Capillary Isoelectric Focusing (cIEF) Analysis of a Monoclonal Antibody under a Denatured and Reducing Condition

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Introduction

Capillary isoelectric focusing (cIEF) is the best tool for protein charge heterogeneity characterization. Common sources of charge heterogeneity for monoclonal antibodies include heavy chain C-terminal lysine heterogeneity, deamidation, and sialyation associated with the glycosylation sites on the antibodies. Under the denatured and reduced condition, disulfide bonds in the antibodies can be reduced and the monoclonal antibodies are broken into heavy chains and light chains. The heavy chain and light chain are expected to have different pI values, thus, they can be separated from each other by IEF. In this way, the contributions of the heavy chain and light chain to the entire antibodies' charge heterogeneity can be observed by IEF analysis.

In this presentation, monoclonal antibodies are analyzed by cIEF under a 8 M urea (denatured) and DTT condition. In the example shown in the presentation, the heavy chain and light chains are well separated and the charge heterogeneity of both is observed.

Experimental

Instrument

ProteinSimple's iCE280 IEF Analyzer is used for the cIEF analysis.

Reagents

The protein sample is a monoclonal antibody, anti-a1-antitrypsin, purchased from Calbiochem. Carrier ampholytes are obtained from GE Healthcare.

Sample Preparation

The final sample concentration is 0.2 mg/mL in 0.35% methylcellulose, 1% pH 3-10 Pharmalytes, 3% pH 5-8 Pharmalytes, pI markers 5.85 and 7.65.

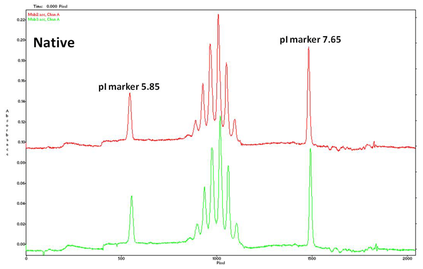
In the denatured condition, the final urea concentration is 8 M. In the reducing condition, the final DTT concentration is 20 mM and the final urea concentration is 8 M.

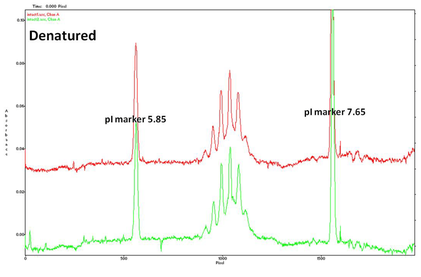
In the sample preparation for the reducing condition, all components are added to the final sample solution except the carrier ampholytes and the pI markers. The sample solution is incubated for 20 min at 37C (with 20 mM DTT in the solution). Then the sample solution is cooled down to room temperature and carrier ampholytes and pI markers are added.

Results and Discussion

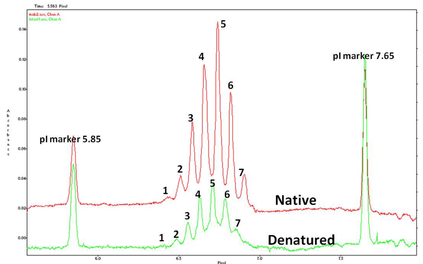
Native and Denatured Conditions

The antibody is run under native and denatured conditions. As shown below under both conditions, the results are reproducible.





The e-grams obtained under native and denatured conditions are calibrated in pI values by the internal pI marker peaks and overlaid, as shown below:



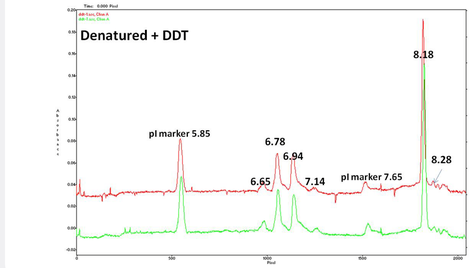
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
Native	6.43	6.51	6.58	6.66	6.74	6.82	6.91
Denatured	6.41	6.49	6.56	6.63	6.71	6.79	6.85

It is interesting to see that the pI values of the 7 peaks are identical in both native and denatured conditions. Generally, under the denatured condition the protein should be denatured and all its charges will expose to the solution. Consequently, the pI values in the denatured condition more closely reflect the total charges of the protein. In this case, no difference in the charges is observed compared to the native condition.

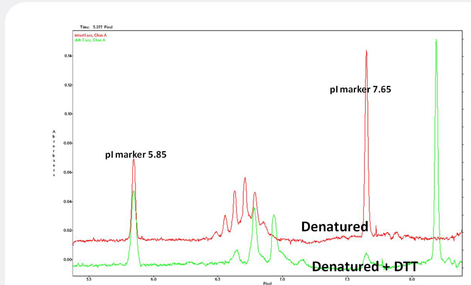
The poorer resolution of the denatured condition is understandable due to the unfolding of the protein molecules.

Reducing Condition

Under the denatured and reduced conditions, the peak pattern changes, as shown in the figure below. Other than a group of peaks around the pI 6.7 region, which should correspond to the heavy chain of the antibody, a new peak is observed at around the pI 8.1 region, as shown below. This high pI peak should be the light chain of the antibody, which has very different pI values from the intact antibody as well as heavy chain.



When the intact form (denatured) of the antibody is compared to the reduced form (as shown in the figure below), we can see that the heterogeneity observed on the intact form of the antibody (both native and denatured) comes mainly from its heavy chain.



Conclusions

The iCE280 IEF Analyzer is a useful tool to analyze monoclonal antibodies under native and denatured conditions.

Under the denatured + reducing condition, the charge heterogeneity on the heavy chain and light chain of an antibody can be well observed.