Electrical Conductivity as a Tool to Detect Salt in Clinical Proteomics Samples

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Abstract Clinical proteomics encompasses the study of the proteins in the human body at different settings to understand the various physiological and pathological pathways. The processing of the samples for electrophoresis based proteomics is a challenge to any researcher. Salt in particular can have an array of effects during the electrophoretic separation of proteins. There is a definite need to determine the concentration of salts in the samples and the effectiveness of salt removing protocols on small volume samples. A simple-cost effective technique to know the salt concentration in the clinical proteomics samples has been highlighted in the report. The application will be of value in a developing country such as India.

Keywords Salt · 2D electrophoresis · Clinical proteomics

Introduction

Clinical Proteomics pertains to the study of proteins in different physiological and pathological states. This necessitates the extraction of proteins from an array of fluids and tissues in the human body. The processing of each sample presents its own set of challenges to the researchers. The most common and universally acknowledged problem has been the presence of excessive salts in the samples. Salts have been known to have a significant bearing on the dynamics of iso-electric focusing (IEF) [1] which is crucial in the gel based proteomics. The effect and the result of salts have been enumerated in Table 1. The clinical samples,

particularly, the body fluids, are subjected to techniques such as ultrafiltration, dialysis and precipitation-washing to remove the salt. In all the above mentioned processes, there remains a very small margin where the right balance between optimum amount of protein and minimum amount of salt is obtained. Invariably, the high salt concentration is inferred when high levels of current are reached during the initial part of IEF. There are certain escape routes during an IEF for samples with high salt [2]. But there is no definitive way to confirm the presence of salts in the small volumes of samples. The concentration of the salt at the time of rehydration of immobilized pH gradient (IPG) strips remains always an unknown parameter. The need to know the amount of salt is of paramount importance because of (1) precious nature and paucity of the clinical samples, (2) enormous effort and time of the researcher in procuring and processing the samples, (3) high costs of the consumables used in 2D electrophoresis and (4) good separation can never be achieved with high salt sample. Though there have been a lot of ways of estimating the protein concentrations in the samples, there is no emphasis and method to determine the amount of salt in the samples.

Materials and Methods

250 µl aliquots of lysis solution (8 M urea, 2 M thio urea, 4% CHAPS) containing 100 µg of protein (bovine serum albumin) and varying concentration of salt (0 M–200 mM) was taken in triplicate in a 96-well microtiter plate. The voltage and power of the Electrophoresis Power Supply unit (EPS 601, Amersham Biosciences) were set at 10 V and 10 W, respectively. The electrodes were placed in the wells of the plate such that 0.5 cm of the tips were below the surface of the solutions. The current flow was measured

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Table 1 Effect and result of high salt concentrations on 1D/2D electrophoresis

Effect	Result
Precipitation of proteins	Streaking in gels
Increased conductivity	Heating and burning of IPG strips
Movement of water	Drying/swelling of the strips
Creation of salt font	Termination of IEF

Fig. 1 Graph showing the conductivity in the samples. Data of three experiments with duplicate readings are shown. Trendline ($y = 0.2019x + 13.027$, $R^2 = 0.9201$) for the plotted readings is shown

as displayed. The readings were done in triplicate for reproducibility and statistical validation. Graph of current (mA) versus salt concentration (mM) was plotted (Fig. 1).

Results and Discussion

The results clearly show a linear correlation between the conductivity and salt concentration. As proteins themselves

being charged molecules are good conductors of electricity, a protein concentration of 100 μg was used in all samples to eliminate the bias. The residual current in the control (0 mM salt) is due to the conductivity of the protein in the sample.

This methodology provides a very simple and robust way to detect salts in samples for gel based proteomics experiments. Standard plots can be derived for a particular set of samples and chemicals. The other controlling parameters such as the distance between the electrodes can be studied and standardized as a laboratory specific condition. The volume considerations can be worked out using a siliconised glass (microscope) slide for small volumes of solutions. Just as protein quantification is important, it could be made mandatory to estimate the salt concentration of samples before proceeding for 2D electrophoresis. This simple and cost effective technique illustrated here can be carried out on a regular basis to know the effectiveness of certain salt removing protocols and to know the concentration of salts in clinical samples.

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