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Note

Comparison of high-performance ion-exchange chromatography and gel electrophoresis in protein separations

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Currently, high-performance liquid chromatography is an important technique to separate proteins. In particular, ion-exchange chromatography seems very useful for both analytical and preparative purposes because it can provide high resolution without denaturation of proteins. On the other hand, gel electrophoresis is most commonly adopted in protein separation, especially for analytical purposes such as purity tests, mainly due to its high resolution. Accordingly, we have compared high-performance ion-exchange chromatography and gel electrophoresis for the separation of crude superoxide dismutase.

EXPERIMENTAL

Ion-exchange chromatography was carried out on a TSK gel DEAE-5PW column (75 × 7.5 mm I.D.) (Toyo Soda) with a high-speed liquid chromatograph Model SP8700 (Spectra-Physics) equipped with a variable-wavelength UV detector Model UV-8 (Toyo Soda) operated at 280 nm. Superoxide dismutase (Sigma) (0.4 mg) was separated by use of a 60-, 120-, 240- or 480-min linear gradient of sodium chloride or sodium acetate from 0 to 0.5 *M* in 0.02 *M* piperazine–HCl buffer (pH 6.0), 0.02 *M* Tris–acetate buffer (pH 7.5) or 0.02 *M* Tris–HCl buffer (pH 7.0, 7.5, 8.0 or 8.5) at a flow-rate of 0.5 or 1 ml/min at 25°C.

Isoelectric focusing, selected as the mode of gel electrophoresis which gave highest resolution, was performed with a LKB Multiphor system and ampholine PAG plate in the range pH 3.5–9.5 or 4.0–6.5. Superoxide dismutase (0.02 mg) was focused at a constant current of 25 mA for 30 min and then at 1000 V for 60 min. After the focusing, the proteins were stained with Coomassie brilliant blue and scanned with a dual wavelength TLC scanner CS-910 (Shimazu) at a wavelength of 560 nm.

RESULTS AND DISCUSSION

Since the results of high-performance ion-exchange chromatography greatly depend on the elution conditions[1–4], the effects of eluent pH and composition, gradient steepness and flow-rate were investigated first. The effect of eluent pH is shown in Fig. 1. According to the enzymatic activity, two peaks corresponded to superoxide

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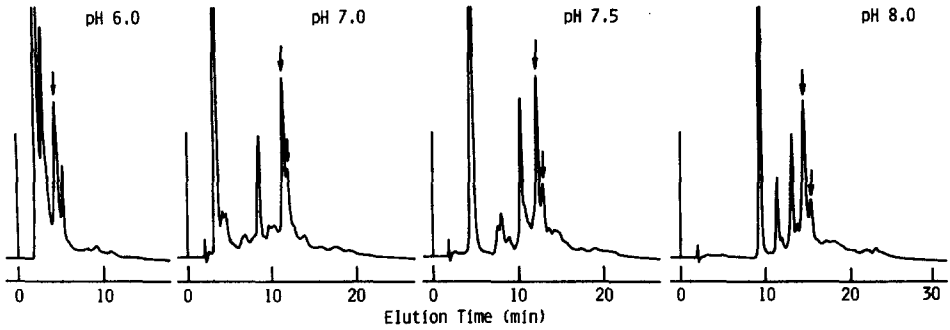

Fig. 1. Chromatograms of commercial superoxide dismutase obtained with a 120-min linear gradient from 0 to 0.5 *M* NaCl in 0.02 *M* piperazine–HCl buffer (pH 6.0) and 0.02 *M* Tris–HCl buffers (pH 7.0, 7.5 and 8.0) at a flow-rate of 1 ml/min. The two peaks in each chromatogram indicated by arrows showed superoxide dismutase activity.

dismutase. A pH around 7.5 seems best for the separation between these two superoxide dismutase isomers and also between the isomers and impurities. At pH < 6.0, all components were eluted earlier and were poorly separated. At pH > 8.0, the separation was again inferior because all components tended to elute in a narrow range of elution volumes although their retention increased. Fig. 2 shows the effect of eluent composition. Tris–HCl and Tris–acetate buffers which can be used at pH 7.5 were compared. Counter ions were unified in each buffer system. Tris–acetate buffer provided a better separation between superoxide dismutase isomers, while superoxide dismutase isomers and impurities eluting before the isomers were better separated with Tris–HCl buffer. Overall, Tris–HCl buffer seems better. The effect of gradient steepness is shown in Fig. 3. With decreasing gradient steepness, the separation was considerably improved, with a concomitant slightly longer separation time. The effect of flow-rate is shown in Fig. 4. The resolution decreased very slightly

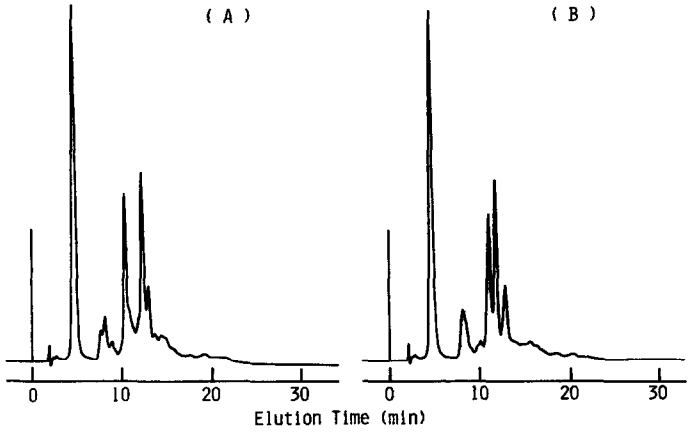

Fig. 2. Chromatograms of commercial superoxide dismutase obtained with a 120-min linear gradient from 0 to 0.5 *M* NaCl in 0.02 *M* Tris–HCl buffer (pH 7.5) (A) and with a 120-min linear gradient from 0 to 0.5 *M* sodium acetate in 0.02 *M* Tris–acetate buffer (pH 7.5) (B) at a flow-rate of 1 ml/min.

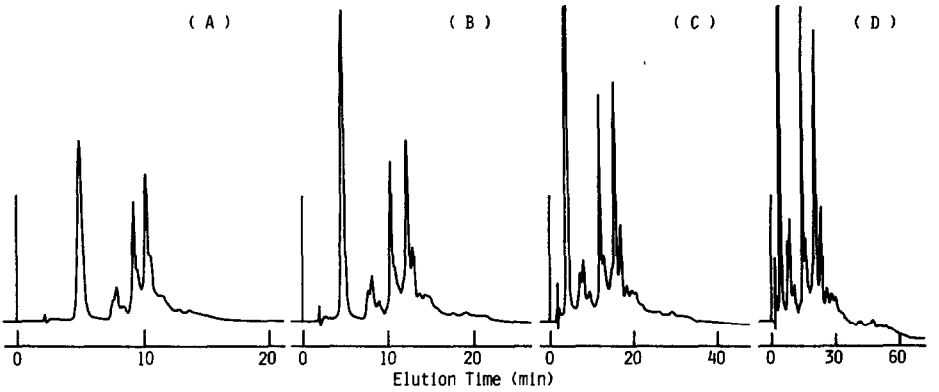

Fig. 3. Chromatograms of commercial superoxide dismutase obtained with 60-min (A), 120-min (B), 240-min (C) and 480-min (D) linear gradients from 0 to 0.5 *M* NaCl, which correspond to gradient steepnesses of 8.3, 4.2, 2.1 and 1.0 m*M* NaCl per min, in 0.02 *M* Tris–HCl buffer (pH 7.5) at a flow-rate of 1 ml/min.

with decreasing flow-rate. Lower flow-rates also resulted in an appreciable increase in separation time.

Superoxide dismutase was focused in the range pH 3.5–9.5 first. All bands were found between pH 4.7 and 6.3. Therefore, the sample was then focused in the range pH 4.0–6.5 in order to attain maximum resolution. The results are shown in Fig. 5.

In high-performance ion-exchange chromatography under appropriate conditions such as C and D in Fig. 3, more than 25 peaks and shoulders were observed. Isoelectric focusing in the range pH 4.0–6.5 also resolved the sample into about 25 components. Therefore, it can be concluded that these two techniques provide comparable resolution.

High-performance liquid chromatography is generally advantageous over gel electrophoresis as regards rapidity, ease of recovery of sample components after separation, reproducibility, quantitation and ease of scaling up, as has already been discussed[5,6]. Consequently, high-performance ion-exchange chromatography may be

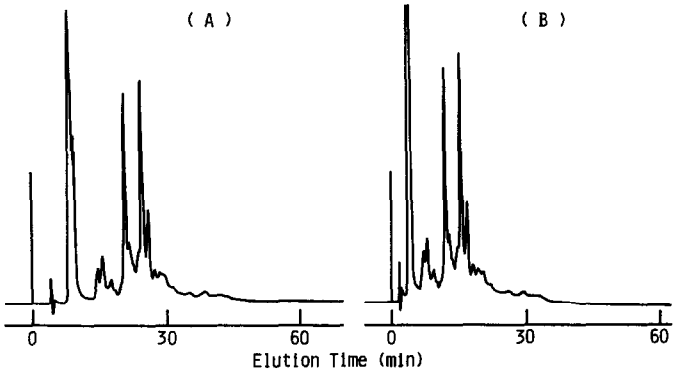

Fig. 4. Chromatograms of commercial superoxide dismutase obtained with a 240-min linear gradient from 0 to 0.5 *M* NaCl in 0.02 *M* Tris–HCl buffer (pH 7.5) at flow-rates of 0.5 ml/min (A) and 1 ml/min (B).

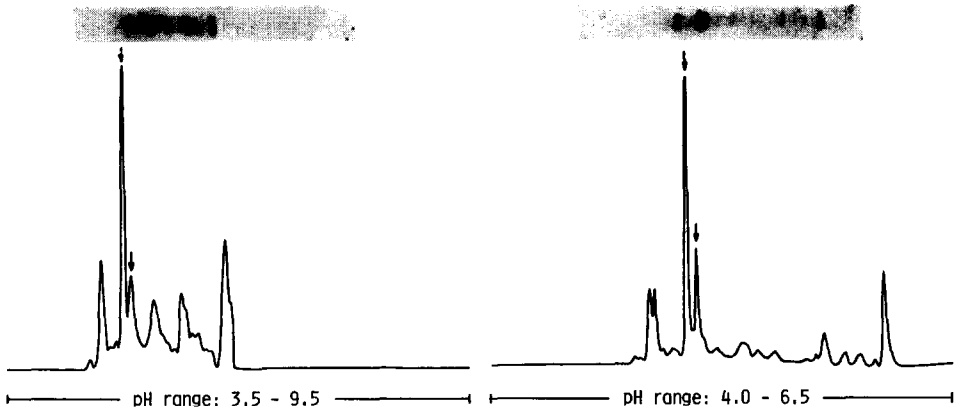

Fig. 5. Isoelectric focusing patterns of commercial superoxide dismutase obtained in the ranges pH 3.5–9.5 and 4.0–6.5. Peaks indicated with arrows are due to superoxide dismutase.

employed successfully as an alternative to gel electrophoresis in both analytical and preparative separations of proteins.

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