C]xyl or UDP-[^{14}C]ara for 1 hour at 37°. There was absolutely no transfer of monosaccharide onto TCA, ether ethanol insoluble material material under these conditions.

Purification and characteristics of human platelet glycosyl transferases. The data in Tables I and II indicate that when exogenous acceptor was added to the platelet homogenate transfer of monosaccharide onto the acceptor occurred. Activity for the collagen:glc, collagen:gal, polypeptide:galNAc, and glycoprotein:gal transferases was found in human platelets; there was no fetuin:glcNAc activity. This result clearly demonstrates that the endogenous acceptors and not the transferases are limiting in the endogenous assay. Purification of 8 to 17 fold with 54 to 80% recovery (Table I) was found for

Table I. Purification of Human Platelet Glycosyl Transferases. Details of purification scheme are given in Materials and Methods. Starting material was human platelets from 1 liter of blood. Complete systems are given in Materials and Methods.

	Collagen: Glc	Collagen: Gal	Fetuin: GlcNAc	Polypeptide: GalNAc	Glycoprotein: Gal
Crude homogenate					
Endogenous activity (cpm/mg protein)	0	0	0	0	0
Total activity (cpm x 10^{-4})	339	1196	0	92	541
Total protein (mg)	170	170	170	170	170
10,000 x g supernatant					
Total activity (cpm x 10^{-4})	306	961	--	82	407
Total protein (mg)	64	64	--	64	64
Sephadex G-150					
Total activity (cpm x 10^{-4})	271	841	--	79	316
Total protein (mg)	9	8	--	9	14
Purification factor	15	14	--	17	8
Recovery (%)	80	70	--	85	54
Platelet plasma membrane					
Total activity (cpm x 10^{-4})	308	1051	0	84	519
Total protein (mg)	1.7	1.7	1.7	1.7	1.7
Purification factor	110	112	--	109	104
Recovery (%)	90	89	--	91	94

Table II. Characteristics of Human Platelet Glycosyl Transferases. Data are cpm per mg protein x 10^{-3}. Experiments were performed with the purified Sephadex G-150 fractions as given in Materials and Methods. All data are for complete systems as given in Materials and Methods.

System	Collagen: Glc	Collagen: Gal	Polypeptide: GalNAc	Glycoprotein: Gal
Complete	301	1051	87	225
0 time	4	21	11	20
0°	6	27	3	38
Minus enzyme	2	1	4	2
Minus acceptor	0	0	0	0
Minus ion[a]	14	18	7	18
Minus enzyme plus boiled enzyme	4	2	1	8
Minus labeled precursor	0	0	0	0
Radioactivity recovered as:	glc 99%	gal 94%	galNAc 97%	gal 100%
pH optimum	5.8	8.0	7.2	7.0

[a]Minus ion refers to assays performed without the divalent cation indicated for the particular complete system in Materials and Methods.

the transferases using the procedure outlined in Materials and Methods. However, as shown in Table I, a much greater purification of the transferases occurred (104-112 fold), simply by preparing platelet plasma membranes. The data indicate that glycosyl transferases are rather exclusively in the platelet

Table III. Effects of Cations on Human Platelet Glycoprotein Transferases. Data are cpm per mg protein x 10^{-3}. All ions were present at 1 mM as the chloride and EDTA was present at 0.5 mM.

System	Collagen: Glc	Collagen: Gal	Polypeptide: GalNAc	Glycoprotein: Gal
Complete	301	1051	87	225
Minus ion	14	18	7	18
Minus ion plus EDTA	2	0	4	0
Minus ion plus EDTA plus:				
Mn^{2+}	300	1060	90	228
Mg^{2+}	116	106	21	19
Ca^{2+}	32	91	8	26
Co^{2+}	135	1116	24	72
Hg^{2+}	9	0	0	4
Ba^{2+}	108	0	2	8
Pb^{2+}	21	0	8	17
Cd^{2+}	14	322	8	19

Table IV. Specificity of Human Platelet Glycosyl Transferases. All experiments were performed with the complete system as given in Materials and Methods except that 1 mg of the indicated acceptor was present instead of the signified acceptor. Data are cpm x 10^{-3} per mg enzyme protein.

Acceptor	Terminal Monosaccharide	Collagen: Glc	Collagen: Gal	Polypeptide: GalNAc	Glyco protein: Gal
Guinea pig skin collagen	glc	0	0	0	0
Guinea pig skin collagen minus					
glc	gal	360	21	0	1
glc, gal	none	7	1160	0	14
Fetuin	NANA	0	0	0	4
Fetuin minus					
NANA	gal	0	6	0	2
NANA, gal	galNAc	1	11	0	225
NANA, gal, glcNAc	man	1	3	0	5
BSM	NANA	0	4	0	3
BSM minus					
NANA	galNAc	2	1	2	17
NANA, galNAc	none	1	0	106	0
PSM	NANA	0	3	0	0
PSM minus					
NANA	fuc	1	0	0	1
NANA, fuc	gal	1	0	0	2
NANA, fuc, gal	NGN	0	1	0	19

plasma membrane and are tightly bound to this membrane. The data in Table II indicate the enzymes were dependent on incubation time and temperature, non-heat denatured enzyme and that radioactivity for each transferase was recovered as the monosaccharide of the UDP-[^{14}C]monosaccharide precursor. pH optimums of the enzymes ranged from 5.8 to 7.2. The reactions were linear from 0 to 100 µg (as protein) of enzyme and from 0 to 1.5 mg (as protein) of acceptor.

Cation effects. Each of the four platelet glycosyl transferases was dependent on a divalent cation for activity, as shown in Table III. Each of the enzymes had greatest activity with Mn^{++} except for the collagen:galactosyl transferase which surprisingly had greater activity with Co^{++}.

Acceptor specificity. The data in Table IV indicate that the platelet glycosyl transferases are highly specific for the designated macromolecular acceptor of the reaction. In addition none of the following small molecules functioned as acceptors: glc, gal, man, fuc, glcN, galN, glcNAc, galNAc, NANA, ara, xyl.

Discussion

This communication describes four human platelet glycoprotein:glycosyl transferases. In their characteristics these enzymes are rather similar to glycoprotein:glycosyl transferases from other sources with one major important exception: no endogenous acceptors at all exist. Platelets synthesize proteins (28, 29) and contain glycoproteins (30, 31) and so the enzymes may be responsible for the synthesis of glycoproteins for the platelet biogenesis. This synthesis may be important only in immature platelets and hence endogenous acceptors might not be expected in mature platelets.

However, platelets do not synthesize collagen and synthesis of protein occurs only in young platelets (28, 29). Thus the presence, in particular, of the two very specific collagen:glycosyl transferases is unexplainable. A very intriguing supposition is that the collagen:glycosyl transferases do not function at all as enzymes but rather function as mediators of platelet:collagen adhesion. Similarly a function for the other glycoprotein:glycosyl transferases would be platelet:platelet adhesion. Such suppositions would be in line with the hypothesis of Roseman (14) that glycosyl transferases mediate intercellular adhesion. Thus, pieces of evidence presented herein support the suppositions that (1) the platelets contain glycoprotein:glycosyl transferases, (2) the platelets contain absolutely no endogenous acceptors, and (3) the transferases are located in the plasma membrane (external surface?) and not in intracellular membranes.

Acknowledgments. I thank Dr. Michael Weintraub for drawing the blood used in

these experiments. For technical assistance I gratefully thank Mr. Kenneth R. Case, Miss Melinda B. Shea, and Mrs. Gerilyn Z. Pike.

References

1. Schacter, H., Jabbal, I., Hudgin, R. L., Pinteric, L., McGuire, E. J., and Roseman, S., J. Biol. Chem. 245, 1090 (1970).
2. Spiro, R. G., New Eng. J. Med., 231, 1043 (1969).
3. Spiro, R. G., New Eng. J. Med., 231, 991 (1969).
4. Morré, D. J., Merlin, L. M., and Keenan, T. W., Biochem. Biophys. Res. Comm. 37, 813 (1969).
5. Bosmann, H. B., Eur. J. Biochem. 14, 33 (1970).
6. Hagopian, A., Bosmann, H. B., and Eylar, E. H., Arch. Biochem. Biophys. 128, 387 (1969).
7. Bosmann, H. B., Hagopian, A., and Eylar, E. H., Arch. Biochem. Biophys. 130, 573 (1969).
8. Bosmann, H. B., Hagopian, A., and Eylar, E. H., Arch. Biochem. Biophys. 128, 470 (1968).
9. Bosmann, H. B., Hagopian, A., and Eylar, E. H., J. Cell Physiol. 72, 81 (1968).
10. Molnar, J., Chao, H., and Markovic, G., Arch. Biochem. Biophys. 134, 533 (1969).
11. Robinson, G. B., Biochem. J. 115, 1077 (1969).
12. Hagopian, A., and Eylar, E. H., Arch. Biochem. Biophys. 129, 515 (1969).
13. Barber, A. L. and Jamieson, G. A., Fed. Proc. 30, 540Abs (1971).
14. Roseman, S., Chem. Phys. Lipids 5, 270 (1970).
15. Zucker, M. B. and Lundberg, A., Anesthesiology 27, 385 (1966).
16. Barber, A. L. and Jamieson, G. A. J. Biol. Chem. 245, 6357 (1970).
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).
18. Jeanloz, R. W., Glycoproteins, A. Gottschalk (editor) (Vol. V Biochim. Biophys. Acta Library), Elsevier Press, Amsterdam, 1966, pp. 362-394.
19. Spiro, R. G., J. Biol. Chem., 235, 3860 (1960).
20. Katzman, R. L., and Eylar, E. H., Biochem. Biophys. Res. Commun. 23, 769 (1966).
21. Katzman, R. L., and Eylar, E. H., Arch. Biochem. Biophys. 117, 623 (1966).
22. Bosmann, H. B., and Eylar, E. H. Biochem. Biophys. Res. Commun. 30, 89 (1968).
23. Bosmann, H. B., and Eylar, E. H. Biochem. Biophys. Res. Commun. 33, 340 (1968).
24. Kessel, D., and Bosmann, H. B., Cancer Res. 30, 2695 (1970).
25. Bosmann, H. B. and Eylar, E. H., Nature 218, 582 (1968).
26. Bosmann, H. B. Exp. Cell Res. 61, 230 (1970).
27. Bosmann, H. B. Life Sciences 8, 737 (1969).
28. Steiner, M. and Baldini, M., Blood 33, 628 (1969).
29. Boyse, F. M. and Rafelson, M. E., Biochim. Biophys. Acta 166, 689 (1968).
30. Pepper, O. S. and Jamieson, G. A., Biochemistry 8, 3362 (1969).
31. Mullinger, R. N. and Manley, G., Biochim. Biophys. Acta 170, 282 (1968).