is to be separated from the tail of a large peak, the effect of dead volume must be taken into consideration.

In the manufacture of the manifolds, it is difficult to control the resulting performance in advance; by choosing from several products, however, it is easy to obtain one which is satisfactory.

4 Conclusion

The manifolds proposed in this paper have very small dead volume, relatively high inertness (in comparison with manifolds made from metals), and low thermal mass. They are very easy to make, easily fitted with capillary columns of different diameters, and inexpensive. Using this technique, it is possible to make integrated manifolds with arbitrarily chosen flow patterns and good chromatographic performance. Further work is being performed to study the effect of their adsorptive and catalytic activity on the analysis of active compounds.

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Applications of Capillary Electrophoresis to Some Redox Proteins

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1 Introduction

One of the most powerful means of resolving proteins is sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE). This technique, however, despite its high resolving power, has some drawbacks: SDS often denatures and inactivates functional proteins and the whole process, including preparation of the gel, performing the electrophoresis, and staining and destaining for detection, takes quite long time. Capillary zone electrophoresis (CZE) has been shown to be useful for resolving peptides and proteins [1, 2]. In this paper, we report CZE separations of some of electron carrier proteins which occur loosely bound to membranes, *i.e.* cytochrome c, ferredoxin, and plastocyanin. It has been found that the reduced forms of the proteins could be separated from the oxidized forms and that the two isozymes of ferredoxin, which could not easily be separated by conventional techniques, were also clearly resolved by CZE.

2 Experimental

CZE was performed with a Jasco Model CE-800 capillary electrophoresis system [1] comprising a high voltage power supply (model 890-CE), an on-column detector, a UV/VIS detector (model 875/870-CE), and a data-processing integrator (model 807-IT). The instrument was fitted with 0.05 mm i.d. (0.37 mm o.d.) fused silica capillary tubing of effective length 50 cm which was washed before and after each measurement by using a hand-operated vacuum pump (MityVac, Nalgen) to suck water (1 min), 0.1 N hydrochloric acid (1 min), water (1 min), 0.2 N sodium hydroxide solution (1 min), water (1 min), and, finally, the running buffer used for electrophoresis (2 min) through the tubing.

Samples were introduced into the capillary by siphoning: the end of the capillary was dipped into a sample tube which was then lifted 10 cm above the detector level for a short period. The amount of sample injected could be varied by changing the siphoning time, usually between 5 and 15 s. The column temperature was *ca* 25 °C.

UV/Vis spectra of samples were measured with either a Jasco model Ubest-35 double-beam spectrophotometer or a Jasco Model Ubest-55 double monochromator spectrophotometer.

Ferredoxins prepared from pokeweed (*Phytolacca americana*) and spinach leaves were kindly provided by *Professor K. Suzuki* [3]. Plastocyanin was prepared from spinach according to the method

of *Katoh* [4]. Horse heart cytochrome c was purchased from Sigma (St Louis, Mo., USA) and zwittergents from Calbiochem (La Jolla, CA, USA).

3 Results and Discussion

Untreated (horse heart) cytochrome c preparation gave a single peak. On addition of a small amount of a reductant, dithiothreitol (DTT), another peak appeared and as the concentration of DTT was increased the original peak was gradually replaced by this new peak, indicating the new peak to be that of reduced cytochrome c. By checking the absorption spectrum of each sample, we confirmed that the original and new peaks were indeed those of oxidized and reduced cytochrome c, respectively (data not shown).

Ferredoxin, an extremely electronegative electron carrier protein with an iron–sulfur cluster as the prosthetic group, occurs loosely bound to chloroplast thylakoid membranes. It is known to occur in plants and algae [3, 5, 6] as two different forms (isozymes) with slightly different amino acid sequences. The separation of the isozymes has so far been achieved by derivatization of the proteins [5], by HPLC [6], or by non-denaturing gel electrophoresis [3].

We were able to prepare a CZE medium with which the isozymes could readily be separated. **Figure 1** shows that under the conditions used the migration time of Form II of ferredoxin from pokeweed is much shorter than that of Form I. An electropherogram of the crude ferredoxin preparation (Figure 1C) shows clear separation of the two forms. Much less sample and time are

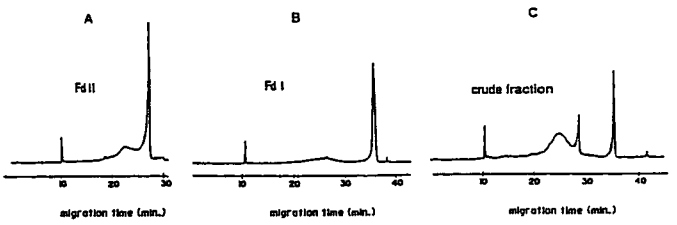

Figure 1

Electropherogram of ferredoxins from *Phytolacca americana* (pokeweed): buffer, 20 mM sodium phosphate, pH 7.0, containing 200 mM zwittergent 3-08; sample concentration, approximately 0.1 mg/ml; voltage, 214 V/cm; average current, 0.01 mA; detection, 200 nm; A, ferredoxin II; B, ferredoxin I; C, crude ferredoxin.

needed for CZE analysis than for methods used hitherto, and the analysis time could be further reduced, still with satisfactory resolution, by use of a shorter column. Spinach ferredoxin was also separated into two forms with this system (data not shown). As the reduced form of ferredoxin is known to be quite unstable, no attempt was made to separate the reduced and oxidized forms.

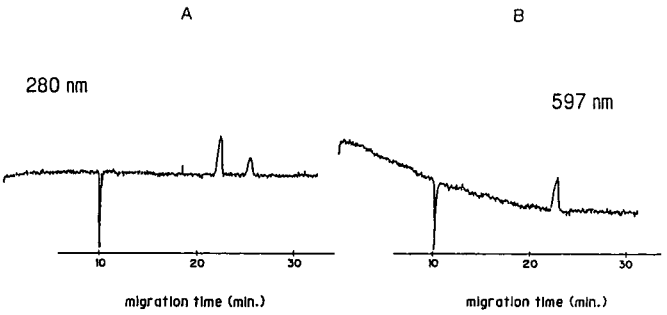

Figure 2

Electropherogram of plastocyanin from spinach: buffer, 20 mM sodium phosphate, pH 7.0; sample concentration, 0.5 mg/ml; voltage, 214 V/cm; average current, 0.025 mA.

Plastocyanin, a copper-containing photosynthetic electron carrying protein, is also bound only loosely to membranes and, similar to ferredoxin and cytochrome c, becomes water soluble once it is released from the membrane [4]. The oxidized form has a distinct blue color characterized by an absorbance maximum at 597 nm, whereas the reduced form does not absorb in the visible region [4]. We found that the oxidized and reduced forms of plastocyanin could be separated by CZE. **Figure 2A** shows two peaks, major followed by minor, obtained from an untreated preparation in which the majority of the plastocyanin was known to be in the reduced form. The major and minor peaks were assigned to the reduced and oxidized forms, respectively, by running, separately, preparations either reduced by ascorbate or oxidized by ferricyanide (data not shown). Further confirmation was obtained by changing the detector wavelength to 597nm, where only the oxidized form has absorbance (**Figure 2B**).

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