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**[Original]**

# Studies on the Preparation of Radioactive Iodine Labelled Concanavalin-A, Lectin Extracted from Korean Native Plant "Banha", and Their Conjugation Products and the Hemagglutination Tests of These Labelled Compounds in Vitro.

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**Abstract**

Concanavalin-A, lectin extracted from Korean native plant "Banha" (Pinellia Termata) and their conjugation products with tyrosine and 5-iodo-6-aminouracil were labelled by radioactive iodine-125, and these labelled compounds were subjected to a hemagglutination test in vitro. The labelling procedures were presented with respect to labelling yield and the trends on the conjugation reaction of lectin with various amino acids were discussed. The preliminary results on the hemagglutination tests against cancer tissues were presented and the potential clinical applications of these labelled compounds were discussed.

**요 약**

Concanavalin-A, 한국산 반하(半夏)로부터 추출된 Lectin 및 이들과 tyrosine 또는 5-iodo-6-aminouracil을 결합시킨 화합물들을 각각 방사성 요오드-125로 표지하였으며 표지된 화합물들을 사용하여 생체외부 시험법으로 혈액응고 시험을 실시하였다. 표지반응에 관하여 표지수율과 함께 그 실험조건을 보고하였으며 Lectin과 여러 아미노산계통 화합물들과의 결합반응성을 논의하였다. 암세포에 대한 혈액응고 시험결과를 보고하였으며 이들 표지화합물들의 임상적 실용전망에 관하여서도 논의하였다.

## Introduction

Various lectins, cell-agglutinating and sugar specific proteins have been known to provide a new tool for studying malignant cell surface such as a cancer cell[1]. Especially, concanavalin-A extracted from the jack bean meal (2.5 to 3.0 percent by weight) has extensively been studied to ascertain its specific agglutination properties towards a malignant cell, the cell transformed by DNA tumor viruses or chemical carcinogens[2]. On the other hand, a kind of glycoprotein extracted from the Korean native plant "Banha" (Pinellia Ter-

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nata) had clinically been observed to afford a specific hemagglutinating property on the malignant cell, which is expected to be useful to locate cancer cells or to be applied for a cancer therapy[6].

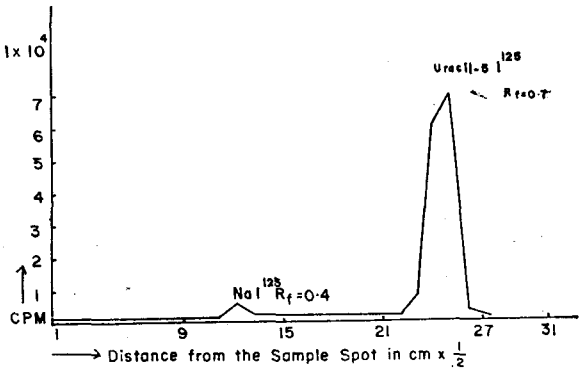
In this research the labelling procedure of concanavalin-A and "Banha" extracted lectin was at first investigated to afford the radioactive labelled compound for the cancer research with respects to the specific agglutinating property of lectins as described above. The extract from "Banha" is to be purified and the effective main component of the extract will be separated and identified. The concanavalin-A and the purified "Banha" lectin will be labelled by $^{125}I$ according to the procedures reported in the previous investigations and reaction parameters of affording a high labelling yield will further be investigated. The labelled compound would be subjected to the clinical investigation at the Cancer Hospital for the purpose of evaluating the feasibility of the labelled compound for the clinical applications.

Crosslinking products of various proteins themselves have been applied for preparing an effective immuno-adsorbent[3], and coupling products of proteins and enzyme have also been achieved by means of glutaraldehyde and they were proved to be useful fon identifying an antigen or an antibody[4]. Especially, conjugation products of concanavalin-A and ferritin have been used for ascertaining the topology of cell surface[5], which may eventually be useful to understand the specific agglutinating properties of a lectin. These trends of researchs seemed to be very promising to investigate the clinical applicability of lectins for cancer therapy or diagnosis, since the binding site between lectins and the malignant cell in a agglutinating reaction is expected to be concerned with the topology or chemical moiety of the cell surfaces. It is, therefore, considered to be worthwhile to investigate the conjugation reaction between "Banha" lectin and one of such anticancer durgs as antimetabolites, which may give a modified or specific agglutinating property towards a malignant cell and results some local uptakes of the anticancer drug itself by the malignant cell surface when the conjugation products are administered and hydrolysed in vivo on the surface of a cell. The conjugation reaction would better be studied through a radioactive tracer method, since the reaction is of a micro scale order. The relevant compounds may be labelled by radioactive iodine and the conjugated products may be subjected to a clinical investigation in vitro to evaluate the particular agglutinating behaviors for a cancer cell.

## Experiments

### 1. Preparation of 5-iodouracil labelled by $^{125}I$.

A catalytical amount of hydrogen peroxide




Developing Solvent: n-Butanol saturated with $H_2O$ at 20°C by descending system
Paper: Whatmann #1

**Fig 1. Paper Chromatogram of the Uracil-5 $^{125}I$**

**Table 1. The Labelling of Uracil, 6-Aminouracil, and Tyrosine with radio isotopes.**

| Compounds | quantity of reagent(mg) | quantity of KI(mg) | quantity of Chl-T* or $H_2O_2$** | Labelling yield***% | Radio nucleide | Remarks |
|---|---|---|---|---|---|---|
| Uracil | 20 | 1 | 0.1 ml | 90 | $^{125}I$ | in acetate Buff solution. (PH 4.8) at 100°C. |
| Uracil-5-Br | 20 | 1 | 0.1 ml | 95 | 〃 | 〃 |
| 6-Aminouracil | 1 | 0.01 | 0.1 ml | 60 | 〃 | 〃 |
| D-Tyrosine | 0.8 | 0.01 | 0.1 mg | 80 | 〃 | in saline solution (PH8-9) |
| Uracil | 56 | 59 | 0.1 ml | 20 | $^{82}Br$ | 〃 |
| Uracil-5 Br | 200 | | | 4.3 | $^{82}Br$ | recoil reaction |

*Chloroamine-T

**30% $H_2O_2$

***The labelling yield was estimated by comparing the areas of the peaks of the unreacted iodnie and product on a PPC.

was added dropwise to a boiling aqueous solution of uracil or 5-bromouracil, Na $^{125}I$, and acetate buffer(PH 4.8), and the resulting mixture was further refluxed for 1 hour. The compositions of the reaction mixture was checked by a descending PPC and the labelling yield of the product was evaluated by checking the radioactivity of the peak of the reported $R_f$ value[7]. The reaction parameters, PPC, and the labelling yield were given in the table 1 and the Fig. 1.

### 2. Preparation of 5-bromonuracil labelled by $^{82}Br$.

The same procedures as above were applied using $K^{82}$ Br. The $K^{82}$ Br was prepared by neutrilizing an aqueous solution of $NH_4$ $^{82}Br$ with 5% KOH solution and evaporate the solution to dryness. The results were shown in the table. 1.

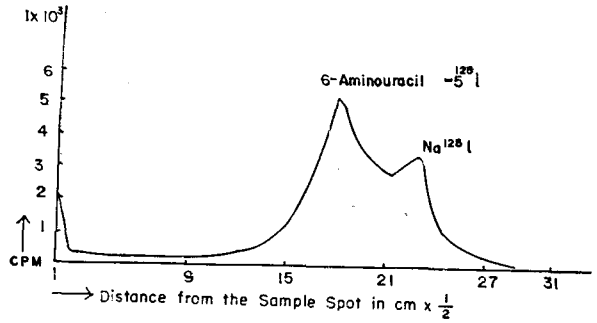
### 3. Preparation of 6-aminouracil

The reported procedure for the preparation of diaminouracil hydrochloride[8] was adopted. In a three necked round bottom flask equipped with a stirrer, reflux condenser, and glass-col mantle, 2.0 g of metallic sodium was dissolved in 50 ml. of absolute ethanol. To the solution, 4.6 ml. of ethyl cyanoacetate and 2.6 g. of urea were added and the mixture was heated at 80°C under stirring for 4 hrs. The reaction mixture was slowly dissolved in hot water (80°C) and the solution was heated for 20 minutes. This hot solution was neutralized by glacial acetic acid to give a frothing. The frothings were separated from the mixture by centrifuge and the separated solid product was washed several times with distilled water. The dried solid product melted at 360°C. The yield was about 60%. The chemical structure of the product was identified by means of IR and UV spectrophotometry.

### 4. Preparation of 6-aminouracil labelled by $^{125}I$.

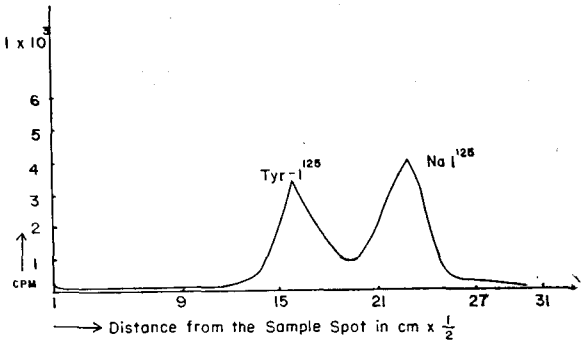
The procedure described for labelling 5-iodouracil was applied. The labelled product was identified by PPC(See Fig. 2). The reaction parameters and labelling yield were shown in the table 1.

### 5. Preparation of tyrosine labelled by $^{125}I$.




Developing Solvent: 75% Methanol in Water at 20°C
Paper: Whatmann #1

**Fig. 2. Paper Chromatography of 6-Aminouracil-5 $^{125}I$.**




Developing Solvent: 75% Methanol in $H_2O$ at 20°C
Paper: Whatmann #1

**Fig. 3. Paper Chromatography of Tyrosine-$^{125}I$**

The chloromine-T procedure[9] was adopted. In the saturated aqueous solution of tyrosine an adequate amount of sodium bicarbonate solution was added to adjust the PH of the entire solution at 8-9. To this solution, 0.1 mg of potassium iodide and Na $^{125}I$ solution were added and the reaction mixture was stirred at room temperature for 1 hr. The reaction mixture was examined by PPC to identify the product (See Fig. 3). The reaction parameters and labelling yield were shown in the table 1.

### 6. Preparation of glutaraldehyde

According to the reported proccedure[10], the mixture of acrolein (0.5 mole) and ethyl vinylether (0.55 mole) was reacted in a stainless bomb at 140-150°C for 12 hrs. The reaction mixture was distlilled under reduced pressure. The fraction boiled at 38°C (8 mm/Hg) was collected and hydrolysed with 62.5 ml of distilled water acidified by 5.2 ml of hydrochloric acid. The hydrolysed mixture was extracted with ethyl ether. The ether extract was dried under anhydrous calcium chloride and the ether was distilled off. The residue was distilled under reduced pressure to obtain the product boiled at 50°C (6 mm/Hg). The yield was averaged 38-40%.

### 7. Separation of lectin portion from the "Banha" extract.

a. The preparation of Banha extract.

The dried root of "Banha" plant (Pinellia Ternata) was pulverized in a fine powder[11]. 100 g of the powder was suspended in 500 ml of 0.15 M NaCl solution and the suspension was stirred at 4°C for 2 hrs. The resulting suspension was filtered and the filtrate was centrifuged using a refrigerated centrifuge equipped with a SS-34 Rotor. (11,000-12,000 r. p. m., 145,000-150,000g) The yellowish supernatant liquid was separated and it was saturated with 30% solution of ammonium sulfate. The saturated solution was neutralized using ammonium hydroxide solution and stirred for 4 hrs. The solution was again centrifuged and the supernatant liquid was separated. The supernatant solution separated was again saturated with 80% solution of ammonium sulfate and it was centrifuged. The precipitate was separated and dissolved in 150 ml of cold distilled water. The solution was dialysed against distilled water and it was again dialysed against 1 M solution of sodium chloride. The dialysed product was centrifuged and

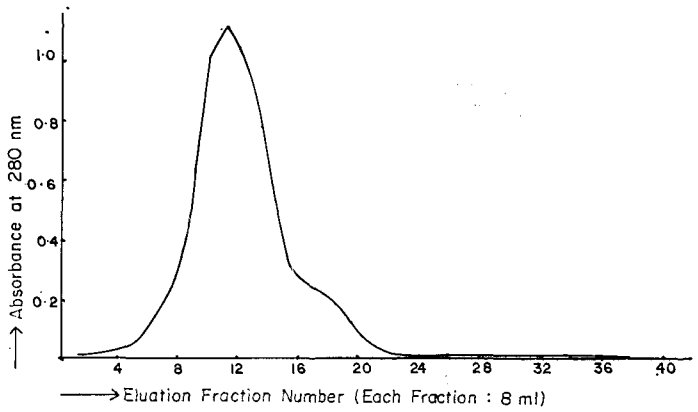
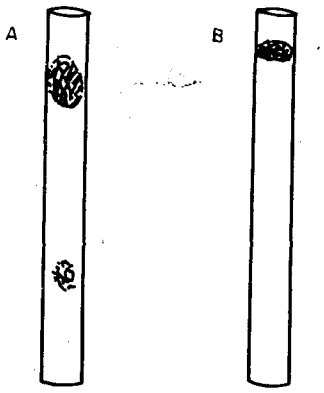


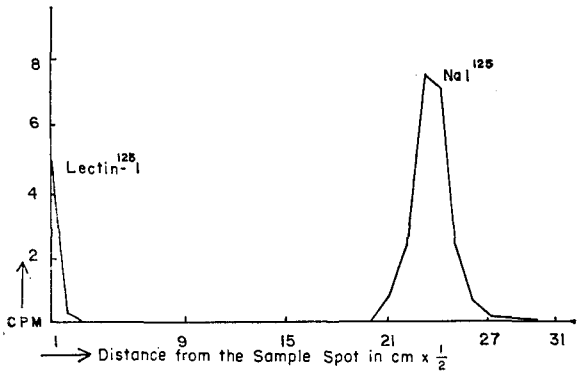

**Fig 4. Elution Curve of the purified lectin solution by Shephadex G-120 column Chromatography**




Amidoblack 10B Staining PAS Staining

**Fig. 5. 5% SDS-polyacrylamide gel electrophoregram[20] of purified lectin from Banha root stained for protein and glycoprotein.**

**Eiectrophoresis; Const. Current, 8 ma per gel.**




Developing Solvent: 75% Methanol in Water at 20°C

Paper: Whatmann #1

**Fig. 6. Paper Chromatography of the Lectin-$^{125}$I.**

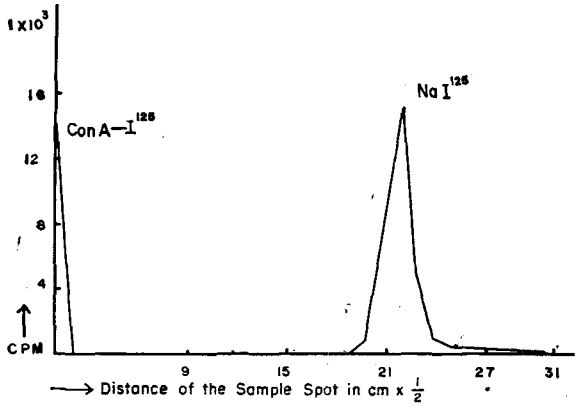
the clear supernatant liquid was separated as the purified extract.

b. The separation of lectin portion from the extract.

The purified extract was chromatographed at the flow rate of 35-40 ml/hr. through a Shephadex G-120 column presaturated by 1 mol. solution of sodium chloride. The eluate from the column was collected 8 ml interval and each fraction was examined for its UV absorption at $\lambda$ max 280 nm (See Fig. 4), and the fractions showing absorption at 280 nm were collected as the active lectin portion of the extract. The collected fractions were dialysed against distilled water. The dialysed extract was stored at 4°C as a stock solution of "Banha" lectin or lyophilized as a dried powder.

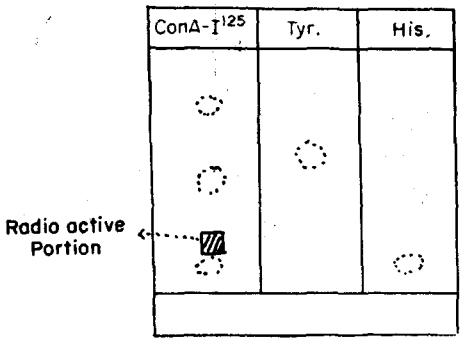
### 8. Determination of molecular weight of "Banha" lectin.

According to the Gel electrophoresis procedure for polyacrylamide[12] a phosphate buffer (pH 7.2) solution of "Banha" lectin lyophilized as above was subjected to a gel




Developing Solvent: 75% Methanol in Water at 20°C
Paper: Whatmann #1

**Fig. 7. Paper Chromatography of the Con-A-$^{125}$I**




Developing Solvent: Butanol: Acetic Acid: Water (4 : 1 : 5 V/V)
Paper: Whatmann #1
Solvent Front: 20 cm

**Fig. 8. Paper Chromatography of the hydrolyzed lectin**

electrophoresis in a tube (ID 5mm, Length, 10 cm) (See. Fig. 5). It was found that the molecular weight of the lectin amounted to 150,000 when the porition of the spot was compared with that of Con-A of known mcolecular weight[20].

### 9. Preparation of "Banha" lectin labelled by $^{125}$I.

The reported procedure for labelling concanavalin-A[13] was applied. The purified lectin was acetylated and the acetylated product was dialysed against distilled water. The dialysed solution (2 ml) was mixed with Na $^{125}$I, (desired amount of radio activity) potassium iodide (0.1 mg) and the PH of the resulting solution was adjusted at 8.0 by means of a sodium bicarbonate solution. To this solution, 0.5 ml of chloroamine-T (2 mg/ml) was added dropwise and the reaction mixture was reacted for 1 hr at 20C°. The reaction mixture was examined by PPC to determine the labelling yield (35%) (See. Fig. 6). The labelled lectin was separated from the reaction mixture by means of a dialysis.

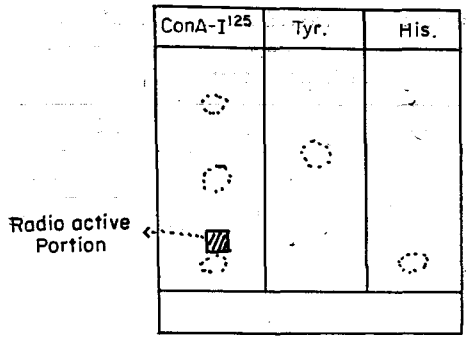
### 10. Preparation of concanavalin-A labelled by $^{125}$I.

The concanavalin-A (Signa Chem, Co. USA) was acetylated and the acetylated product was labelled by the procedure described above. The PPC of the labelled mixture was shown in the Fig. 7. The labelling yield was 50%. The labelled product was separated by dialysis.

### 11. Hydrolysis of labelled lectin and concanvalin-A

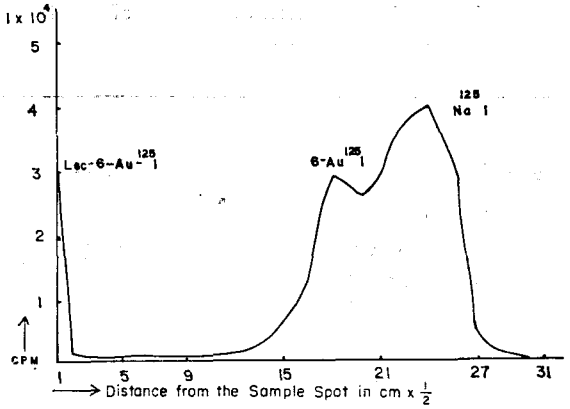
a. Hydrolysis of labelled lectin

2 ml. of labelled lectin and 10 ml. of 6N-HCI were mixed together in a round bottomed flank equipped with a reflux condenser and the resulting mixture was refluxed for 20 hrs. The resulting solution was concentrated to a small volume and was examined for it's amino acid compositions by PPC. The $R_f$ values of each amino acid components on the PPC were studied both by radioactivity counting and coloring reagent such as ninhydrin. Separately, a standard solution of tyrosine and histidine was prepared and their $R_f$ values on the PPC were examined by means of coloring rea-



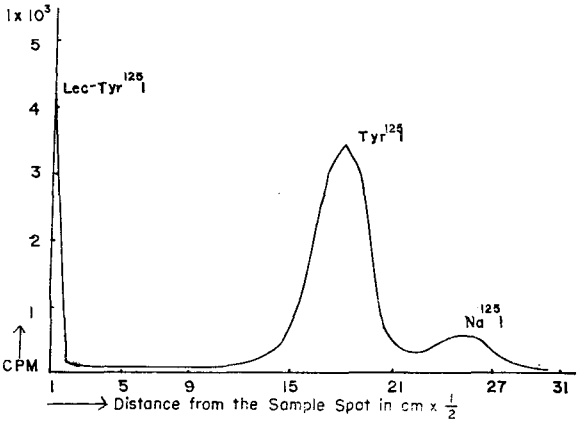

Developing Solvent; Butanol : Acetic Acid : Water (4 : 1 : 5 V/V)
Paper: Whatmann #1
Solvent Front : 20 cm

**Fig. 9. Paper Chromatography of the hydrolyzed Con-A-$^{125}$I**




Developing Solvent: 75% Methanol
Paper: Whatmann #1

**Fig. 10. Paper Chromatography of 6-Aminouracil-$^{125}$I Conjuated Lectin.**




Developing Solvent: 75% Methanol in $H_2O$ at 20°C
Paper: Whatmann #1

**Fig. 11. Paper Chromatography of Tyr-$^{125}$I conjugated Lectin**

gent such as ninhydrin. The obtained $R_f$ values on both PPC were compared and the compositions of the hydrolyzed product of labelled lectin were evaluated. (See Fig. 8)

b. Hydrolysis of labelled concanavalin-A.

The same procedure described in a, was followed. The result was shown in Fig. 9.

### 12. Preparation of the conjugated lectins.

a. Conjugated product between lectin and labelled 6-aminouracil.

The glutaraldehyde conjugation procedure[5] was applied for the present system. 1.0 ml of the solution of 6-aminouracil labelled (0.1 M acetate buffer solution, PH= 4.8) 5 mg. of the lectin, and a small amount of NaCl were dissolved to make up a solution and 0.1 ml of 1% aqueous solution of glularaldehyde was further added to this solution. The resulting solution was kept at room temperature (20—22°C) for 2 hrs. The reaction mixture was examined for it's conjugated product by means of PPC as shown in Fig. 10. The conjugation reaction solution was dialysed against distilled water to remove the low molecular weight components and the dialyzed product was examined again for its purity by PPC and the radioactivity of pure conjugated product was measured to check the overall labelling yield, which amounted to 15.0%.

b. Conjugated product between lectin and labelled tyrosine.

The same procedur described in a was followed. The overall labelling yield was 18.0%. The PPC data was given in Fig. 11.

### 13. Hemagglutination tests (in vitro) for labelled "Banha" lectin and conjugated lectins.

**Table. 2. Agglutination test of $^{125}I$-labelled glycoprotein extracted from "Banha" Plant.**

| | Total (cpm) | | | Sediment (cpm) | | |
|---|---|---|---|---|---|---|
| organ / case | RBC | WBC | | RBC | WBC | |
| | | Lym. | Granulocyte | | Lym. | Granulocyte |
| C3Hf | 1182 | 2149 | 1880 | 780 | 1026 | 975 |
| Hartley | 2181 | 1911 | 2493 | 1464 | 1315 | 1382 |
| Strain 2 | 3127 | 3129 | 2087 | 1941 | 1998 | 945 |
| Case A | 2505 | 1838 | 2656 | 731 | 1248 | 1072 |
| Case B | 1398 | — | 1869 | 505 | — | 1158 |

**Table. 3. Agglutination test of $^{125}I$-labelled glycoprotein extracted from "Banha" Plant.**

| Radioactivity / Case | Total (cpm) | | Sediment (cpm) | |
|---|---|---|---|---|
| Ehrlich | 9909 | | 5716 | |
| Sarcoma-180 | 7992 | | 4338 | |
| organ / Case | RBC | WBC | RBC | WBC |
| Strain 2 | 10346 | 9082 | 1523 | 5296 |
| .. .. .. | 10058 | 8822 | 2852 | 4839 |
| Case A | 11183 | 9488 | 1231 | 4079 |
| Case B | 9101 | 9063 | 1354 | 4196 |

**Table. 4. Agglutionation test of 6-Aminouracil and Tyrosine conjugated Lectin**

| Radioactivity / Case | | Total (cpm) | | Sediment (cpm) | |
|---|---|---|---|---|---|
| | | RBC | WBC | RBC | WBC |
| Case A | Heparin | U* 12851 | 14059 | 4229 | 1540 |
| | | Tyr** 17917 | 18225 | 3234 | 2078 |
| | EDTA | U 15297 | 13958 | 5075 | 1851 |
| | | Tyr 16363 | 16248 | 2845 | 2307 |
| Case B | Heparin | U 13428 | 14737 | 4821 | 2222 |
| | | Tyr — | 11015 | — | 1567 |
| | EDTA | U 13602 | 13502 | 5469 | 2686 |
| | | Tyr — | — | — | — |

* 6-Aminouracil-5 $^{125}I$ conjugated lectin
** Tyrosine-$^{125}I$ conjugated lectin

Standard procedure-The blood samples from hartly, strain 2 guinea pig, C3Hf mice, and leukemia cases were separated for their RBC and WBC components, and the WBC portion was further separated for Lym. and Granulocyte. The separated portions were mixed with a definite amount of labelled lectins or labelled conjugated

**Table. 5. Agglutination test of 6-Aminouracil and Tyrosine conjugated lectin.**

| Case \ Radioactivity | | Total (cpm) | | Sediment (cpm) | |
|---|---|---|---|---|---|
| Ehrlich | U.* | 33265 | | 18180 | |
| | Tyr.** | 26336 | | 4337 | |
| Sarcoma-180 | U. | 31600 | | 26052 | |
| | Tyr. | 24485 | | 4504 | |
| Case \ organ | | RBC | WBC | RBC | WBC |
| C3Hf | U. | 36837 | 34825 | 21333 | 6460 |
| | Tyr. | 27271 | 25203 | 6694 | 3067 |
| Strain 2 | U. | 31567 | 35877 | (42620) | 14422 |
| | Tyr. | 24863 | 26946 | 5235 | 3569 |
| Hartly | U. | 33295 | 42009 | (35297) | 16797 |
| | Tyr. | 25901 | 25438 | 4247 | 4291 |

* 6-Amino Uracil-5 $^{125}I$ Conjugated lectin
** Tyrosine-$^{125}I$ Conjugated lectin

lectins and the mixture was shaked vigorously to complete the hemagglutination reaction. Each system was examined for the hemagglutination by a microscope.[19] After being ascertained the hemagglutination of a sample system, the system was centrifuged and the radioactivities of both supernatent liquid and precipitate were counted. The results were summarized in table. 2. In cases of Ehrlich and Sarcoma-180, the hemagglutination reaction for a system was completed at first and the resulting mixture was sedimented. The radioactivity of the sedimented fraction of each system was measured and evaluated. The results were summarized in table 3, 4, and 5, respectively.

## Results and Discussion

As shown in table 1, labelling of uracil, 6-aminouracil, and D-tyrosine could be achieved with relatively high labelling yields and purities by means of chloroamine -T[9] or $H_2O_2$[9] procedures. In case of labelling an uracil by the radioactive bromine-82, a rapid direct bromination of an uracil could be achieved by means of the reactor produced $K^{28}Br$, whereas the recoil labelling procedure could give only the low labelling yield (20%) together with many kinds of decomposed products. The prepared products are all examined by PPC, which showed the same composition of the labelled products as reported in literatures (See Figs 1-3). Generally, the hydrogen peroxide procedure could give the better labelling yields in cases of uracil derivatives than those of the chloroamine-T procedure. Probably, the hydrogen peroxide could act as the better oxidzing agent in the present reaction systems. Even though the labelled products prepared are mainly utilized as the tracer compounds for the precursor purposes in this paper, they may independently be used for the basic research of the clinical applications for detecting tumours of any kinds in this country, as

many literatures reported their potential applications in the clinical works.

Since the saline extract of a "Banha" root could clinically show a selective hemagglutination reaction towards various tumour cells,[6] it was considered that the extract would contain some sorts of lectin equivalent to concanavain-A or the similars, which had been known to show a selective hemagglutination reaction[1] Therefore, the saline extracts of Banha are prepared and they are subjected to the separation scheme adopted for the separation of con-A from jack-meal bean as reported.[11] As shown in Fig. 4 the eluate from a Shephadex cloumn did not show the UV adsorption as the later parts of the elution fractions which are the fractions eluated by a D-glucose solution. The fractions showing UV adsorption at $\lambda$ max 280nm belong to the fraction numbers of from 4 to 24. These parts of the fractions were considered as the inactive protein for the hemagglutination reaction in case of the separation of the concanavalin-A[11], but the present portions could give the distinct agglutinating activity when they are subjected to an agglutination test as shown in tables 2-3. The lyophilized product from the fractions (2.0g from 100g of the dried Banha root) gave the broad bands near at the region of molecular weghts 120,000-170,000 and showed some tails at those of the lower region when they are examined through a polyacrylamide gel electrophoresis. (see Fig. 5) However, they showed the distinct deep coloring of a glycoprotein by the PAS staining in the cloumn B as shown in Fig. 5. Furthermore, the purified extract showed a distinct sugar reaction towards the chemical tests and the hydrolyzed product of it had shown the amino acid residues on the PPC (see Fig 8. and 9). Since the concanavalin-A was proved to be a non glycoprotein which showed an average molecular weight of 80,000 and lectin separated from the lotus tetragonolohus[1] showed three different molecular weights, the glycoprotein separated as above from the Banha would be a mixture of the glycoproteins, molecular weights of which are 120,000-170,000. Further quantitative identifications for the purity of the glycoprotein would be necessary to ascertain whether it is composed of a pure lectin or not, as concanavalin-A was proved to be 98% pure lectin.[11] But the extract from the Banha could not show an affinity to the Shephadex gel as described above and hence there were no fractions showing UV absorption at the D-glucose elution stage. On basis of these facts, the glycoprotein from Banha could be considered qualitatively to be a lectin of an average molecular weight of 150,000. The hydrolyzed product of the Banha lectin labelled by $^{125}I$ was compared to those of the concanavalin-A labelled by $^{125}I$ through PPC. Both of them showed the same $R_f$ value of their amino acid residues on PPC, which are more closed to the histidine residue. It could, therefore, be considered that the glycoprotein separated from the Banha root was labelled at the same amino acid residue as compared those of concanavalin-A. Though the presence of the subunit or impuities in the Banha lectin should, quantitatively, be proved, the lectin from the Banha would contain the amino acid residue resembled to that of the concanavalin-A.

The labellings of the concanavalin-A and the lectin separted from the Banha are

proceeded smoothly by means of a modification of the $H_2O_2$-lactoperoxidase procedure[18] of labelling the concanavalin-A. The increase of labelling yield would further be expected when the concentration of the reaction mixture is adjusted depending on their application in vitro.

The agglutination tests by means of labelled Banha lectin thus prepared gave interesting results as listed in tables 2 and 3. Generally, the high radioactivity was ovserved in the agglutination mixture, but the sedimented fractions of the mixture could not retain all of the initial radioactivity. Expecially, RBC portions could retain less than 50% of the initial radioactivity in their sedimented fractions, which indicated that the binding of the Banha lectin with a RBC is not substantial enough to keep their binding in the sedimented particles. In cases of the WBC portions the initial radioactivity of agglutination mixture could be retained in the sedimented fraction of the lymphocytes of WBC, whereas the retain of the radioactivity was greatly decreased in case of the granulocyte of the WBC. The agglutination tests for case A (Leukemia) could show a considerable retention of the radioactivity in the sedimented particles of the WBC fraction, which would be substantial enough to identify a high agglutination behavior of the Banha lectin for the case.

Further investigations utilizing the lectin conjugated with a biologically active amino acid such as tyrosine or uracil derivatives showed a decreased retention of the radioactivity in the sedimented fraction of a WBC, whereas it was slightly increased in case of a RBC fraction. These observations would indicate that the binding sites of the Banha lectin towards a agglutination reaction with the RBC and the WBC was decreased, because of the conjugation between the lectin and it's counterpart. Furthermore, the agglutination by Banha lectin is specifically dependent on the binding moiety of the RBC or the WBC. Various conjugation partners for the Banha lectin were searched, but the tyrosine and the 6-amino 5-iodouracil could only be able to give their conjuation products, whereas 5-iodouracil or equivalents had failed to conjugate with the lectin.

In conculusion, the Banha lectin, when it is labelled by $^{125}I$, could show a quantitative profile for the agglutination test and its binding of the WBC portion was substantial enough to identify such case as a leukemia. The binding sites of the Banha lectin with the blood cell were decreased when it was conjugated with an amino acid or an uracil derivative, which would predict the Banha lectin has the specific binding potentialities towards blood cells, especially such a malignant WBC as a leukemia.

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