Protein sample pretreatment: weak ion exchange methods based on magnetic particle separation

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ABSTRACT

Due to the complexity of protein samples, fractionation steps are frequently used during protein purification. Prior to the actual purification process it is highly useful and time-saving to evaluate the behavior of the protein(s) of interest with different separation methods. QuickPick™ CM and DEAE kits can be used as an aid in designing the correct fractionation strategy for multiple applications. The QuickPick methods are especially convenient for small sample volumes with fast and material-conserving analysis in mind.

PROBLEM

Proteins are much more complex than nucleic acids. They are built from twenty different amino acids with multiple types of functional side groups instead of four bases with similar properties. Many types of functional side groups (phosphates, sugars) can be added to some of these twenty amino acids by posttranslational modifications. The purification of proteins is based upon their similarities and differences. Protein similarity is used to separate them from the non-protein contaminants. Differences, for example size, shape, charge, hydrophobicity, solubility and biological activity, are used to fractionate one protein from another.

Standard protein purification/separation methods rely on conventional liquid chromatography strategies based on chromatographic or spin columns. However, these methods are time-consuming and complex. Due to the extremely high trial and error nature of protein purification it is efficient and time-saving to evaluate the behavior of the protein(s) of interest with different fractionation methods prior to the actual purification process.

QuickPick weak ion exchange methods

Available from BN Products & Services are methods used in the fractionation of proteins, based on weak ion exchange. They are designed to help in the evaluation of the protein fractionation prior to actual purification. Described below are methods for weak cation exchange using carboxymethyl particles (CM), and weak anion exchange using diethylaminoethyl (DEAE) particles. The methods are based on the use of PickPen magnetic particle transfer device.

SOLUTIONS FOR PROTEIN FRACTIONATION

Protein fractionation / screening

QuickPick CM and DEAE ion exchange kits for proteins can be utilized for different purposes:

Testing of fractional behavior of a protein sample based on proteins' overall charges at different pH values or salt concentrations.

Fractionation of protein samples before 2D gel electrophoresis.

Concentration of proteins expressed in low amounts.

In a method comparison QuickPick methods for CM and DEAE take 5 minutes per preparation while conventional methods take from 30 min to several hours. In combination with the PickPen magnetic particle transfer device, the QuickPick CM and DEAE methods provide rapid and efficient assessment of different protein samples.

MATERIALS AND METHODS

As an example of the weak ion exchange methods available as QuickPick kits, the fractionation procedure (Fig. 1a-e) for neutral and basic proteins (weak cation exchange) is described. A protein mixture consisting of aprotinin, albumin and β-lactoglobulin was used as a sample. The carboxymethyl (CM) magnetic particles were first suspended in the Regeneration buffer (a) and then transferred into the Wash buffer (b). The equilibrated particles were incubated for 2 min in the protein mixture solution (c), washed once in the Wash buffer (d) and to conclude the bound proteins were eluted out by incubating the particles in the Elution buffer (e) for 1 min.

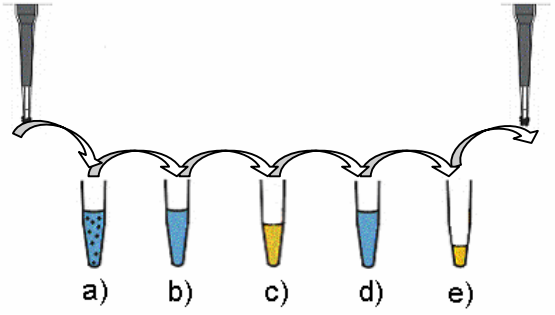

Fig. 1

The QuickPick CM kit reagents from BN Products & Services were used in the purification.

RESULTS

To demonstrate the fractionation properties of CM magnetic particles with PickPen technology, the original protein mixture, the protein mixture after magnetic particle treatment and the final elution solution were analyzed (Fig. 2a-c, respectively). The chromatograms show the binding and elution behavior of proteins during the procedure. The transferring efficiency of CM magnetic particles has been shown to be over 60 µg aprotinin per preparation.

a) Chromatogram of protein mixture containing aprotinin, albumin and β-lactoglobulin

b) Chromatogram of protein mixture after removal of aprotinin with magnetic particles

c) Chromatogram of aprotinin released from magnetic particles and eluted into buffer

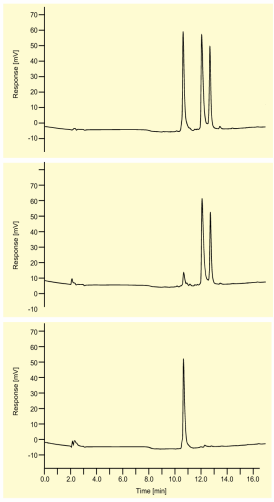

Fig. 2

The PickPen technology using CM particles has several advantages in comparison to other methods, some of the most significant being speed and ease of use. The time needed for single purification is only 5 minutes.

The method is optimized for small sample volumes. However, with samples containing low protein concentration volumes up to 1 ml can be applied. In fact, PickPen technology also serves as a method to concentrate the target protein and thereby facilitate, for example, the detection of proteins expressed in low levels.

CONCLUSIONS

QuickPick CM and DEAE kits together with the PickPen transfer technology serves as a fast and cost-effective alternative to tedious and time-consuming protein purification methods commonly available on the market. The excellent fractionation properties of the magnetic particles are especially optimized for use with PickPen.