Monitoring Chromatography Separations With a Real-Time Bioassay

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Introduction

Chromatography plays a major role in the purification of proteins, so tools that automate and simplify the purification process are highly desired. Traditionally, sample elution is monitored with a UV detector at 214 or 280 nm. However, a UV detector cannot identify which peak corresponds to the protein of interest in crude samples or whether the protein is active. In this paper we present a novel chromatography monitoring system that uses a real-time bioassay to identify proteins as they are eluted from a column. To demonstrate the usefulness of this monitoring system, we have used it to monitor the purification of bovine alkaline phosphatase.

Methods

Crude bovine alkaline phosphatase (100 µg/ml; Sigma catalog #P 7640) was dissolved in 25 mM Tris, pH 8.1 and filtered to remove insoluble debris. Chromatography was performed using a BioLogic DuoFlow™ chromatography system and an UNO™ Q1 anion exchange column (Bio-Rad). Experiments were performed at 2 ml/min with a 5 ml (500 µg) sample load and a 20 ml linear salt gradient (0–0.5 M NaCl).

Both a BioLogic DuoFlow UV detector (280 nm) and a bioassay monitoring system were used to monitor protein elution (Figure 1). The bioassay monitor was composed of an Econo™ gradient pump, Econo gradient pump splitter valve, and a BioLogic QuadTec™ multiple wavelength detector (Bio-Rad). The splitter valve was placed immediately after the UV detector and was used to divert 10% of the column effluent for the activity assay. The diverted column effluent was then mixed with an equal volume of 2x assay reagent (alkaline phosphatase yellow (pNPP) liquid substrate, Sigma catalog #A 3469) delivered by the Econo gradient pump (0.2 ml/min). The reaction mixture was then allowed to flow through an incubation loop at ambient temperature into a QuadTec detector, where the breakdown of 4-nitrophenyl phosphate (pNPP) was monitored at 405 nm. (Specialized incubation loops as long as 5 m have been employed that provide a 1 ml hold-up volume without introducing appreciable peak dispersion.) The incubation loop selected (50 cm, 100 µl) provided adequate time (15 sec) for the bioassay mixture to react before it reached the detector.

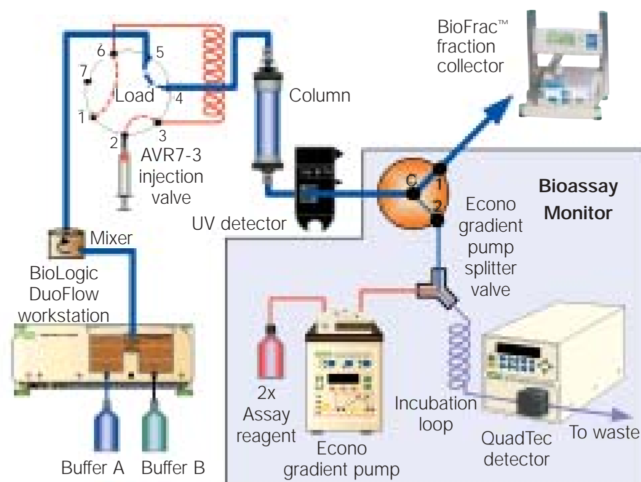

Fig. 1. Bioassay monitor plumbing diagram.

Results

The chromatogram obtained from the bovine alkaline phosphatase purification showed that only a small portion of the material injected onto the column contained active protein (Figure 2). Furthermore, the chromatogram showed two active alkaline phosphatase peaks (eluting at 8.1 and 12.4 min).

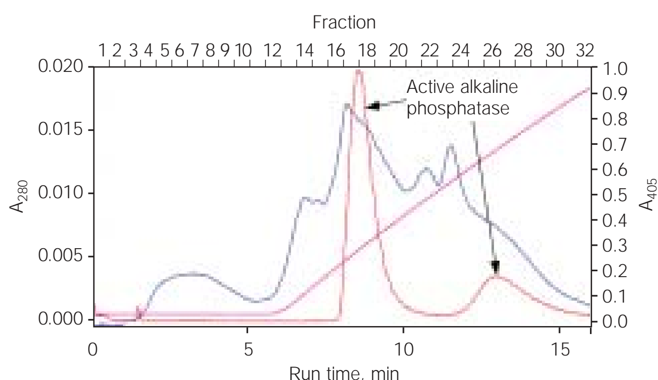

Fig. 2. Chromatogram showing detection of two active alkaline phosphatase peaks. Blue trace, UV absorbance; red trace, bioassay monitor signal (A_{405}); pink trace, conductivity.

Conclusions

In-line detectors are typically more sensitive than cuvette spectrophotometers. Consequently, much shorter reaction times are permissible with in-line assays, as demonstrated in this study. The bioassay system successfully monitored the purification of bovine alkaline phosphatase and allowed identification of two active phosphatase peaks that would have been difficult to identify from the A_{280} trace alone.
This bioassay monitor may incorporate any assay that can be monitored using either a UV/Vis detector, such as the QuadTec detector, or a fluorescence detector.

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