Automated Protein-Expression Profiling System using Crude Protein Direct Blotting Method

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Abstract: Proteome research in the medical field is expected to accelerate the understanding of disease mechanism, and to create new diagnostic concept. For protein profiling, this paper proposes a new methodology named CPDIB (Crude Protein Direct Blotting). In the CPDIB procedure, crude protein sample is directly immobilized on a membrane and the expression of protein molecules in the sample are analyzed quantitatively by using a special device called ImmobiChip, where the membrane is used as a field of the immune reaction. The over-all structure of the ImmobiChip is based on the conventional Slot blot device. Mechanical improvement in the air-tightness of the case holding the membrane realizes the direct blotting and results in high performance of stability in the immune reaction. In the measurement of multiple proteins, a dispensing robot is used for increasing the efficiency of handling of liquid. Cooperation of the dispensing robot with the ImmobiChip for immobilizing proteins realizes automated and stable performance of the CPDIB procedure. This paper shows the evaluation of the air-tightness of the ImmobiChip, the ability of analyzing proteins using the CPDIB procedure and the performance of the automated equipment.

Keywords: protein profile, membrane, blotting, protein expression, crude protein

1. INTRODUCTION

After the draft sequencing of the human genetic code was completed on 2000, there is an increasing interest in exploiting proteome analysis as an interface between the genomic data and biological function. This proteome analysis is expected to support clarifying the mechanism of disease. Therefore, the proteomic technology that is applicable for clinical practice, such as individualized medicine, should now be developed.

Several methods for proteome analysis have been proposed. The ELISA [1] and Slot blot [2] are used to detect and/or to quantify proteins in biological samples. The Sandwich ELISA [3] that repeats the immune reaction twice is the most widely used method: a primary antibody is immobilized on the inside of a vessel in advance of bonding with a target protein in a sample solution, then a secondary antibody labeled with fluorescent material reacts with the bonded target protein. The expression of the protein is measured as the intensity of fluorescence caused by exciting the fluorescent label. However, this method is not suitable for analysis of multiple proteins, because two separate specific antibodies are required to measure one protein.

Slot blot that also applies the immune reaction, uses an apparatus called "blotter" that is composed of two templates. Between the upper and lower templates, a membrane to adsorb proteins and filter papers are inserted. The sample solution including the target protein is poured through several openings made on the upper template and the protein is immobilized to the membrane directly. For the immune reaction the membrane is removed from the blotter and soaked in the antibody solution labeled with the fluorescent material because the blotter cannot keep the solution until the reaction is completed. Therefore, the bothersome handling of the membrane makes Slot blot only the laboratory use and unsuitable for clinical use.

The goal of our study is to realize a platform for automated profiling of multiple proteins, where preparation of sample solution, reaction with antibodies, detection of target proteins, and quantitative analysis of the proteins are processed automatically. For our goal, Slot blot is suitable because one target protein can be detected with only one specific antibody. Disadvantage of Slot blot for automated processing can be eliminated by improving the air-tightness of the blotter. We improve the shape and the machining accuracy of the upper and lower templates for better air-tightness, and propose a new methodology where the immobilization of proteins and the immune reaction with antibodies can be completed without disassembling the membrane from the templates. We call this method Crude Protein Direct Blotting.

To automate protein analysis, we use a dispensing robot for pouring samples and reagents to the membrane. The order of dispensing is arranged so as to avoid contamination from mixing the liquids poured from one opening to the next. According to the number of proteins to be measured and the time for the immune reaction indicated by an operator, the dispensing robot accommodates the sequence of dispensing. The flow valves are driven for aspiration of poured liquid automatically.

This paper shows the evaluation of the air-tightness of the improved blotter, the ability of analyzing proteins using the procedure of Crude Protein Direct Blotting and the performance of the automated equipment.

2. PROPOSAL OF CRUDE PROTEIN DIRECT BLOTTING (CPDIB) METHOD

The procedure of the proposed Crude Protein Direct Blotting method is illustrated in Figs. 1 and 2. In order to detect multiple proteins with CPDIB method at a time, a sample solution including several proteins is poured into openings of a membrane placed between two templates, and then the proteins are immobilized to the binding sites of the membrane directly (Fig. 1(a)). After immobilization, empty binding sites of the membrane are blocked with the protein (BSA: Bovine Serum Albumin) having no effect on the later reactions to prevent unspecific binding to the primary antibodies. This process is called "blocking", as shown in Fig. 2. For the first immune reaction, a primary antibody that specifically bonds to the target proteins (1) in Fig. 1(b) is poured to one opening, and another primary antibody is poured to another opening to detect the target proteins (2), and so on. The number of the primary antibodies is equal to the number of the proteins to be detected, however these

antibodies can be originated from the same origin (for example, from rabbit). For the second immune reaction, a common secondary antibody labeled with the fluorescent material is poured to all openings and reacts with the primary antibodies, because the antigen for the secondary antibody is the rabbit protein, the origin of all primary antibodies. Therefore, the cost of developing specific antibodies for target proteins becomes less than that for the sandwich ELISA's where two specific antibodies are needed for one target protein. Finally, as shown in Fig. 1(c), only the target proteins in each opening are labeled with the fluorescent material, and the quantity of the target proteins in the opening can be measured as the intensity of fluorescence caused by exciting the fluorescent label. When the intensity of fluorescence is not enough, as shown in Fig. 2, a reagent labeled with the fluorescent material is added after the second immune reaction. Then, the secondary antibody bonds with a number of molecules labeled with fluorescence material and the intensity of fluorescence increases when excited [4-6]. During the immobilization and these binding reactions, the membrane is kept between the templates.

There are two conditions to achieve CPDIB method. The first condition is the isolation of each opening. The samples and antibodies should not be blended among openings. The second is the air-tightness of the templates. The antibody solution should be kept in the openings of the template for the time of immune reaction. To satisfy these conditions, we designed a new type of blotter called "ImmobiChip" to immobilize proteins.

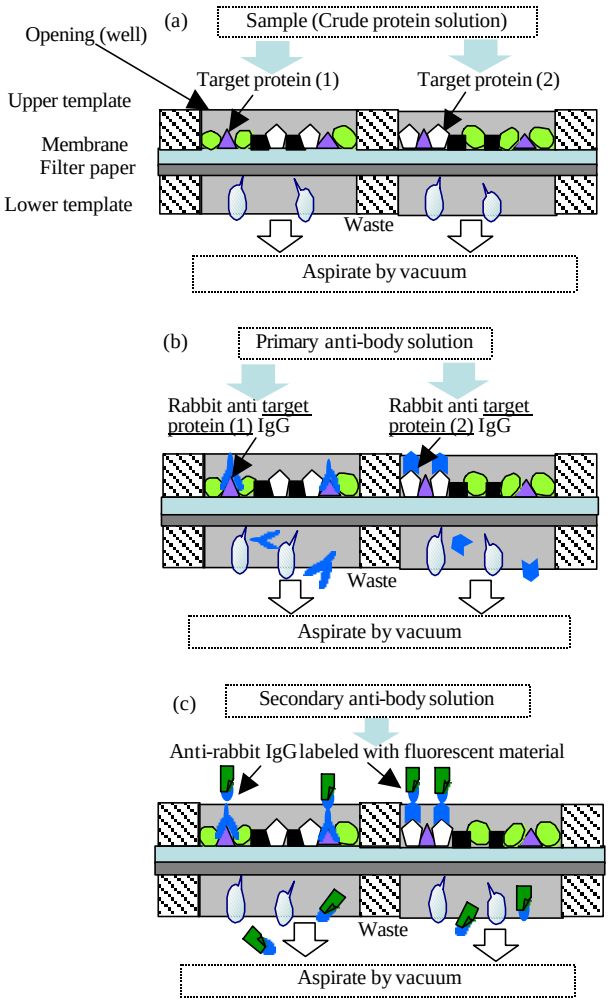

Fig. 1 Illustrated CPDIB assay method

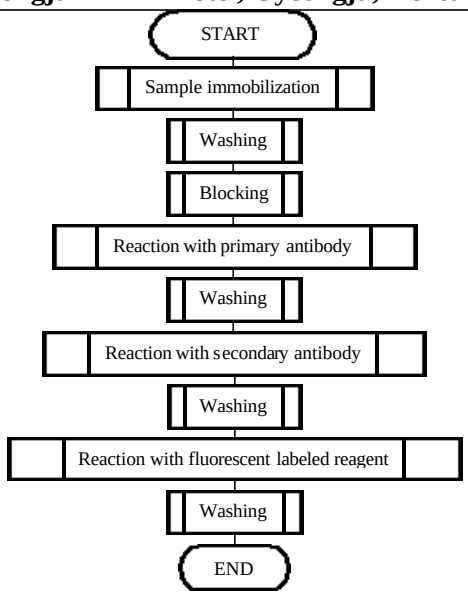

Fig. 2 The assay procedure of CPDIB method

3. AUTOMATED PROTEIN-EXPRESSION PROFILING SYSTEM

3.1 ImmobiChip for immobilizing protein

Fig. 3 shows the configuration of the reaction-part of the system involving the prototype ImmobiChip made of polyvinyl chloride resin. The ImmobiChip shown in the upper left of the figure is composed of an upper and a lower templates, a membrane and filter papers. The membrane (Immobilon-PSQ Transfer Membranes, Millipore) is hydrophobic, and made from PVDF (Poly Vinylidene Fluoride). Pore sized of the membrane used in the CPDIB method is 0.2 μm, which is suitable for immobilizing our target protein with low molecular weight. The protein poured to this membrane creeps in pores and is adsorbed with hydrophobic and/or electrostatic bond.

The area of the membrane to immobilize our sample (the volume is 50 μl) including protein is calculated to be 12 mm^2 (1.5-fold of safety factor). This value is based on the maximum and experiential concentration of protein poured into the well (200 μg/ml) and the property that the PVDF membrane with 0.45 μm of pore size can adsorb 1.3 $\mu g/mm^2$ protein (BSA) [7].

To measure multiple proteins at a time, the membrane (51 mm × 32 mm) is divided into many areas by the openings of the upper and lower templates. Solutions are stored in these openings, therefore, they are called wells. The area of one well should be about 12 mm^2, (previously described) and the wells should be arranged so as to be dispensed samples and antibody solutions effectively by using a commercially available dispensing robot with multiple pipettes. As a result, the shape of well is designed to be ellipse (3 mm × 5 mm) with the area of 13 mm^2, and the wells are arranged in a 10 × 4 rectangular configuration with 4.5 mm pitch. This pitch is a half of distance between the pipettes of the dispensing robot, which can pour liquid into every other column of wells. The top edge of each well has a fillet with 0.5 mm to guide the apex of the pipette into the well. The membrane and the filters are tightly pressed between the upper and lower templates. For positioning of openings, the upper template has a rectangular groove (32.5 mm × 51.5 mm) where the protrusion of the lower template is inserted. The tolerance zone of this fitting is defined to be p6/H7.

The thickness of the membrane distributes from 0.180 mm to 0.220 mm, and the total thickness of one membrane and two sheets

of filter paper is 0.92 mm with 0.1 mm of fluctuation. To accommodate such fluctuation in the thickness, the membrane and the filters are pressed between both templates by an exclusive jig with enough pressure. The surfaces of the upper and lower templates contacting with the membrane and filter papers are finished with the surface roughness of about Ra = 1.6 μm and the flatness below 30 μm. Consequently, pores in the membrane are crushed at the edges of the openings and the air-tightness of the wells is realized. The surface of each template contacting with gaskets is also finished with the surface roughness of about Ra = 1.6 μm and allows good air-tightness at each surface. Such air-tight structure of the templates allows the wells to keep the antibody solutions for the time of the binding reaction without mixing them in the other wells.

Prototype ImmobiChip is composed of machined parts and is disassembled when bringing out the membrane for observing fluorescence. The improved ImmobiChip under consideration will allow detecting fluorescence without disassembling and lower the production cost by molding the templates with membrane.

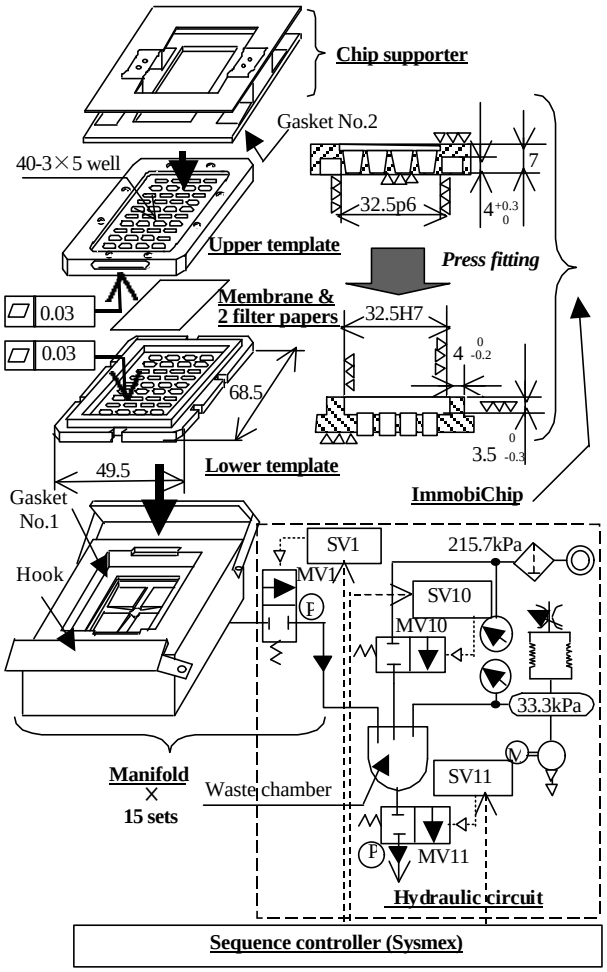

Fig. 3 Configuration of CPDIB module **SV**: solenoid valve, **MV**: master valve, **P**: pinch valve, **M**: motor for vacuum pump.

3.2 Manifold and electronic flow control

We developed a manifold to aspirate solution with constant flow volume from wells of each ImmobiChip (lower left of Fig. 3). The manifold has a rectangular concave portion to mount an ImmobiChip and an aspiration port connected to the vacuum pump in the center. When the ImmobiChip is aspirated, it is pulled downward and pressed against the gasket No.1 which is made of silicon rubber and placed at the step around the concave portion. Because the position of the aspiration port is located at the center of ImmobiChip and the direction of aspiration almost coincides with that of immobilization, the proteins are immobilized on the membrane uniformly. We joins 15 sets of the manifold and ImmobiChip each other, and realize a module that can measure the expressions of 15 proteins at a time. This is named "CPDIB module".

As shown the hydraulic circuit under Fig. 3, the line coming from the vacuum port of air supply is connected to the waste chamber via the bellows for adjusting the vacuum presser with 33.3kPa. The waste is aspirated from the ImmobiChip and stored the chamber. The flow of air and liquid is switched by the solenoid valves (SV), and these valves and lines are connected to 15 manifolds respectively. The valves are controlled by the sequence controller (Sysmex controller).

3.3 Automated sample dispensation and protein analysis

On the CPDIB module, there are 600 wells where samples and antibody solutions will be poured. We used a commercially available dispensing robot (GENESIS RSP 100, TECAN) for effective pouring. As shown in Fig. 4, GENESIS has quadruple pipette which able to put and release disposable tips, a X-Y-Z mechanism to move around the worktable (418 mm × 745 mm), and syringe pumps to aspirate and dispense the liquid. The motion of GENESIS is programmed by using an exclusive software (GEMINI, TECAN) and controlled with a personal computer (TECAN controller). On the worktable, a cooling unit and micro-switches are mounted. The cooling unit controlled separately keeps the temperature of mounted reagents at 4 ^{o}C, and prevents from degradation. When the micro-switches are pushed by the tip of the robot, they send trigger signals to the Sysmex controller. Then the CPDIB module begins to aspirate the liquid in the wells of ImmobiChip. Thus the TECAN controller and Sysmex controller become to cooperate.

Figure 5 shows the whole procedure of the protein-expression analysis using this automated equipment. At first, reagents are set in position by an operator. The reagents are the samples, the washing solution, the antibody solutions, and standard samples to make standard line which used for converting the detected fluorescence intensity into the concentration of the target protein. The arm of GENESIS begins to move after the number of proteins to be measured and the times for binding reactions are indicated to this equipment by the operator. According to these parameters, this equipment accommodates the sequence of measurement. In the next step, the standard samples are diluted automatically into five concentration levels for the later process. The order of dispensing by the robot is arranged so as to avoid contamination from mixing samples and reagents poured into wells. The disposable tips are always replaced when GENESIS begins to aspirate some reagent different from the former one. Then, the procedure of CPDIB described in section 2 is executed. After this CPDIB procedure finishes, the ImmobiChip is ejected from the equipment and is disassembled with a jig. The membrane bringing out is dried out at 65 ^{o}C, and the intensity of fluorescence from the fluorescent material with the target protein is measured by the fluorescence imager (Molecular Imager FX, BIO RAD). In this imager, the fluorescence caused by exciting with 488nm of wavelength is led through the band pass filter (530nm) and detected by the PMT (Photo Multiplier Tubes) [8]. The unit of fluorescence intensity is arbitrary depending on the imager. In this paper, the unit is expressed CNT. Finally, the standard curve is plotted against the fluorescence intensity of the standard samples immobilized under the same procedure as the target protein, and the concentration (expression) of the target protein in the sample can be determined by using this regression curve.

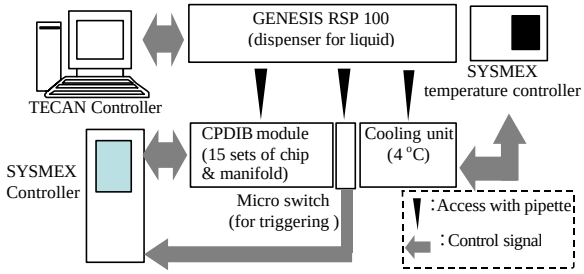

Fig. 4 Configuration of automated protein-expression profiling system

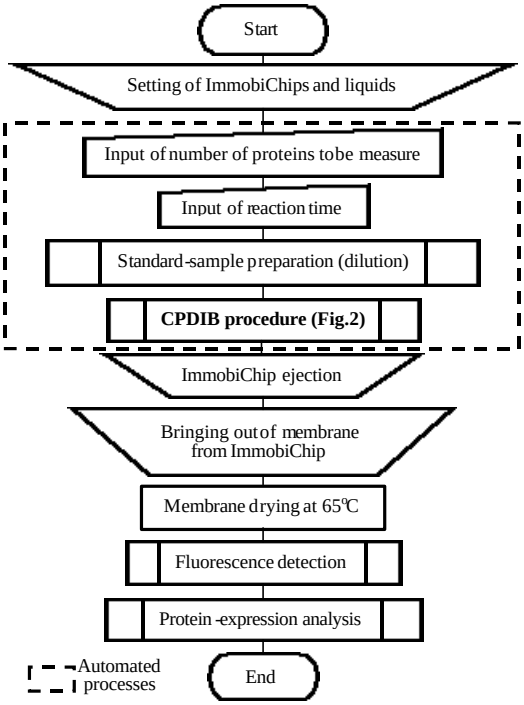

Fig. 5 Whole procedure of protein-expression analysis

4. PERFORMANCE OF CPDIB MODULE

4.1 Retention of liquid

We compared the ImmobiChip with conventional blotter (Bio Dot SF, BIO RAD) [2] on the ability of keeping poured solution. For clear comparison, the pore size of membrane in the ImmobiChip coincides with that used in the blotter (Immobilon-P Transfer Membranes, Millipore, pore size 0.45 μm). The BPB (Bromphenol Blue) solution, reagent of dye, was poured into wells of the ImmobiChip mounting in the manifold and openings (slots) of blotter with 50 μl. After 30 minutes, the remaining liquid was too little to quantify the volume. Those liquid in the wells and slots was removed to a micro-plate, and measured absorbance. In order to measure it, we used the plate reader (VERSAmax, Molecular Devices) and measured at 600nm of wavelength. The absorbance was converted into the value per unit area because the area of well (13 mm^2) was different from the area of slot (7.2 mm^2). As a result, the average of absorbance in the slots (0.006AU (Absorbance Unit)) was less than 1/10 of absorbance in the wells (0.069AU). These absorbance demonstrated that the volume of liquid remaining in the slot was much less than that of well. Therefore, it was obvious that the ImmobiChip was superior to the blotter in keeping liquid.

4.2 Suction of liquid

The aspiration volume of the manifold affects the immobilizing protein to the membrane, the aspiration of remaining antibody after the binding reaction, and the washing in the procedure of CPDIB. The insufficient washing increases the background fluorescence and decreases the signal to noise ratio in the detection. To evaluate the aspiration volume of the manifold, we measured the volume of aspirated water in a given time.

The one end of Teflon tube (inside diameter 4 mm, outside diameter 6 mm, length 795 mm) was put in the beaker filled with 800ml water and fixed at the position keeping constant distance from the bottom face. At first, the total-weight of the beaker, the tube and water was measured. Another end of the tube was connected to the inlet for aspiration of a manifold. The water was aspirated with 33.3kPa for 25 seconds. Then, the total-weight was measured again after the tube was disconnected from the manifold. The decrease in weight between two total-weights became the aspirated volume of the manifold. The mean volume among 15 manifolds was 334.8ml (coefficient of variation was 0.7%), that was over 150 times as much as the total volume (2ml) poured into the 40 wells of ImmobiChip. Therefore, it was shown that the aspirating ability of manifolds was enough for CPDIB module.

5. PERFORMANCE OF PROTEINS ANALYSIS

5.1 Analysis of protein immobilization in membrane

It is presumable that the protein poured to the membrane has the highest concentration on the superficial layer of the membrane and is distributed with a concentration gradient across the membrane's vertical dimension. As illustrated in Section 3.1, there is a maximum amount of protein which can bind to the unit area of the membrane, and the protein exceeding this maximum value will flow out of the membrane. It is also presumable that fluorescence is generated from the fluorescent material present within the area accessible for the excitation light from the fluorescence imager. We examined the range of protein concentration levels which can be immobilized in our membrane and the range of concentrations which allows the excitation light from the fluorescence imager to reach the entire immobilized protein.

To create a situation resembling after the second immune reaction in the CPDIB method, a mixture of protein (BSA) solutions and antibody (FITC anti-rabbit IgG) labeled with FITC (fluorescein isothiocyanate; absorption wavelength 494 nm and emission wavelength 518 nm) was poured to each well in a volume of 50 μl and immobilized on the double-layered membrane. The protein (BSA) solutions were prepared in 10 concentration levels, ranging very widely from 0 to 2400 μg/ml. The concentration of the antibody was 1/500 of the protein concentration, and BSA does not bind to FITC-labeled antibody. TBS (Tris buffered saline) containing 0 μg protein/ml was added to the wells used for background correction. The membrane is then removed and dried. The intensity of the fluorescence from the membrane is measured.

Figure 6 shows differences in intensity of fluorescence from upper and lower membranes. In this figure, ordinate is the intensity of the fluorescence from the FITC-labeled anybody contained in the mixture and abscissa is the concentration of the protein poured to the well. The fluorescence intensity shown here is the level after background correction, namely subtraction the fluorescence intensity in the well containing 0 μg protein/ml from the intensity recorded at a given protein

concentration. Linear relationship between the protein concentration in the upper membrane and the fluorescence intensity can be seen in Fig.6, when the protein concentration was between 0 and 150 μg/ml. This suggests that the excitation light from the fluorescence imager reached the whole protein poured. As the protein concentration exceeded 150 μg/ml, this linear relationship was lost and the fluorescence intensity became saturated at protein concentrations of 300 μg/ml or over. Following saturation of the fluorescence intensity, the lower membrane began to emit fluorescence, suggesting that the protein began to flow out of the upper membrane into the lower membrane. These results indicate that the maximum concentration of protein which can be immobilized in each well of the ImmobiChip, is 300 μg/ml. However, when the protein concentration exceeds 150 μg/ml, fluorescence cannot be obtained from all immobilized FITC because a portion of the poured protein is immobilized in the layer deeper than the maximum depth which the excitation light can reach. Therefore, the protein concentration to be poured to each well needs to be limited to below 150 μg/ml.

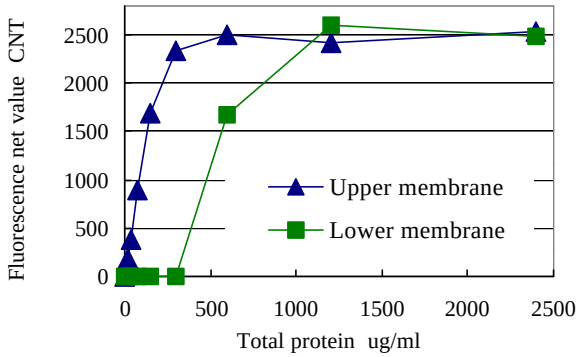

Fig. 6 Ability of membrane to immobilize protein

5.2 Stability of immobilization and immune reaction

The automated protein expression measuring system shown in Fig. 4 was used to immobilize protein and to evaluate variation in the immobilizing among different wells. After 0.2 μm membranes were inserted into the ImmobiChips, the chips were mounted to 8 of the 15 manifolds (every second manifold). By using GENESIS, rabbit antibody solution (100 ng/ml of rabbit antibody mixed in TBS solution), was dropped onto 20 wells (every second well), followed by dropping of the TBS solution without the antibody to the remaining wells. This rabbit antibody solution is used as the primary antibody for CPDIB. This process was repeated on 8 ImmobiChips. The volume of the sample added to each well was kept constant at 50 μl. Then, the sample was immobilized on the membrane by aspiration with the CPDIB module, and the material which failed to be immobilized was washed out of the well.

Upon completion of immobilization, secondary antibody (biotinylated anti-rabbit IgG in TBS 1% BSA, 4 μg/ml) which reacts with the rabbit antibody in the sample was added to each well of the chip. Then the chip was left standing for 30 minutes while the immune reaction progresses. After washing of wells, streptavidin reagent (streptavidin FITC in TBS 1% BSA, 10 μg/ml) was added to react with the secondary antibody. Because four molecules of this streptavidin can bind to each molecule of biotinylated secondary antibody, the use of this reagent can obtain higher intensity of fluorescence than the use of the secondary antibody directly labeled with fluorescent material. The membrane was then removed from each ImmobiChip and dried for 20 minutes, and fluorescence intensity was measured.

Figure 7 shows a fluorescent image of the membrane taken out of one of the 8 ImmobiChips. Thanks to the effects of fluorescence augmentation, the signal wells, containing rabbit antibody, emitted strong fluorescence (appearing black in the image), making good contrast to the background wells containing TBS alone. The mean fluorescence intensity was 869 CNT for the signal wells, while the mean fluorescence for the background wells was 276 CNT, i.e., below 1/3 of the mean fluorescence for the signal wells. This indicates that the leakage of solution including antibody from the signal wells was negligible. The coefficient of variation within the ImmobiChip was about 3% for both the signal wells and the background wells. The mean fluorescence intensity of signal wells in each of the remaining 7 ImmobiChips ranged from 760 to 880 CNT, with a coefficient of variation below 6%. The mean fluorescence intensity of background wells in each of the 7 ImmobiChips ranged from 240 to 290 CNT, with a coefficient of variation below 7%. This figure shows that the difference among wells is little and that immobilization and immune reactions were achieved in a reliable manner thanks to the adequate air-tightness of the CPDIB module. Thus, individual processes, ranging from sample immobilization to reaction with FITC-labeled antibody, can be measured automatically and in parallel for 8 ImmobiChips constituting this system. More reliable measurements will be achieved when the conditions for sample application, etc., are optimized.

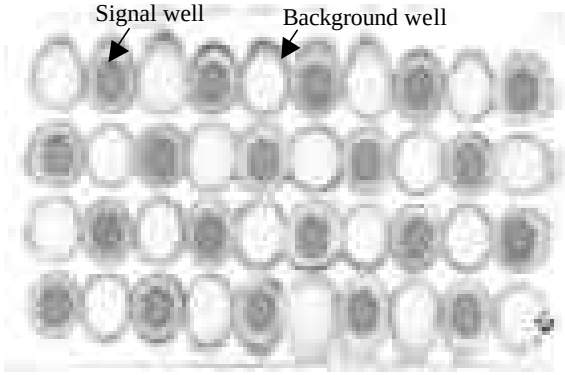

Fig. 7 Result of immobilization of rabbit IgG

5.3 Quantitative analysis for protein content

In our system using the CPDIB module, creation of a standard curve will allow conversion of measured fluorescence intensity into the expression level of the targeted protein. As shown in Fig. 6, this conversion can be linear when the protein concentration is below 150 μg/ml where the fluorescence intensity is proportional to the protein concentration. Assuming a situation where multiple proteins are measured simultaneously, we tried to determine the gradient of the standard curve and its variation for 7 standard protein samples. For these samples, the range of concentration showing a linear relationship between protein concentration and fluorescence intensity had been revealed in the preliminary experiment.

For each standard sample, solutions in 5 concentration levels were prepared, and these solutions were poured to the separate wells of ImmobiChips. After aspiration, the immobilized proteins were reacted with rabbit-derived 7 primary antibodies, specifically binding to each of 7 antigens. Then the proteins were reacted with the common secondary antibody which binds to the rabbit antibody. After being exposing to

FITC-labeled streptavidin for obtaining more intense fluorescence, the membranes were taken out of each ImmobiChip and dried, and fluorescence intensity was measured.

The result of measurement for CDK2 is shown in Fig. 8, where the standard curve was obtained as a regression curve for fluorescence readings at 5 concentration levels. The coefficient of correlation was 0.99. Such a high value endorses that immobilization of the protein, subsequent immune reactions and measurement of fluorescence were done in a stable manner. Table 1 shows the gradients of the regression curves for each of the 7 standard samples determined in the same way. Because the relationship between fluorescence and protein concentration varied among different samples, the mean gradient differed greatly among different samples (an about 100-fold difference at maximum). However, the coefficient of variation of the gradient of the standard curve was below 17% for all samples except for p27. Thus, reproducibility was high. The high coefficient of variation for p27 is probably attributable to the low affinity of the p27 standard sample for the primary antibody. Despite such exceptional cases, the results indicate that the Protein-Expression Analyzing system including CPDIB module is useful as a measuring device which allows quantitative analysis of the concentration of multiple types of protein.

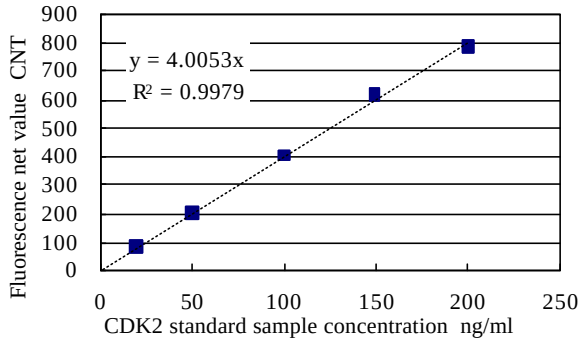

Fig. 8 Standard line for CDK2

Table 1 Reproducibility of gradient of standard lines

Protein	CDK1	CDK2	CDK4	CDK6	Cycline B1	Cycline D1	p27
N	9	8	14	7	6	5	14
AVG [CNT*ml/ng]	1.47	3.97	5.80	3.88	47.52	111.35	5.50
STDEV	0.2	0.5	1.0	0.5	6.2	6.1	1.6
CV	10.5%	13.0%	16.5%	13.0%	13.1%	5.4%	29.6%

6. CONCLUSIONS

Toward the goal of developing a clinically applicable automated measuring system equipped with all functions of pretreatment, reaction, detection and analysis for multiple protein assay, this paper proposed the use of CPDIB as a reaction method for this system. The ImmobiChip needed for this method and the automated device for the system were also proposed, and following basic features are obtained. (1) The liquid-retaining ability of the ImmobiChip precisely finished is one-digit higher than that of conventional devices and is high enough to allow satisfactory immobilization and immune reactions of protein samples. (2) The mean capability of the manifold to aspirate the sample in 25 seconds is 335 ml, more than 150 times the necessary level. The variance in this capability among 15 points of the device is minimal, indicating that the aspiration system of this device has adequate mechanical stability. (3) The maximum protein concentration which can be immobilized on each well of the ImmobiChip is 300 μg/ml. When fluorescence is detected with an imager, there is a range in which a linear relationship exists between the concentration of the target protein and the intensity of fluorescence. (4) This protein-expression measuring device, equipped with a sample applying arm mechanism, allows full-automated immobilization of protein on the ImmobiChip wells. The results are stable, and no mixture of liquids from different wells takes place. The coefficient of variation in each well is 7% or less. The fluorescence obtained has adequate intensity. (5) By Using the CPDIB module, the linear relationship between the concentration of protein and the intensity of fluorescence is examined repeatedly for 7 protein samples. The reproducibility of the gradient of the curve obtained from multiple measurements is satisfactorily high (coefficient of variation below 17%). This indicates that this module will allow quantitative analysis of samples containing multiple proteins.

As shown above, the ImmobiChip (designed for automated operation and manufactured in high accuracy) and the CPDIB (suitable for multiple measurement) allow full-automated measurement of protein expression when they are used in combination with the sample applying arm mechanism. With a pretreatment unit, a detector, etc., the proposed system will be applicable as a platform for protein analysis in various fields.

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