CCD-BASED SYSTEM OFFERS RAPID METHOD FOR DETECTING GLYCOSYLATED AND NON-GLYCOSYLATED PROTEINS

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Glycosylation is the most common form of post-translational modification of human and other eukaryotic proteins. Glycoproteins have vital roles in many physiological and pathological reactions and are also responsible for molecular recognition, cell signalling and protein stability, as well as protein folding and structure. Therefore, identifying glycoproteins is often of critical importance in life science research.

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To detect glycoproteins, scientists traditionally use lectins that bind to specific glycans, but recently stains such as Pro-Q® Emerald 300 (Invitrogen) have been introduced which will selectively stain glycoproteins to produce a fluorescent signal.

Imaging fluorescent dyes can be difficult as it requires the use of laser-based scanners, many of which are large, expensive and limited to fluorescence imaging applications.

To overcome these challenges, this article describes how CCD-based technology can be used and the levels of sensitivity that can be achieved imaging Pro-Q Emerald 300, a popular fluorescent dye for the labelling of glycoproteins.

MATERIALS AND METHODS

Into three 1-D polyacrylamide gels (12% polyacrylamide gel with a 5% stacking gel), the following mixture of glycosylated and non-glycosylated proteins, bovine serum albumin (66 kDa) (non-glycosylated), chicken ovalbumin (45 kDa) (glycosylated), bovine β-casein (24 kDa), (non-glycosylated) bovine pancreatic trypsinogen (23 kDa) (non-glycosylated) and soybean trypsin inhibitor (20 kDa) (non-glycosylated) was loaded in the following amounts: 1000, 400, 100, 40, 20, 10, 4, 2, 1, 0.4, 0.2ng.

Figure 1. The Dyversity Imaging System

The gels were run under standard electrophoresis conditions and were stained according to manufacturers' protocols with

Pro-Q Emerald 300 (Invitrogen, Paisley, UK), Sypro® Ruby (Invitrogen) or a standard visible dye, Coomassie Blue (GelCode Blue, Perbio Science, Cramlington, Northumberland).

The Dyversity 4, multi-functional CCD-based analyzer was chosen for imaging. This has its own light-tight cabinet containing a state-of-the-art 16 bit camera with 4 mega pixel resolution in a 2048 x 2048 pixel format, ensuring separate imaging of bands and spots that are close together. The system can be fitted with a range of precision made filters, as well as UV and visible lighting modules making Dyversity 4 versatile enough to allow imaging of any commercially available protein stain. To produce an optimum image of the gels stained with Pro-Q Emerald 300 and Sypro Ruby, Invitrogen's recommended emission filter and UV illumination conditions were used in the Dyversity 4.

The gel stained with Coomassie Blue was placed on a 302nm transilluminator with a NovaGlo visible light converter and an image of the gel was captured with a neutral density filter using an optimum exposure time to maximise the dynamic range of the Dyversity 4 camera. Using the same settings an image of the visible light converter was then captured and using the neutral fielding correction method in the GeneSnap software of Dyversity 4, the image was normalised using algorithms for light illumination. Normalisation was applied to the gel image so that uneven illumination from the light source was corrected.

RESULTS AND DISCUSSION

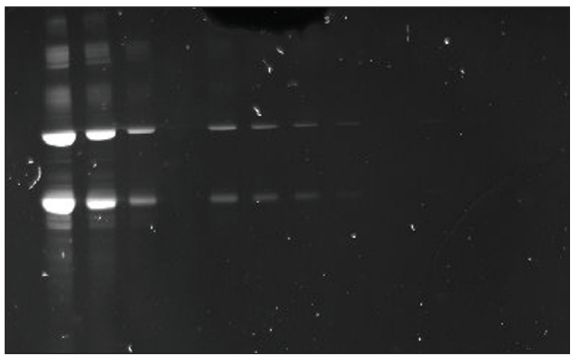

Figure 2. 1-D polyacrylamide gels stained with Pro-Q Emerald. Showing protein mixture of bovine serum albumin, (top band), chicken ovalbumin (second band), bovine-casein, bovine pancreatic trypsinogen (third and fourth non-visible bands) and soybean trypsin inhibitor (bottom band). From left to right the gel contains the following: protein sample mix 1000ng, 400ng, 100ng, sample buffer, 40, 20, 10, 4ng, sample buffer, 2, 1, 0.4, 0.2ng, sample buffer.

Using transillumination (302nm) and a short pass UV filter on Pro-Q Emerald 300 stained gels allows visualisation of 2ng of chicken ovalbumin *(Figure 2)*. The bovine serum albumin and trypsin inhibitor also produce a strong signal despite not being glycosylated proteins, while the other non-glycosylated proteins bovine β-casein and bovine pancreatic trypsinogen remain unstained. This non-specific staining could be due to these proteins producing false positives.

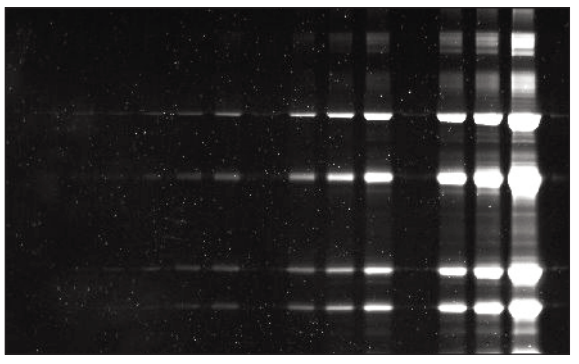

Figure 3. 1-D polyacrylamide gels stained with Sypro Ruby. Showing a protein mixture of bovine serum albumin, (top band), chicken ovalbumin (second band), bovine -casein and bovine pancreatic trypsinogen (third and fourth bands respectively) and soybean trypsin inhibitor (bottom band). From left to right the gel contains the following: 0.2, 0.4, 1, 2, sample buffer 4, 10, 20, 40 sample buffer, 100, 400, 1000ng. The gel image was captured, using 302nm transillumination and a UV mid pass filter.

According to the manufacturers, Pro-Q Emerald 300 dye is very non-selective when any SDS remains in the gels but can be removed with a more vigorous fixation step prior to staining, this was not done but could offer a method of eliminating these false positives. Also some fluorescent speckling is seen especially near the edges of the gel but according to the dye's manufacturer, this is an intrinsic property of the stain and does not affect sensitivity.

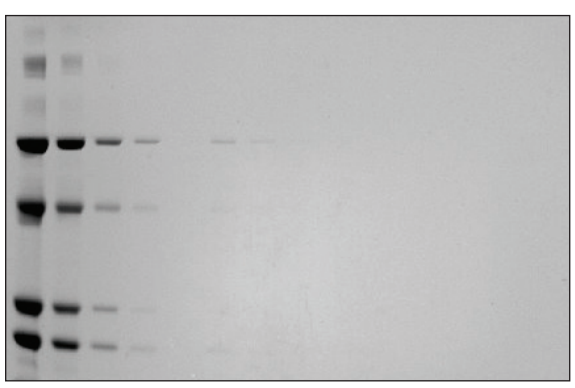

Figure 4. 1-D polyacrylamide gels stained with Coomassie Blue. Showing a protein mixture of bovine serum albumin, (top band), chicken ovalbumin (second band), bovine -casein and bovine pancreatic trypsinogen (third and fourth bands respectively) and soybean trypsin inhibitor (bottom band). From left to right the gel contains the following: 1000, 400, 100, 40, sample buffer, 20, 10, 4, sample buffer, 2, 1, 0.4, 0.2. The gel image was captured using visible light, neutral density filter and a neutral fielding correction.

The gel image was captured using a medium wave transilluminator and short pass filter. For the gel stained with Sypro Ruby, 302nm transillumination and a mid pass UV filter allows visualisation of 0.4ng of all five protein bands *(Figure 3)*.

Conditions used for capturing Coomassie Blue stained gel images permits imaging of 20ng of five protein bands *(Figure 4)*.

Table 1. Summary of imaging conditions for Dyversity 4:

Protein stain	Excitation source	Emission filter	Exposure time	Number of Bands visible
Coomassie Blue	302nm transilluminator with NovaGlo converter	Neutral density filter, neutral fielding	5 seconds	5 (100-20ng)
Pro-Q Emerald	302nm transilluminator	Short pass UV filter	2 seconds	3 (100-4ng) bands bovine serum (top band), chicken 300 ovalbumin (second band), and soybean trypsin inhibitor (bottom band). 2 (2ng) bands bovine serum albumin and chicken ovalbumin 1 (1ng) band bovine serum albumin
Sypro Ruby	302nm transilluminator	Mid pass UV filter	170ms	5 (40-0.4ng)

Using these conditions, Dyversity 4 can detect as little as 2ng of glycosylated protein stained with Pro-Q Emerald 300 or 0.4ng of protein stained with Sypro Ruby in less than 2 seconds. This is at least 10-50 times the sensitivity of Coomassie Blue stained gels and is within the published range of sensitivity limits for both fluorescent stains.

CONCLUSIONS

Imaging fluorescent dyes is achievable with CCD-based systems containing a high performance camera that can detect a wide range of dyes with UV excitation peaks. Additionally, the system requires a good range of UV and visible light, and a range of emission filters, to achieve the right excitation and emission conditions, respectively, for a range of protein dyes.

Since Dyversity 4 offers these capabilities, it enables researcher to rapidly image fluorescent proteomics dyes such as Pro-Q Emerald 300 and Sypro Ruby with excellent levels of sensitivity. The system is so flexible that users can upgrade it for different applications, as well as add the filters and lighting they need to analyse new dyes as they become available. In summary, this level of flexibility and sensitivity makes the Dyversity 4 an excellent, rapid method of imaging any commercially available fluorescent proteomics stains.

ACKNOWLEDGEMENTS

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