Elucidation of Lectin Receptors by Quantitative Inhibition of Lectin Binding to Human Erythrocytes and Lymphocytes†

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ABSTRACT: The binding to normal and sialidase-treated human erythrocytes and lymphocytes of four ^{125}I-labeled lectins [*Maackia amurensis* hemagglutinins (MAM and MAH), *Ricinus communis* hemagglutinin (RCH), and *Bauhinia purpurea* hemagglutinin (BPH)] was studied in detail. The quantitative inhibition assays against the lectin binding to the cells were also performed with various glycoproteins and glycopeptides as inhibitors. The comparison of the inhibition constants of the inhibitors thus obtained with the association constants of the lectins to the cells permitted estimation of the relative receptor activities of cell surface glycoproteins toward the lectins.

Lectins have recently been extensively investigated because of several peculiar biological activities (Sharon and Lis, 1972; Nicolson, 1974). These activities are assumed to stem from the initial binding of the lectins to certain receptor sites of carbohydrate nature of the cell surface.

To elucidate the mechanism of the biological activities of lectins and to apply them effectively to the detection of sugar moieties on normal and neoplastic cell surfaces, it is important to define the carbohydrate binding specificities of lectins. The carbohydrate binding specificities of lectins have generally been studied by hemagglutination inhibition assays with sugars and glycopeptides as hapten inhibitors. However, precise quantitative comparison of the inhibitory activities of the haptenic sugars is almost impossible in these hemagglutination inhibition assays. Therefore, we sought a more quantitative method for the elucidation of carbohydrate binding specificities of lectins and the nature of lectin receptors on the cell surface. Radioactively labeled lectins have been utilized by many investigators to obtain information on the number of lectin receptors and strength of binding to the cell surface (Sharon and Lis, 1975).

In this paper, we present a method for the quantitative assessment of the cell surface receptors for lectins by the inhibition of radioactively labeled lectin binding to the cell surface with various glycoproteins and glycopeptides and the comparison of their inhibition constants thus obtained with the association constants of lectins to the cell surface.

Experimental Section

Lectins. Maackia amurensis mitogen (MAH)[1] and *Maackia amurensis* hemagglutinin (MAH) were purified from *M. amurensis* seeds (purchased from F. W. Schumacher, Sandwich, Mass.) according to the method previously described (Kawaguchi et al., 1974a). *Ricinus communis* hemagglutinin (RCH) was prepared from commercially available *R. communis* seeds by the method of Tomita et al. (1972). *Bauhinia purpurea* hemagglutinin (BPH) was purified from *B. pupurea* seeds (purchased from F. W. Schumacher, Sandwich, Mass.) by the method previously described (Irimura and Osawa, 1972). The homogeneity of these purified lectins was ascertained by ultracentrifugal analysis and electrophoresis on polyacrylamide gel.

Glycoproteins and Glycopeptides. PAS-1 glycoprotein of human erythrocyte membranes was isolated as described previously (Fukuda and Osawa, 1973). Band 3 glycoprotein of human erythrocyte membranes was purified by the stepwise selective solubilization of membrane proteins followed by gel filtration on Sepharose 6B in the presence of sodium dodecyl sulfate as described in detail elsewhere (T. Kondo et al., in preparation). Band 3 glycoprotein thus purified was pure in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Fairbanks et al., 1971), and its chemical composition was in good agreement with that previously reported (Yu and Steck, 1975; Furthmayr et al., 1976). The chymotrypsin fragment (Ch-3) from PAS-1 glycoprotein containing only mucin-type sugar chains (Figure 1), described by Jackson et al. (1973) and Tomita and Marchesi (1975), was kindly provided by Dr. M. Tomita, Showa University, Tokyo, Japan. Porcine thyroglobulin was prepared from porcine thyroid glands by the procedure described by Ui and Tarutani (1961). The glycopeptide B (GPB; unit B glycopeptide) from porcine thyroglobulin (Figure 1) was prepared according to the procedure described by Fukuda and Egami (1971). Desialization of glycoproteins and glycopeptides was performed by an acid hydrolysis in 25 mM H_2SO_4 for 3 h at 80 °C in a sealed tube in vacuo. The reaction mixture was neutralized with 1 M NaOH and then desalted by passage through a column of Sephadex G-25.

Iodination of Lectins. The purified lectins were iodinated with ^{125}I by the Chloramine-T method of Hunter (1967) as described previously (Kawaguchi et al., 1974b). This procedure did not affect the hemagglutinating activity of the lectins. The specific radioactivity was $1-3 \times 10^4$ cpm/μg of protein.

Preparation of Purified Erythrocytes and Lymphocytes for Lectin-Binding Studies. Purified erythrocytes and lymphocytes were prepared from human group O venous blood according to the method previously described (Kawaguchi et al., 1974a).

Sialidase and Formaldehyde Treatments of Cells. Sialidase

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[1] Abbreviations are: MAM, strongly mitogenic *Maackia amurensis* hemagglutinin; MAH, strongly hemagglutinating *Maackia amurensis* hemagglutinin; RCH, *Ricinus communis* hemagglutinin; BPH, *Bauhinia purpurea* hemagglutinin; PAS-1, major sialoglycoprotein of human erythrocyte membranes; Ch-3, the chymotrypsin fragment from major sialoglycoprotein of human erythrocyte membranes; GPB, unit-B glycopeptide from porcine thyroglobulin.

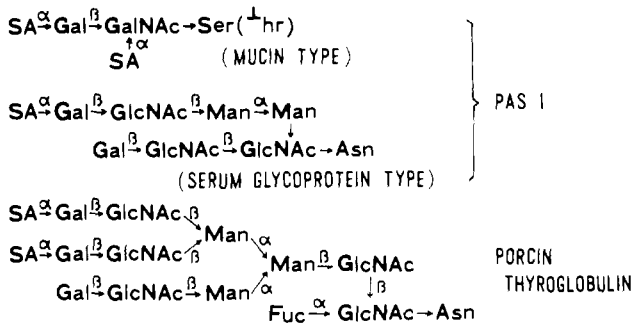

FIGURE 1: Proposed structures of carbohydrate chains of PAS-1 glycoprotein of human erythrocytes (Thomas and Winzler, 1969; Kornfeld and Kornfeld, 1971) and of porcine thyroglobulin glycopeptide B (T. Kondo, M. Fukuda, and T. Osawa, in preparation).

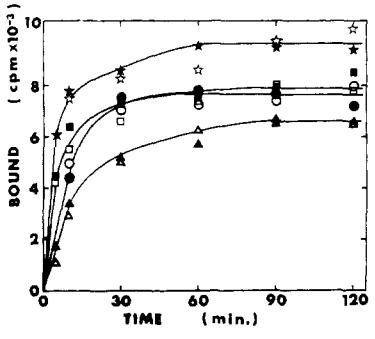

FIGURE 2: Time course of ^{125}I-labeled lectin binding to normal (solid symbols) and formaldehyde-fixed (open symbols) human lymphocytes. (●, ○) [^{125}I]MAM; (■, □) [^{125}I]RCH; (▲, △) [^{125}I]MAH; (★, ☆) [^{125}I]BPH.

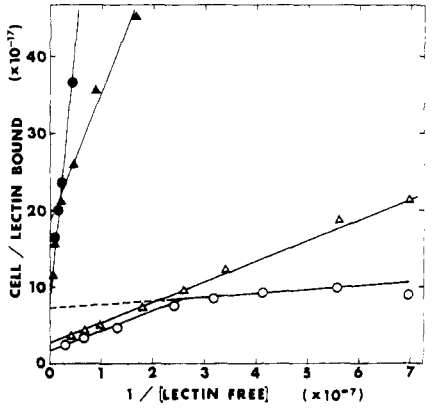

FIGURE 3: Binding of ^{125}I-labeled lectin to sialidase-treated human group O erythrocytes. The binding reactions were performed as described previously (Kawaguchi et al., 1974b). The data were plotted by the method of Steck and Wallach (1965). (●) [^{125}I]MAM; (○) [^{125}I]RCH; (▲) [^{125}I]MAH; (△) [^{125}I]BPH.

treatment of cells was performed as follows. To 10% cell suspension in 0.05 M acetate buffered saline (pH 5.5) was added 0.1 unit of neuraminidase, prepared from the culture filtrate of *Streptococcus* group K according to the method of Kiyohara et al. (1974), per 10^8 cells, and the suspension was gently shaken at 37 °C for 1 h. The cells were then washed five times with 5 mM phosphate buffered saline (pH 7.0)–0.25% bovine serum albumin. Formaldehyde fixation of purified lymphocytes was performed by the method of Inbar et al. (1973).

Hemagglutination Assays. The titration and inhibition assays were carried out according to the method previously described (Matsumoto and Osawa, 1970).

Binding Studies. Binding reactions were carried out according to the method previously described (Kawaguchi et al., 1974b). The reaction mixture contained 6×10^6 erythrocytes or 1×10^6 lymphocytes and 3–500 pmol of ^{125}I-labeled lectin in a final volume of 0.3 ml of 5 mM sodium phosphate buffered saline (pH 7.0)–0.25% bovine serum albumin. In binding inhibition assays with a glycoprotein or a glycopeptide, various amounts (up to 2×10^{-5} mol) of the glycoprotein or the glycopeptide were mixed with the cells just before the addition of ^{125}I-labeled lectin. Since the purified band 3 glycoprotein was insoluble in water and phosphate buffered saline, all of the inhibition studies with band 3 glycoprotein were performed with the glycoprotein solution containing sodium dodecyl sulfate. The final concentration of sodium dodecyl sulfate was less than 0.01%. In this concentration range, sodium dodecyl sulfate did not affect the binding activity of ^{125}I-labeled lectins to cells and the inhibitory activity of PAS-1 glycoprotein against ^{125}I-labeled lectin binding to cells.

Results and Discussion

Time Course and Reversibility of ^{125}I-Labeled Lectin Binding to Normal and Formaldehyde-Fixed Lymphocytes. As in the case of the [^{131}I]lectin binding to human erythrocytes reported in the previous paper (Kawaguchi et al., 1974b), the binding of ^{125}I-labeled MAM, RCH, MAH, and BPH to human peripheral lymphocytes was found to reach a plateau after 60–90 min as shown in Figure 2 under the conditions described in the Experimental Section. Since entirely the same results can be obtained with formaldehyde-fixed human lymphocytes prepared by the method of Inbar et al. (1973), the endocytosis of ^{125}I-labeled lectins is negligible under these conditions. Furthermore, the lectin binding to lymphocytes was specifically reversed by adding PAS-1 glycoprotein to the binding mixture. These experiments were performed in the same fashion as in the case of human erythrocytes (Kawaguchi et al., 1974b). However, the release of lectin from lymphocytes takes a little longer time than from erythrocytes. Thus, after 19 h of incubation with PAS-1 glycoprotein, 72, 75, 60, and 58% of RCH, BPH, MAH, and MAM, respectively, were released from human lymphocytes, and 80, 70, 74, and 64% of RCH, BPH, MAH, and MAM, respectively, were released from formaldehyde-fixed lymphocytes.

Estimation of Binding Constants and Number of Receptor Sites. The binding studies of ^{125}I-labeled lectins to sialidase-treated human erythrocytes, normal human lymphocytes, and sialidase-treated human lymphoccyes were carried out, and the data obtained were plotted according to the method of Steck and Wallach (1965) as shown in Figures 3 and 4. The binding of the ^{125}I-labeled lectins gave biphasic lines in some of these cases. Similar results had previously been obtained with normal human erythrocytes (Kawaguchi et al., 1974b). These results indicate that there exist two kinds of receptor sites on the cell surface for each of these lectins, namely the major receptor sites to which the lectin binds preferentially and the minor receptor sites to which the lectin binds only at high concentrations. The apparent constants for the major receptor sites (K_0) and the average number of major receptor sites per cell (n) were calculated. These values are listed in Table I. K_0 and n values obtained previously for normal human erythrocytes (Kawaguchi et al., 1974b) are also listed in Table I for comparison. Sialidase treatment of cells caused the remarkable decrease of K_0 values for both MAM and MAH, and the marked increase of the n value for BPH. These results suggest that sialic acid residues at the nonreducing end of sugar chains play an important role in the receptor activity for both MAM

TABLE I: Binding Constants of Lectins to Human Erythrocytes and Lymphocytes.

Lectin		Normal		Sialidase Treated[c]	
		K_0[a]	n[b]	K_0[a]	n[b]
MAM	Erythrocyte	0.12×10^8	0.65×10^6	0.013×10^8	0.60×10^6
	Lymphocyte	0.16	9.4	0.006	20
RCH	Erythrocyte	1.2	0.86	1.2	0.90
	Lymphocyte	1.2	10	1.2	10
MAH	Erythrocyte	1.4	1.2	0.11	0.32
	Lymphocyte	1.2	4.2	0.008	4.6
BPH	Erythrocyte	0.15	0.40	0.15	2.0
	Lymphocyte	0.11	1.6	0.12	6.0

[a] Apparent association constant (M^{-1}) for major receptor sites. Average value of triplicate experiments. [b] Number of major receptor sites. Average value of triplicate experiments. [c] Approximately 40% of sialic acids on the cell surface were removed.

and MAH and, in contrast, BPH binds preferentially to an asialosugar chain. On the other hand, both K_0 and n values for RCH-binding to major receptor sites were not changed in this treatment of cells. Adair and Kornfeld (1974) also described that the sialidase treatment of human erythrocytes did not increase the number of major receptor sites for RCH, but Nicolson (1973) observed 1.5–3.0-fold increase of the total number of the receptor sites for RCH after the same treatment of the cells. These facts suggest that the major receptors for RCH on human erythrocytes are possibly the glycoproteins which bear asialosugar chains such as band 3 glycoprotein.

It is of interest to note that K_0 values of these four lectins for human lymphocytes are nearly identical with those human erythrocytes. These results may indicate that the structure of the sugar chains, which serve as receptor sites for these lectins, on the lymphocyte cell surface is the same as or quite similar to that on the erythrocyte cell surface.

The results in Table I also shows that, in contrast to human erythrocytes, human lymphocytes have more receptor sites for MAM and RCH than for MAH and BPH. Since MAM and RCH bind preferentially to the serum glycoprotein-type sugar chains as revealed by the following experiments in this paper, this fact suggests that the serum glycoprotein-type sugar chains are more abundant on the lymphocyte cell surface than on the erythrocyte cell surface.

Hemagglutination Inhibition with Various Glycoproteins and Glycopeptides. The results of hemagglutination inhibition assays of MAM, RCH, MAH, and BPH with glycoproteins and glycopeptides as hapten inhibitors are given in Table II. PAS-1 glycoprotein from human erythrocyte membranes, containing two kinds of sugar chains, namely the serum glycoprotein-type (Kornfeld and Kornfeld, 1971) and mucin-type sugar chains (Thomas and Winzler, 1969) shown in Figure 1, exerted strong inhibitory activity against all of these four lectins. However, the chymotrypsin fragment (Ch-3) of the PAS-1 glycoprotein, which had been reported to contain only the mucin-type sugar chains (Tomita and Marchesi, 1975), showed potent inhibitory activity only against MAH and BPH. On the other hand, porcine thyroglobulin, and its Pronase-digested glycopeptide (glycopeptide B) which has only the serum glycoprotein-type sugar chains (Figure 1), were potent inhibitors only against MAM and RCH. Desialization of these glycoproteins and glycopeptides gave rise to a remarkable loss of inhibitory activity against MAH and MAM, but marked enhancement of inhibitory activity against BPH. Band 3 glycoprotein of human erythrocyte membranes, sugar chains of which had been known to consist mainly of asialo-serum glycoprotein-type ones (Adair and Kornfeld, 1974), exerted

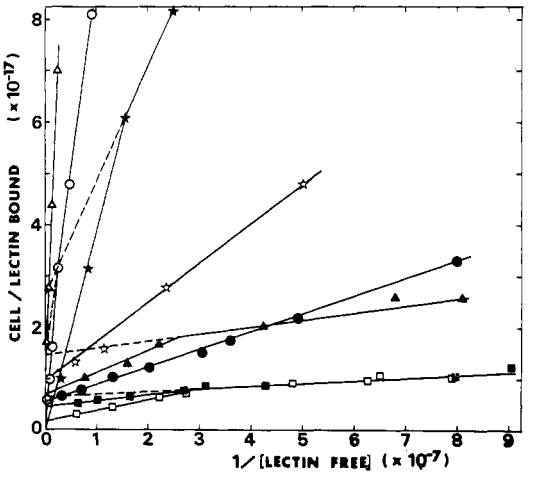

FIGURE 4: Binding of ^{125}I-labeled lectin to normal (solid symbols) and sialidase-treated human lymphocytes (open symbols). The binding reactions were performed as described previously (Kawaguchi et al., 1974b). The data were plotted by the method of Steck and Wallach (1965). (●, ○) [^{125}I]MAM; (■, □) [^{125}I]RCH; (▲, △) [^{125}I]MAH; (★, ☆) [^{125}I]BPH.

marked inhibitory activity only against RCH. These results are in good agreement with our previous assumption (Kawaguchi et al., 1974b; Irimura et al., 1975) that both MAH and BPH bind preferentially to the mucin-type sugar chains, whereas both MAM and RCH bind primarily to the serum glycoprotein-type sugar chains on human erythrocyte membranes. The same conclusion was obtained more definitively by the following more quantitative studies on the inhibition of lectin binding to human erythrocytes and lymphocytes with various glycoproteins and glycopeptides.

Inhibition of Lectin Binding to Cell Surface with Various Glycoproteins and Glycopeptides. The inhibition constants of various glycoproteins and glycopeptides against ^{125}I-labeled lectin binding to the major receptor sites on the cell surface were calculated by eq 12 which was derived as follows. We assume two mutually exclusive equilibria are present between ^{125}I-labeled lectin (L), the inhibitory glycoprotein or glycopeptide (I), and the major receptor site on the cell surface (R), if R and I share a common binding site of L.

$$L + R \rightleftharpoons LR \quad (1)$$

$$L + m'I \rightleftharpoons LIm' \quad (2)$$

In these equilibria, the reversibility of the lectin binding was demonstrated. Then, the following equations describe the

TABLE II: Hemagglutination Inhibitory Activities of Various Glycoproteins and Glycopeptides.

Inhibitor	Mol Wt	Minimum Concn (μM) Completely Inhibiting 4 Hemagglutinating Doses			
		MAM	RCH	MAH	BPH[a]
PAS-1	31 000[b]	0.002	0.0004	0.0008	0.002
Desialized PAS-1	23 000[b]	>0.1	0.0004	>0.1	0.0001
Ch-3 glycopeptide	7 200[b]	>300	>300	3	100
Desialized Ch-3 glycopeptide	4 800[b]	>100	>100	>100	2
Band 3	88 000[c]	>2	0.0005	>2	>2
Porcine thyroglobulin	670 000[d]	0.1	0.02	>2	>2
Desialized porcine thyroglobulin	650 000[d]	0.5	0.02	>2	>2
GPB	3 300[e]	1	300	>3000	>3000
Desialized GPB	3 000[e]	3	200	>3000	>3000

[a] Sialidase-treated erythrocytes were used. [b] Tomita and Marchesi, 1975. [c] Steck, 1972. [d] McQuillan and Trikejus, 1972. [e] Fukuda and Egami, 1971.

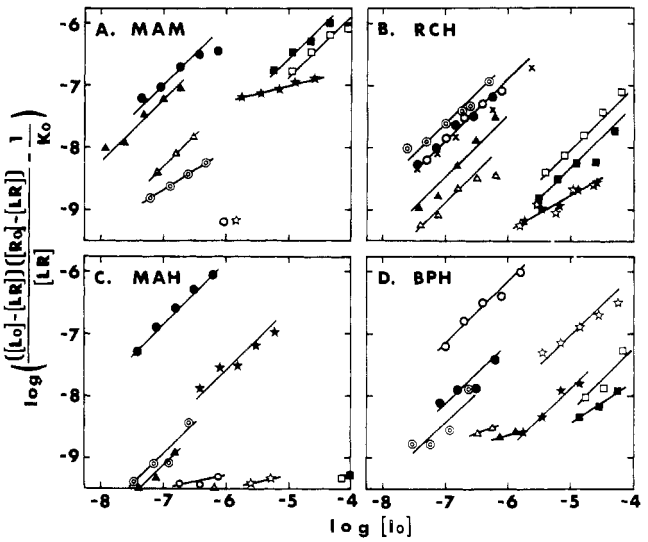

FIGURE 5: Effects of various glycoproteins and glycopeptides on ^{125}I-labeled lectin binding to human erythrocytes and determination of K_I values. Binding of (A) [^{125}I]MAM (3.05×10^{-8} M), (B) [^{125}I]RCH (2.25×10^{-8} M), (C) [^{125}I]MAH (2.86×10^{-8} M) to normal erythrocytes and (D) [^{125}I]BPH (1.65×10^{-8} M) to sialidase-treated erythrocytes was carried out in the presence of various concentrations of inhibitors. From the data of the binding inhibition, $\log \{([([L_0] - [LR])([R_0] - [LR])]/[LR]) - (1/K_0)\}$ values were calculated and shown as a function of $\log [I_0]$ (see eq 12 in the text). K_I values determined are presented in Table III. (●) PAS-1, (○) desialized PAS-1, (▲) porcine thyroglobulin, (△) desialized porcine thyroglobulin, (■) GPB, (□) desialized GPB, (◎) band 3, (★) Ch-3 glycopeptide, (☆) desialized Ch-3 glycopeptide, (X) PAS-1 in the presence of 0.01% sodium dodecyl sulfate.

equilibria:

$$K_0 = [LR]/([L][R]) \quad (3)$$

$$K_I = [LIm']/([L][I]^{m'}) \quad (4)$$

where [L] = free ^{125}I-labeled lectin concentration, [I] = free inhibitor concentration, [R] = concentration of free major receptor site on the cell surface, [LR] = concentration of ^{125}I-labeled lectin–receptor complex, [LIm'] = concentration of ^{125}I-labeled lectin–inhibitor$_{m'}$ complex. Since

$$[R] = [R_0] - [LR] \quad (5)$$

where $[R_0]$ = input receptor concentration calculated by input cell number (per liter) × n (listed in Table I)/Avogadro's number, then

$$K_0 = \frac{1}{[L]} \frac{[LR]}{[R_0] - [LR]} \quad (6)$$

and

$$[LIm'] = [L_0] - [L] - [LR] \quad (7)$$

where $[L_0]$ = input ^{125}I-labeled lectin concentration. By substituting for [LIm'] in eq 4

$$K_I = \frac{[L_0] - [L] - [LR]}{[L][I]^{m'}} \quad (8)$$

then

$$[L] = \frac{[L_0] - [LR]}{1 + K_I[I]^{m'}} \quad (9)$$

By substituting for [L] in eq 6

$$K_0 = \frac{1 + K_I[I]^{m'}}{[L_0] - [LR]} \frac{[LR]}{[R_0] - [LR]} \quad (10)$$

Equation 10 can be rearranged into eq 11 and 12.

$$\frac{([L_0] - [LR])([R_0] - [LR])}{[LR]} - \frac{1}{K_0} = [I]^{m'} \frac{K_I}{K_0} \quad (11)$$

$$\log \left\{ \frac{([L_0] - [LR])([R_0] - [LR])}{[LR]} - \frac{1}{K_0} \right\} = m' \log [I] + \log \frac{K_i}{K_0} \quad (12)$$

Equation 12 is of the form $y = ax + b$. From the data of the binding inhibition

$$\log \left\{ \frac{([L_0] - [LR])([R_0] - [LR])}{[LR]} - \frac{1}{K_0} \right\}$$

values were calculated and showed as a function of $\log [I_0]$ ($[I_0]$ is input inhibitor concentration which is approximately equal to [I]) in Figures 5 and 6. Thus the slope will give m' and the intercept on the ordinate will give $\log (K_I/K_0)$. The m' values of most inhibitors were found to be approximately 1 as shown in Figures 5 and 6. K_I values were then calculated and listed in Table III. The K_I values of PAS-1 glycoprotein toward MAM and MAH are almost identical with the K_0 values for MAM and MAH, respectively. Furthermore, the K_I value of the desialyzed PAS-1 glycoprotein toward BPH was equal to the K_0 value of the same lectin. Moreover, Ch-3 glycopeptide gives meaningfully large K_I values only toward MAH and BPH and, in contrast, porcine thyroglobulin and its glycopeptide (glycopeptide B) give significantly large K_I values

TABLE III: K_I Values of Various Glycoproteins and Glycopeptides toward the Lectin Binding to Human Erythrocytes and Lymphocytes.

Inhibitors	K_I Values[a] in the Competitive Binding with			
	[^{125}I]MAM	[^{125}I]RCH	[^{125}I]MAH	[^{125}I]BPH[b]
Pas-1	1×10^7 (1×10^7)[c]	2×10^7 (3×10^7)[c]	2×10^8 (2×10^8)[c]	6×10^6
Desialized PAS-1	nd[d]	$2 \times$	$<1 \times 10^0$	1×10^7 (8×10^6)[c]
Ch-3 glycopeptide	$<1 \times 10^1$	$<6 \times 10^2$	5×10^6 (5×10^6)[c]	3×10^4
Desialized Ch-3 glycopeptide	nd[d]	$<6 \times 10^2$	$<1 \times 10^0$	2×10^5 (3×10^5)[c]
Band 3	$<4 \times 10^2$	3×10^7	1×10^6	6×10^5
Porcine thyroglobulin	1×10^7 (1×10^7)[c]	4×10^6	1×10^6	$<2 \times 10^0$
Desialized porcine thyroglobulin	6×10^5	2×10^6	$<7 \times 10^4$	$<1 \times 10^2$
GPB	4×10^5 (8×10^5)[c]	6×10^4	nd[d]	$<1 \times 10^2$
Desialized GPB	2×10^5	1×10^5	nd[d]	1×10^4

[a] Average values of triplicate experiments. [b] Sialidase-treated cells were used. [c] The values in parentheses were obtained toward the lectin binding to lymphocytes. [d] Not determined.

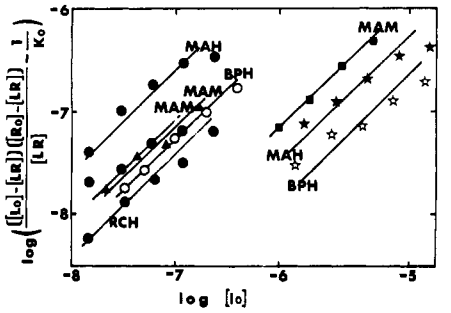

FIGURE 6: Effects of various glycoproteins and glycopeptides on binding of ^{125}I-labeled lectins to human lymphocytes, and determination of K_I values. The binding of ^{125}I-labeled BPH was performed on sialidase-treated human lymphocytes. The concentrations of ^{125}I-labeled lectins were as follows: [^{125}I]MAM, 7.02×10^{-8} M; [^{125}I]RCH, 2.53×10^{-8} M; [^{125}I]MAH, 2.54×10^{-8} M; [^{125}I]BPH, 3.37×10^{-8} M. From the data of the binding inhibition, $\log \{[([L_0] - [LR])([R_0] - [LR])]/[LR] - (1/K_0)\}$ values were calculated and shown as a function of $\log [I_0]$ (see eq 12 in the text). K_I values determined are presented in Table III. (●) PAS-1; (○) desialized PAS-1; (▲) porcine thyroglobulin; (■) GPB; (★) Ch-3 glycopeptide; (☆) desialized Ch-3 glycopeptide.

toward MAM and RCH than toward MAH and BPH. Desialyzation of these glycoproteins and glycopeptides lowered remarkably the K_I values toward MAM and MAH but increased that toward BPH. These results clearly indicate that PAS-1 glycoprotein of human erthrocyte membranes is the major receptor site for MAH, MAM, and BPH. MAH binds preferentially to the sialic acid containing sugar sequence of the mucin-type sugar chains of PAS-1 glycoprotein and MAM binds primarily to the sialic acid containing sugar sequence of the serum glycoprotein-type sugar chains of PAS-1 glycoprotein. On the other hand, BPH possibly binds to the asialomucin-type sugar chains of PAS-1 glycoprotein. In the case of RCH, the highest K_I values were obtained for both PAS-1 and band 3 glycoproteins, but they were still significantly smaller than K_0 value of the same lectin. This may possibly be explained by the fact that band 3 glycoprotein is a mixture of glycoproteins having different structures of sugar chains (Steck, 1974) and RCH can bind to one of these glycoproteins (Kondo and Osawa, unpublished results). Therefore, it could be assumed that RCH binds primarily to the serum glycoprotein-type sugar chains of a glycoprotein of the band 3 group and partly to the same type sugar chains of PAS-1 glycoprotein on human erythrocyte membranes. Adair and Kornfeld (1974) also reported that band 3 glycoprotein was selectively adsorbed to a RCH-Sepharose affinity column from Triton-solubilized human erythrocyte membranes. The fact that the K_I values of PAS-1 glycoprotein toward the lectin binding to human lymphocytes (Table III) are almost equal to the K_0 values of the lectins for the binding to human lymphocytes (Table I) also suggests that carbohydrate chains on human lymphocytes have similar structure to those on human erythrocytes.

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Enzymatic Synthesis of (15*S*)-[15-^{3}H]Prostaglandins and Their Use in the Development of a Simple and Sensitive Assay for 15-Hydroxyprostaglandin Dehydrogenase†

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ABSTRACT: The stereospecificity of swine renal NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase has been determined. It was found that the enzyme is a B-side specific dehydrogenase. (15*S*)-[15-^{3}H]Prostaglandins were synthesized by stereospecific transfer of the tritium label of D-[1-^{3}H]galactose to prostaglandins by coupling 15-hydroxyprostaglandin dehydrogenase with β-D-galactose dehydrogenase, an enzyme of the same stereospecificity. A simple and sensitive assay for 15-hydroxyprostaglandin dehydrogenase was developed based on the stereospecific transfer of the tritium label of tritiated prostaglandins to glutamate by coupling 15-hydroxyprostaglandin dehydrogenase with glutamate dehydrogenase. The amount of prostaglandin oxidized is determined by the radioactivity of labeled glutamate present in the supernatant after charcoal precipitation of labeled prostaglandin. Concurrent assays with the present tritium release method and the thin-layer chromatography method indicated excellent correlation. The assay was employed to study some of the properties of swine renal 15-hydroxyprostaglandin dehydrogenase in crude extract and the distribution of enzyme activity in various tissues of rat. Enzyme activity was linear for the first 10 min studied and was nonlinear with increasing amounts of crude enzyme, indicating the possible presence of endogenous inhibitor(s). Apparent K_m's for PGE_2, $PGF_{2\alpha}$, and PGA_2 were found to be 2.5, 12.5, and 3.9 μM, respectively. The distribution pattern indicated high levels of enzyme activity in gastrointestinal tract, lung, kidney, and spleen. The assay method may prove to be valuable for studying enzyme turnover and enzyme regulation by hormonal and pharmacological agents.

Conversion of the 15(*S*)-hydroxyl group of prostaglandins to a keto function by NAD^+-dependent[1] 15-hydroxyprostaglandin dehydrogenase (NAD^+-15-hydroxyprostanoate oxidoreductase (EC 1.1.1.141)) is considered to be both the initial and major route for their transformation to inactive metabolites (Anggard and Samuelsson, 1964). This enzyme has been shown to be present in most tissues examined and purification of this enzyme from human placenta (Braithwaite and Jarabak, 1975; Schlegel and Greep, 1975), bovine lung (Nagasawa et al., 1975, Matschinsky et al., 1974), swine lung (Anggard and Samuelsson, 1966), chicken heart (Lee and Levine, 1975), and swine kidney (Tai et al., 1974) has been attempted. The methods employed by these workers for the assay of 15-hydroxyprostaglandin dehydrogenase include development of chromophore at 500 nm (Anggard et al., 1971), measurement of the formation of NADH spectrophotometrically, and application of radioimmunoassay. Development of chromophore at 500 nm induced by alkalinization of the reaction product, 15-oxo-PGE or 15-oxo-PGA, provides a simple assay for this enzyme. However, the assay can not be reliably employed in crude stages of the enzyme preparation because of the interference of hemoproteins at 500 nm. Furthermore, the chromophore has only transient stability. Measurement of the formation of NADH spectrophotometrically is allowed only after a certain degree of enzyme purification simply because the interfering enzymes utilizing NADH are also present in the crude preparation. Although radioimmunoassay provides

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[1] Abbreviations used are: PGE_1, prostaglandin E_1 (11α,15α-dihydroxy-9-oxo-13-*trans*-prostenoic acid); PGE_2, prostaglandin E_2 (11α,15α-dihydroxy-9-oxo-5-*cis*,13-*trans*-prostadienoic acid); PGA_2, prostaglandin A_2 (15(*S*)-hydroxy-9-oxo-5-*cis*,10,13-*trans*-prostatrienoic acid); $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$ (9α,11α,15α-trihydroxy-5-*cis*,13-*trans*-prostadienoic acid); 15-oxo-PGE_2, 15-oxoprostaglandin E_2 (11α,15α-dihydroxy-9,15-dioxo-5-*cis*,13-*trans*-prostadienoic acid); 15-oxo$PGF_{2\alpha}$, 15-oxoprostaglandin $F_{2\alpha}$ (9α,11α-dihydroxy-15-oxo-5-*cis*,13-*trans*-prostadienoic acid). NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane.