Application of CHT™ Ceramic Hydroxyapatite for Proteomic Analysis

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Abstract

CHT ceramic hydroxyapatite is a sintered matrix composed of calcium and phosphate. Among its varied interactions, it is known to have strong affinity for phosphoryl residues on proteins. This study demonstrates fractionation of proteins based on relative phosphorylation, and subsequent measurement of phosphorylation in the fractions with a quantitative assay we have developed for protein applications. Data is shown from a variety of commercially available and experimentally modified proteins. The combination of CHT fractionation and a quantitative phosphorylation assay provides a flexible platform for proteomic research and subsequent scale-up.

Introduction

The goal of proteomics is to obtain a global and integrated view of biology by studying all the proteins of a cell rather than each one individually and subsequently. By the very definition of proteomics, complex protein mixtures will be encountered (1). To resolve these protein mixtures into their individual components for visualization, identification and characterization, 2-D electrophoresis, the predominant technology in proteomics, has been employed. As an adjunct to electrophoresis, multidimensional purification techniques have been evaluated (2). But even with high resolution separation techniques, the assumed 100,000 to 1,000,000 proteins in a biological sample cannot be resolved in a single step. Therefore, fractionation of the proteins mix is necessary. Chromatography on hydroxyapatite is a long established technique for selective fractionation of phosphorylated proteins and it offers outstanding potential for their enrichment in proteomic research (3). In this presentation we demonstrate the practical value of this application. We also introduce a convenient quantitative assay for determination of molar phosphorylation on proteins.

Materials and methods

Column

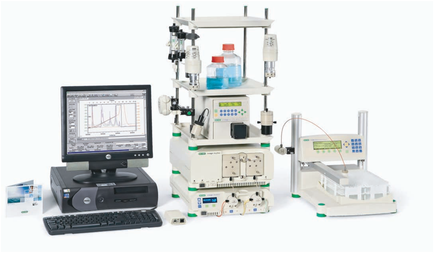
The experiments were conducted on a Bio-Scale MT2 column obtained from Bio-Rad Laboratories (Hercules, CA). The column dimensions were 0.7 x 3.5 cm. The column was dry packed with CHT Type I resin (Bio-Rad Laboratories, Hercules, CA) using a density of 0.60 gm/ml. Each column was wetted in ten column volumes of 20% ethanol before equilibration.

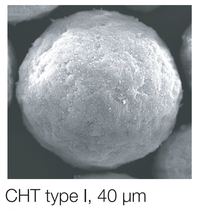
System

All chromatography experiments were automated and executed using a BioLogic DuoFlow™ system and software.

Proteins

Bovine serum albumin, dephosphorylated casein, α-casein, β-casein, ovalbumin, and human serum were purchased from Sigma-Aldrich (St. Louis, MO). Laboratory phosphorylated ovalbumin was prepared by the method of Morefield et. al. (4)





CHT type I, 40 µm
Sinter temperature: 400°C
Pore diameter: ~600-1000Å
Surface area: ~40m2/g

Fig. 1. BioLogic DuoFlow system and CHT matrix used for chromatography of phosphoproteins.

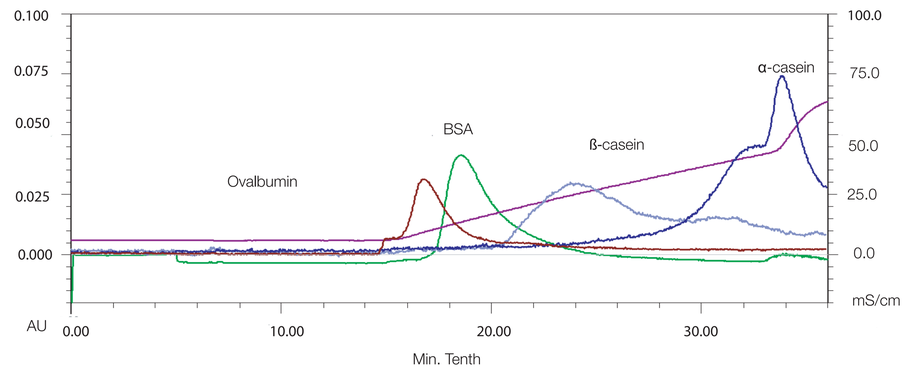


Fig. 2. Differential retention of phosphoproteins on CHT.
To determine the retention of phosphorylated proteins on CHT, elution was carried out with a phosphate gradient at pH 6.5 The equilibration buffer was 0.05 M MES pH 6.5. The elution buffer was 0.5 M KPO4. The experiment was run with the following configuration: equilibrate with 10 column volumes (CV) equilibration buffer, inject 50 microliters of each protein sample, wash 5 CV, elute with a 20 CV linear gradient to 60% elution buffer, strip with 5 CV of 100% elution buffer.

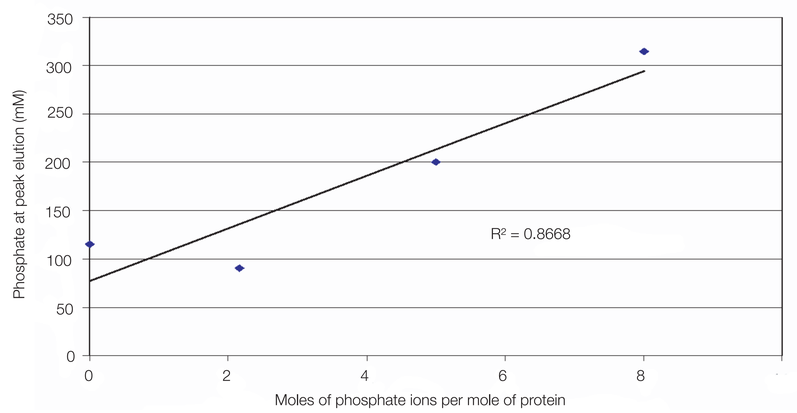


Fig. 3. Correlation of retention vs. phosphate content of proteins.

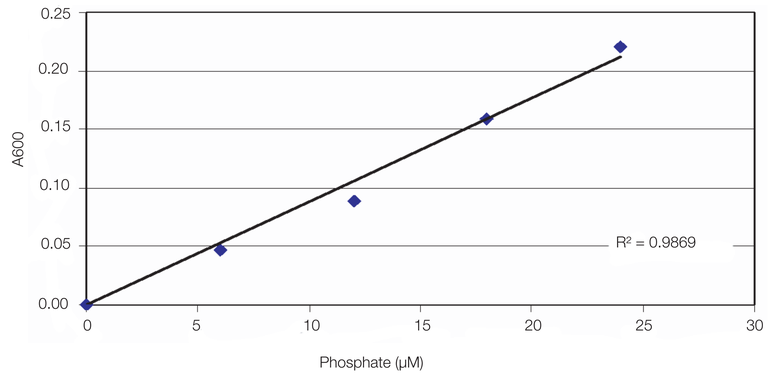


Fig. 4. Determination of inorganic phosphate.

Assay procedure

1. Mix 1 volume of sample and 1 volume of 2 M NaOH at 100˚ C for 7 minutes
2. Neutralize with 1 volume of 4.7 M HCl
3. Quantitate phosphate using 96-well plate by QuantiChrom phosphate assay kit (BioAssay Systems, Hayward, CA)

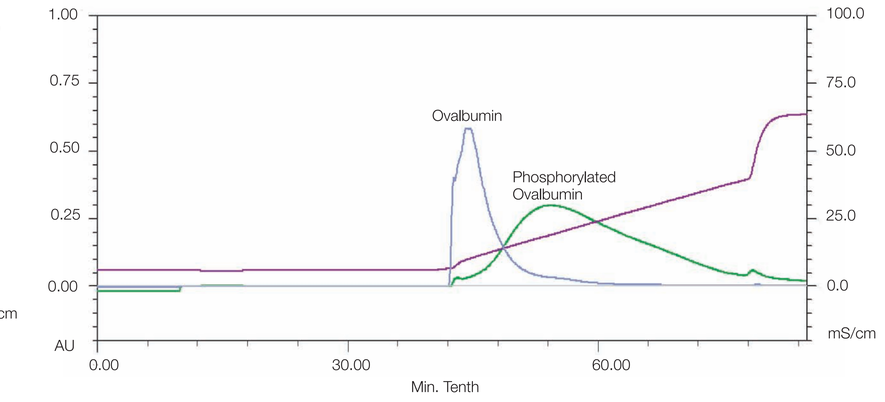


Fig. 5. Resolution of ovalbumin and laboratory phosphorylated ovalbumin.

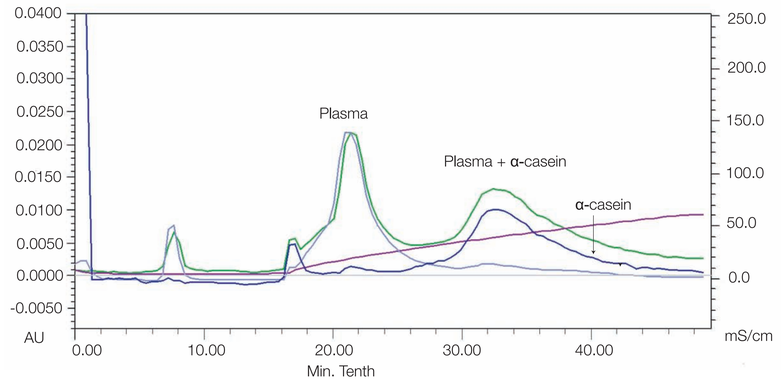


Fig. 6. Separation of a phosphoprotein (α casein) from a mixture of plasma proteins.

Conclusions

We have developed a chromatography system and an improved phosphate assay for the purification and characterization of phosphoproteins. This facilitates the initial purification of a complex protein mixture when specifically studying phosphoproteins. Additional effort is under development, leading to the isolation of proteins substantially free of non phosphorylated proteins.

References

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Acknowledgements

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