A Flexible, High Throughput Method for 2-D Protein Separations

By Ben Herbert, Australian Proteome Analysis Facility, Macquarie University, Sydney, Australia, on secondment from the Wool Research Organization of New Zealand

Two-dimensional electrophoresis (2-D PAGE) is the only method currently available which is capable of simultaneously separating thousands of proteins, and thus, is the heart of proteome technology. The introduction of immobilized pH gradients (IPGs) eliminated the problems of gradient instability and poor sample load capacity associated with carrier ampholyte pH gradients.[1, 7] The availability of commercial premade IPGs in a variety of narrow and broad pH ranges has brought 2-D PAGE out of the realm of the dedicated specialist and to the forefront of high resolution protein separation. Using the commercially available IPGs as the first dimension of 2-D PAGE it is possible to create highly reproducible reference maps, as well as separate milligram quantities of protein for micropreparative purposes.[2, 4] In the second dimension SDS-PAGE gel, a vertical format has a number of advantages over a horizontal system. First, vertical systems are able to run multiple gels in a single apparatus, and second, vertical slabs can be made thick enough to accommodate high protein loads for micropreparative purposes.

For separation in the second dimension, the PROTEAN® II xi system is widely used. In the PROTEAN II xi multi-cell version, the capacity to run up to twelve gels at once combines flexibility with throughput. In a recent upgrade of the PROTEAN II xi unit, Bio-Rad has widened the gel from 16 to 18.5 cm, which enables loading of full length commercially available 18 cm IPGs. The conventional PROTEAN II xi gel format required over 2 cm to be cut from 18 cm IPGs before the strips could be sealed onto the second dimension gels. The loss of more than 2 cm on a 3–10 IPG amounts to at least 1 pH unit on the linear IPG and about 2 pH units on the non-linear IPG. A conversion kit for PROTEAN II xi units, including spacers, gaskets, clamps, and combs is now available. The system is supplied with 1 mm or 2 mm spacers and combs, and is thus applicable to both analytical and preparative protein loads. This article describes an application using the recently modified PROTEAN II xi system.

Methodology

Sample preparation and isoelectric focusing

The eukaryotic slime mould Dictyostelium Discoideum, at the multicellular aggregate (slug) stage, was separated by 2D-PAGE. Slugs (250 µg dry weight) were solubilized in 500 µL of a solution containing urea (8 M), CHAPS (4% w/v), 2 mM Tributyl Phosphine and 40 mM Tris base.[5] After strong mixing, endonuclease (150 units) was added to remove DNA and the sample was left to stand for 2 hours at room temperature. An 18 cm pH 4–7 immobilized pH gradient strip was rehydrated using the 500 µL of sample as previously described.[6, 8] Isoelectric focusing was performed for 80,000 Vh with the initial voltage limited to 300 V for 5 hours and then stepping up to 1,000 V for 1 hour, 2,500 V for 1 hour and a final voltage of 5,000 V. After IEF, the IPG was prepared for transfer to the second dimension PROTEAN II xi gel by soaking, with agitation, for 15 minutes in an equilibration solution (6 M urea, 2% SDS, 20% glycerol, 0.375 M Tris base, 5 mM TBP and 2.5% acrylamide monomer).[5] The equilibrated IPG was embedded on the top of the SDS-PAGE gel in molten 1% (w/v) agarose in cathodic electrode buffer.

SDS-PAGE

Six 18 cm x 20 cm, 8–18% T, 2.5% C polyacrylamide gels, crosslinked with piperazine diacrylamide (PDA), were cast in a Bio-Rad multi-casting chamber. Gel buffer consisted of 0.375 M Tris/HCl, pH 8.8. Electrode buffer was 192 mM Tris/glycine, 0.1% (w/v) SDS, pH 8.3. Gels were run at 10 mA/gel for 1 h, then 20 mA/gel overnight with the cooling water circulating at 5 ºC.

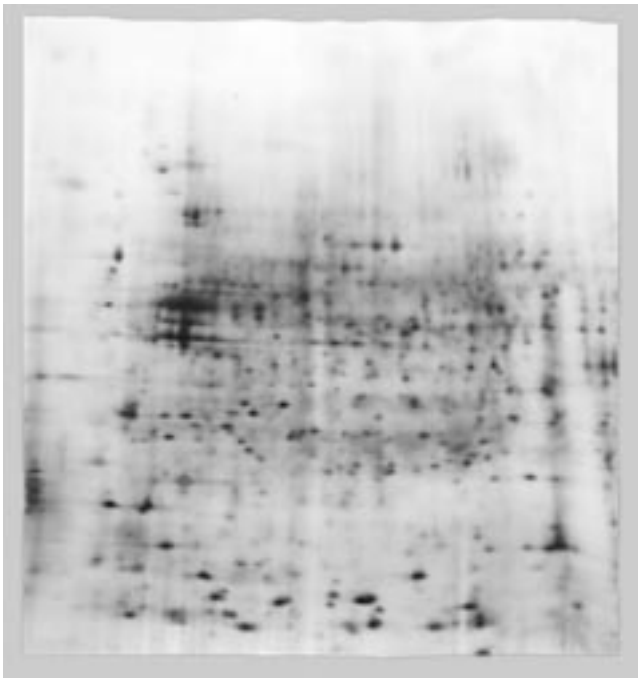

Fig. 1. Silver stained 2-D gel of Dictyostelium Discoideum slug cells. The 18 cm wide PROTEAN II xi gel allowed a full length 18 cm IPG to be embedded on the second dimension gel and thus no proteins were lost from the map.

Silver staining

The 2-D gel of Dictyostelium Discoideum was silver stained using the ammoniacal silver stain as described by Bjellqvist *et al*. (1993a)[2].

Conclusions

The new wider format PROTEAN II xi system increases the number of proteins which can be resolved on a single 2-D PAGE gel, using commercial 18 cm IPGs. The increased resolution is important for proteome analysis where 2-D PAGE is the central tool for separating the protein complement of organisms or tissues.

In the post-genome era, two-dimensional electrophoresis has assumed a very important position in proteome studies. First, 2-D PAGE is essential for displaying proteomes and establishing reference maps such as those available on the World Wide Web (WWW) in databases such as SWISS-2DPAGE at the URL address: http://expasy.hcuge.ch/. Second, micropreparative 2-D PAGE is the method of choice for protein purification for analytical techniques such as amino acid analysis, mass spectrometry, and Edman sequencing.

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PROTEAN II xi IPG Conversion Kit

The new PROTEAN II xi IPG conversion kit allows you to reconfigure the PROTEAN II xi cell to a wider gel format to accommodate commercially available IPG gel strips 18 cm long. The kit consists of narrow spacers, wide gaskets and combs, and notched clamps to expand the width of the gel from 16 to 18.5 cm. Available in two gel thicknesses, the 1 mm kit is ideal for analytical applications, while the 2 mm kit accommodates micropreparative applications.

To convert your PROTEAN II xi cell, order the IPG kit of your choice. To set up a new wide format 2-D electrophoresis system, order an IPG kit and the PROTEAN II xi basic unit. For more information, contact your Bio-Rad Representative.

Ordering Information

Catalog Number	Product Description
165-3183	**PROTEAN II xi Cell IPG Conversion Kit**, 1 mm gel*
165-3184	**PROTEAN II xi Cell IPG Conversion Kit**, 2 mm gel*
165-1834	**PROTEAN II xi Basic Unit**, with casting stand, 20 cm length**
Accessories	
165-1836	**Narrow Spacers**, 1 mm, 4
165-1837	**Narrow Spacers**, 2 mm, 4
165-1838	**Wide 2-D Prep Comb**, 1 mm, 1
165-1839	**Wide 2-D Prep Comb**, 2 mm, 1

* Kit include 2 sets of IPG clamps, 2 sets of 20 x 20 cm glass plates, 4 IPG spacers, 2 IPG 2-D/prep combs, 2 IPG sealing gaskets, 2 casting stand gaskets, an alignment card, and instructions.

** The PROTEAN II xi basic unit does not include spacers, combs, clamps, gaskets, or plates. Combine it with an IPG Conversion Kit for a complete 18.5 cm wide format system.

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