Enrichment of Basic and Low-Abundance Rat Brain Proteins With Ion Exchange Spin Columns Prior to 2-D Gel Analysis

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Introduction

A biological sample potentially contains tens or hundreds of thousands of different proteins, all with concentrations differing by up to 6 or more orders of magnitude. This complexity makes fractionation critical, especially for two-dimensional (2-D) gel electrophoresis. Fractionation helps to reduce sample complexity, which in turn increases resolution of protein spots on a 2-D gel. In addition, fractionation allows the focus to be on a subset of proteins, thereby increasing the load of proteins of interest and enriching low-abundance proteins. Ideally, fractionation methods should be simple, fast, and efficient. Here we examine the use of Aurum™ ion exchange (IEX) mini spin columns, which contain anion (AEX) and cation exchange (CEX) media, as a fractionation method for rat brain proteins.

Methods

Figure 1 summarizes the workflow and products used in these analyses. Compatibility testing has shown that, though the lysis buffers commonly used in IEF can be used with the Aurum IEX columns, the high concentration of Tris typical in these buffers (for example, 40 mM) interferes with column performance. For use in 2-D workflows, the binding buffer provided with these kits must be replaced with one containing 1 mM Tris.

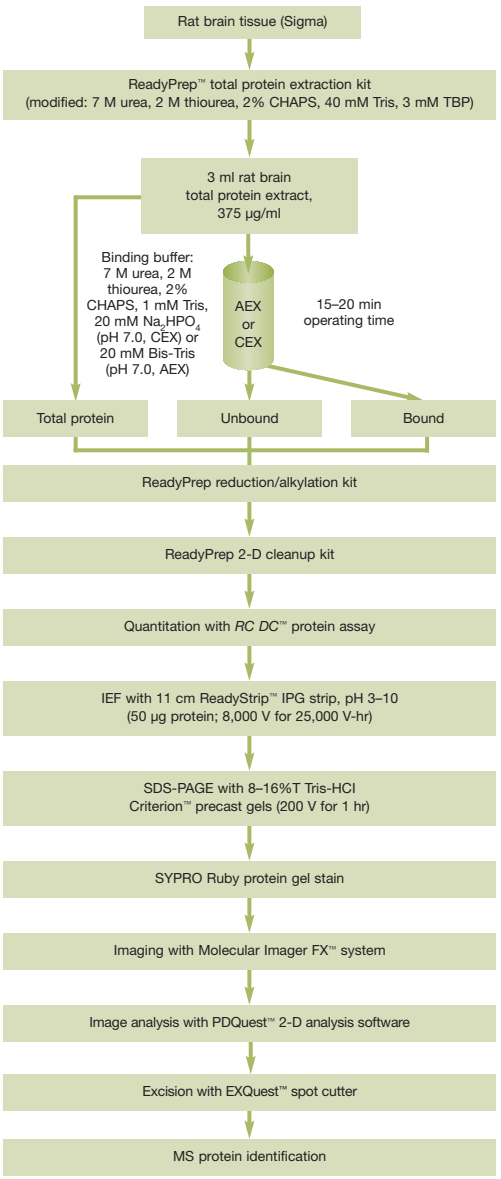


Fig. 1. 2-D analysis workflow using Aurum AEX or CEX columns for fractionation prior to 2-D gel analysis.

Conclusions

- Aurum AEX and CEX mini columns are compatible with the lysis buffers commonly used in 2-D electrophoresis, and fractionation of rat brain tissue with these columns improves detection of low-abundance proteins in 2-D gels.

Results

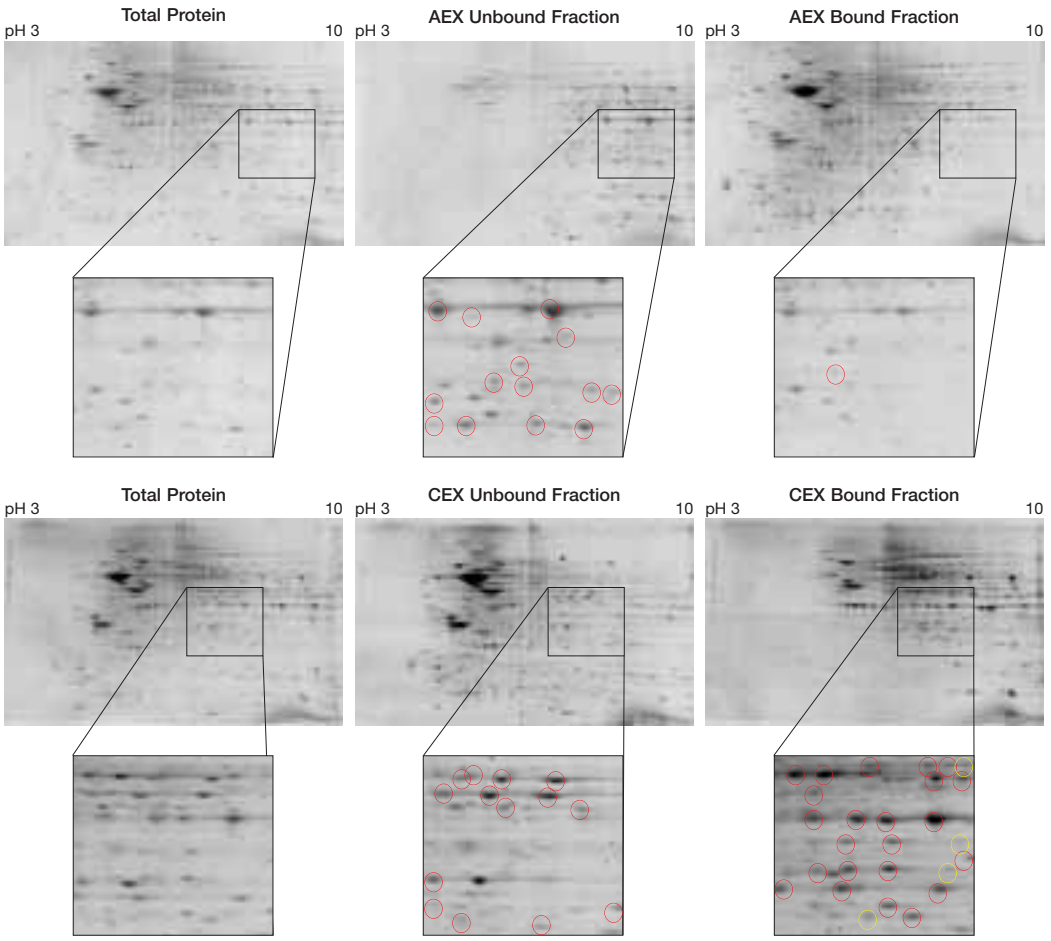
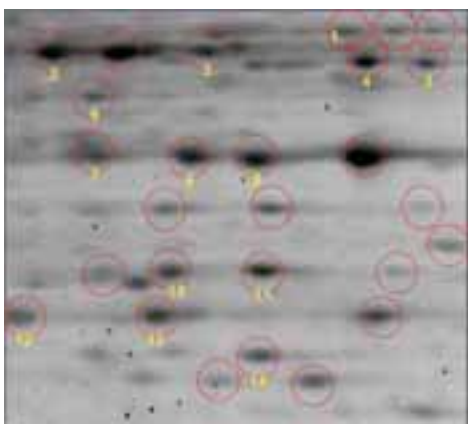


Fig. 2. Enrichment of basic proteins in rat brain after fractionation with Aurum AEX and CEX mini columns at pH 7.0. Proteins in the AEX unbound and CEX bound fractions focused mostly on the basic side of 2-D gels. In contrast, proteins in the AEX bound and CEX unbound fractions focused mainly on the acidic side of the gel. Enlarged views reveal that the intensities of many of the proteins were increased (red circles) compared to the total protein samples. Some proteins that were barely detectable in the total protein gel were easily detected in the CEX bound fraction gel (yellow circles).

Table 1. Summary of numbers of protein spots detected. PDQuest analysis of the gels shown in Figure 2, revealing that 12–30% of the protein spots on the 2-D gels of the AEX or CEX fractions showed at least a 2-fold increase in intensity compared to the total protein gels; 15–24% of the spots were not observed on the total protein gels.

	AEX			CEX		
Sample	**Total Protein**	**Unbound**	**Bound**	**Total Protein**	**Unbound**	**Bound**
Total	557	247	550	428	418	391
2x enrichment	—	34	66	—	61	109
3x enrichment	—	11	22	—	19	46
Bound only	—	—	112	—	—	59
Unbound only	—	66	—	—	69	—

CEX Bound Fraction



Spot	Protein ID	MW	pI	Probability	XC* Score	% AA
1	Citrate synthase	52	8.33	1.1×10^{-10}	100.3	18
2	Septin 2	42	6	2.8×10^{-9}	38.2	7.2
3	Glutamine synthetase	42	6	7.3×10^{-5}	10.2	4.3
4	Creatine kinase	43	8.33	3.6×10^{-6}	78.3	21
5	Casein kinase II	45	7.2	5.0×10^{-12}	40.3	13
6	MAPK 1	41	6.0	4.5×10^{-13}	68.3	21
7	GAPDH	36	8.33	1.9×10^{-7}	54.2	19
8	GAPDH	36	8.33	4.8×10^{-7}	40.2	11
9	GAPDH	36	8.33	1.1×10^{-7}	40.2	15
10	Carbonic anhydrase 2	29	6.0	1.1×10^{-11}	20.2	9
11	Brain Y-box binding protein 1	36	10.7	8.9×10^{-4}	8.1	6
12	Similar to triosephosphate isomerase	27	6.0	5.9×10^{-11}	60.3	42
13	Triosephosphate isomerase	27	7.2	4.6×10^{-10}	20.2	10
14	GTPase Ran	24	6.0	5.2×10^{-6}	20.2	10

Fig. 3. Identification of the proteins enriched in the CEX bound fraction by mass spectrometry. Spots excised from the gel were digested with trypsin, and digests were analyzed by reverse-phase LC-MS using an Agilent 1100 series capillary HPLC coupled to a Finnigan LTQ linear ion trap mass spectrometer equipped with a nanoES ionization source. Proteins were identified by SEQUEST search of the rat.fasta database. * XC = SEQUEST cross-correlation score.

- Aurum AEX and CEX mini columns can enrich basic proteins in rat brain samples at pH 7.0 for 2-D gel analysis. These columns can also be applied to enrich acidic proteins by adjusting the pH of the binding buffer to a low pH value (for example, pH 5.0). The combination of AEX and CEX mini columns may allow enrichment of proteins at any pI for 2-D gel analysis.
- At 15–20 min per fractionation, AEX and CEX mini columns provide a quick, convenient, and viable sample preparation method for use in a complete 2-D gel-based expression proteomics workflow.