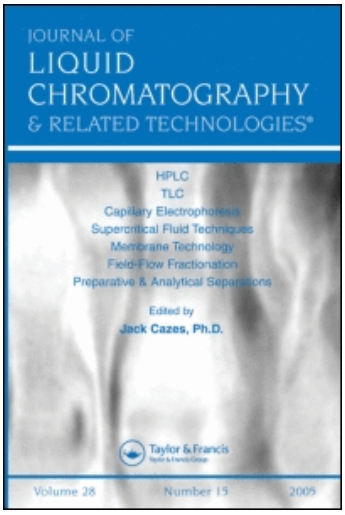

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High-Performance Hydrophobic Interaction Chromatography of Proteins on TSKgel Phenyl-5PW Preparative Column

Yoshio Kato[a]; Takahashi Kitamura[a]; Tsutomu Hashimoto[a]
[a] Toyo Soda Mfg. Co., Ltd., Yamaguchi, Japan

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HIGH-PERFORMANCE HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF PROTEINS ON TSKgel Phenyl-5PW PREPARATIVE COLUMN

Yoshio Kato, Takahashi Kitamura, and Tsutomu Hashimoto

Toyo Soda Mfg. Co., Ltd.
Tonda, Shinnanyo, Yamaguchi, Japan

ABSTRACT

TSKgel Phenyl-5PW preparative column of 200 x 55 mm I.D. was evaluated with respect to resolution, sample loading capacity and applications to the purification of enzymes. The preparative column provided similar separations as analytical column (75 x 7.5 mm I.D.) and 150 x 21.5 mm I.D. preparative column. The sample loading capacity was 200 - 1000 mg depending on the sample. If the slight decrease in resolution is acceptable, much more samples could be applied. Lipoxidase, phosphoglucose isomerase and lactate dehydrogenase could be purified to a great extent with high recovery of activity (more than 80 %).

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INTRODUCTION

Hydrophobic interaction chromatography has become increasingly popular for the separation and purification of proteins. Although it was originally a slow technique, rapid separations have become possible by the introduction of microparticulate supports (1-15). TSKgel Phenyl-5PW (Toyo Soda, Tokyo, Japan) is one such support and it has been reported that proteins can be separated rapidly with high resolution without denaturation on analutical columns (75 x 7.5 mm I.D.) and 150 x 21.5 mm I.D. preparative columns of this support (3,5,6,8,12,14). In this paper, the results of protein separations on a 200 x 55 mm I.D. preparative column of the Phenyl-5PW are described. The resolution, sample loading capacity and applications to enzyme purifications have been investigated.

MATERIALS AND METHODS

All chromatographic separations on the preparative column were carried out at 25°C with a high-speed liquid chromatograph Model HLC-837 (Toyo Soda) equipped with a variable-wavelength UV detector Model UV-8 (Toyo Soda) operated at 280 nm.

Lipoxidase was purchased from P-L Biochemicals (Milwaukee, WI, U.S.A.). All other proteins were from Sigma (St. Louis, MO, U.S.A.).

The resolution of the Phenyl-5PW preparative column was studied by separating a mixture of myoglobin, ribonuclease and lysozyme with a linear gradient of decreasing ammonium sulfate concentration from 1.8 M to 0 in 0.1 M phosphate buffer (pH 7.0). The flow rate and gradient time were varied to investigate the effects of these parameters.

The sample loading capacity of the Phenyl-5PW preparative column was studied by separating crude samples of lipoxidase, phosphoglucose isomerase and lactate dehydrogenase at various loadings. The sample concentrations were kept constant (ca. 20 mg/ml) and the injection volumes were varied. Lipoxidase was separated with a 120-min linear gradient of ammonium sulfate from 1.5 M to 0 in 0.1 M phosphate buffer (pH 7.0). Phosphoglucose isomerase was separated with a 60-min linear gradient of ammonium sulfate from 1.5 M to 0 in 0.1 M phosphate buffer (pH 7.0). Lactate dehydrogenase was separated with a 120-min linear gradient of ammonium sulfate from 1.8 M to 0 in 0.1 M phosphate buffer (pH 7.0). The flow rate was 40 ml/min.

The crude samples of lipoxidase (1 g), phosphoglucose isomerase (500 mg) and lactate dehydrogenase (200 mg) were separated under the same conditions as above and the peaks corresponding to each enzyme were fractioned. The fractions were examined for the recovery of enzymatic activity and purity. The enzymatic activity was determined according to the procedures described in reference 16. The purity was tested by high-performance

liquid chromatography (HPLC). Hydrophobic interaction chromatography was performed on an analytical Phenyl-5PW column with 60-min linear gradients of the same eluents as in the preparative separations. Reversed-phase chromatography was performed on a TSKgel Phenyl-5PW RP column (75 x 4.6 mm I.D.) with a 2-min linear gradient of acetonitrile from 5 to 20 % followed by a 48-min linear gradient of acetonitrile from 20 to 80 % in 0.05 % trifluoroacetic acid (TFA). Gel filtration was performed on a TSKgel G3000SW column (600 x 7.5 mm I.D.) in 0.05 M phosphate buffer containing 0.2 M sodium chloride (pH 7.0). Ion-exchange chromatography was performed on a TSKgel DEAE-5PW column (75 x 7.5 mm I.D.) with a 60-min linear gradient of sodium chloride from 0 to 0.5 M in 0.02M Tris-HCl buffer (pH 8.0). All these purity tests were performed at a flow rate of 1 ml/min with a high speed liquid chromatograph Model SP8700 (Spectra-Physics, San Jose, CA, U.S.A.) equipped with UV-8 operated at 280 nm except in reversed-phase chromatography where it was operated at 220 nm.

RESULTS AND DISCUSSION

Fig. 1 shows the dependence of resolution on the flow rate at a constant gradient time of 60 min. The resolution increased, although slightly, with flow rate up to 40 ml/min. The dilution of samples during separation decreased almost in proportion to the flow rate, while the

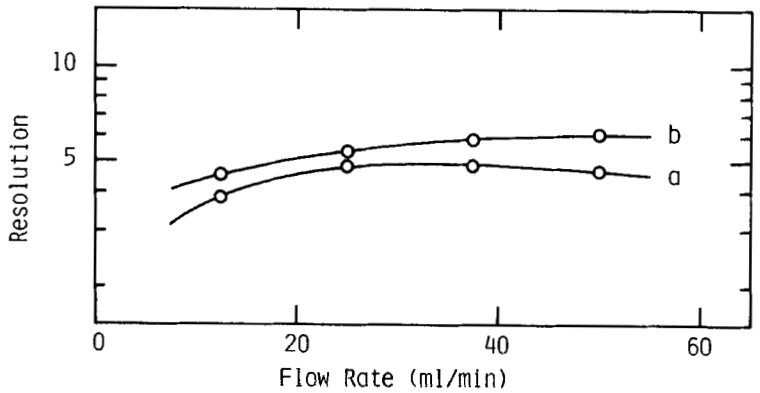

FIGURE 1 Dependence of resolution on the flow rate at a constant gradient time (60 min) in hydrophobic interaction chromatography on a Phenyl-5PW preparative column of 200 x 55 mm I.D.. The resolution was calculated from the peak widths and elution volumes of the pairs, myoglobin and ribonuclease (a) and ribonuclease and lysozyme (b).

separation time increased slightly with decreasing flow rate. Accordingly, a flow rate around 40 ml/min seems to be a good choice for the 200 x 55 mm I.D. preparative column.

Fig. 2 shows the dependence of resolution on the gradient time at a constant flow rate of 40 ml/min. The resolution greatly increased with increasing gradient time up to 60 min. However, this effect was insignificant at gradient times longer than 120 min. Because the separation time became longer and the dilution of samples increased almost in proportion to the gradient time, gradient times of 60 - 120 min are supposed to be a good compromise.

In the separation of lipoxidase with various sample loadings, almost identical patterns were observed with

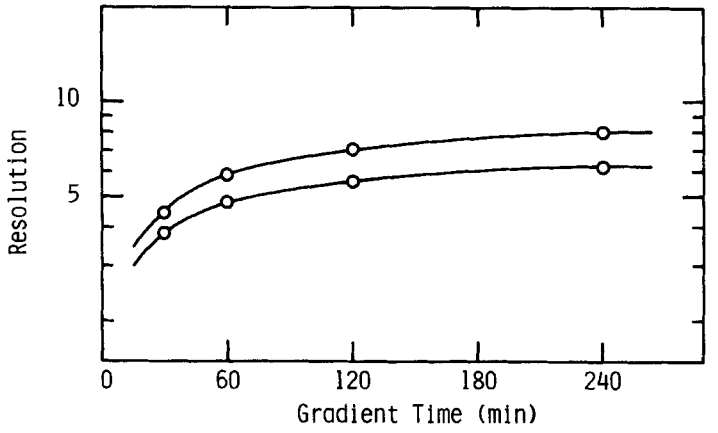

FIGURE 2 Dependence of resolution on the gradient time at a constant flow rate (40 ml/min) in hydrophobic interaction chromatography on a Phenyl-5PW preparative column of 200 x 55 mm I.D.. Resolutions as in Fig. 1.

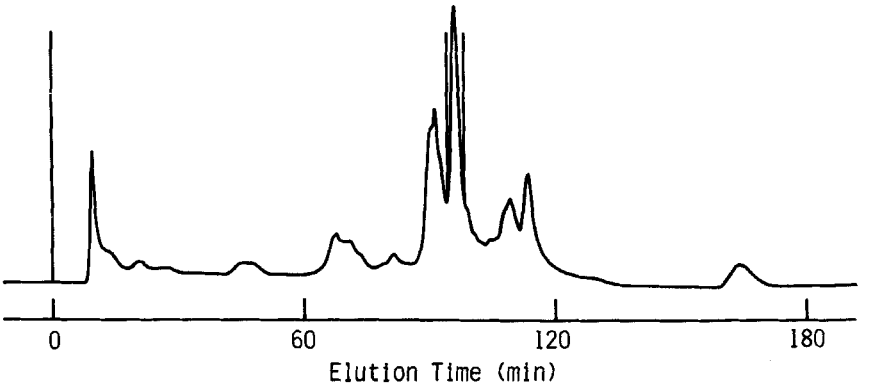

FIGURE 3 Separation of 1 g lipoxidase on the Phenyl-5PW preparative column of 200 x 55 mm I.D..

sample loadings up to 1 g. Phosphoglucose isomerase could be applied up to 500 mg without loss of separation efficiency. In the case of lactate dehydrogenase, almost the same separations were obtained up to 200 mg. However, the resolution decreased gradually with sample loadings above these amounts. Consequently, the maximum sample loadings of the 200 x 55 mm I.D. preparative column which permit the highest resolution may be said to be 200 - 1000 mg depending on the sample. If a slight decrease in resolution is acceptable, much more samples can be applied.

Fig. 3 shows a separation of 1 g crude lipoxidase. The peak corresponding to lipoxidase between the two vertical lines was fractioned. The recovery of enzymatic activity in the fraction was 84 %. The results of purity tests of the fraction by HPLC are shown in Fig. 4. One major peak and several very small peaks are seen in each chromatogram of the fraction. Because the major peaks showed enzymatic activity (except in the case of reversed phase chromatography), they must correspond to lipoxidase. This means that considerably pure lipoxidase was obtained from commercial lipoxidase containing large amounts of impurities.

Fig. 5 shows a separation of 500 mg crude phosphoglucose isomerase. In this separation, enzymatic activity was found in several peaks. Of the applied phosphoglucose isomerase activity, 74 % was found in the peak between the two vertical lines and 25 % was found in the peaks eluted between 64 and 70 min. Therefore, total

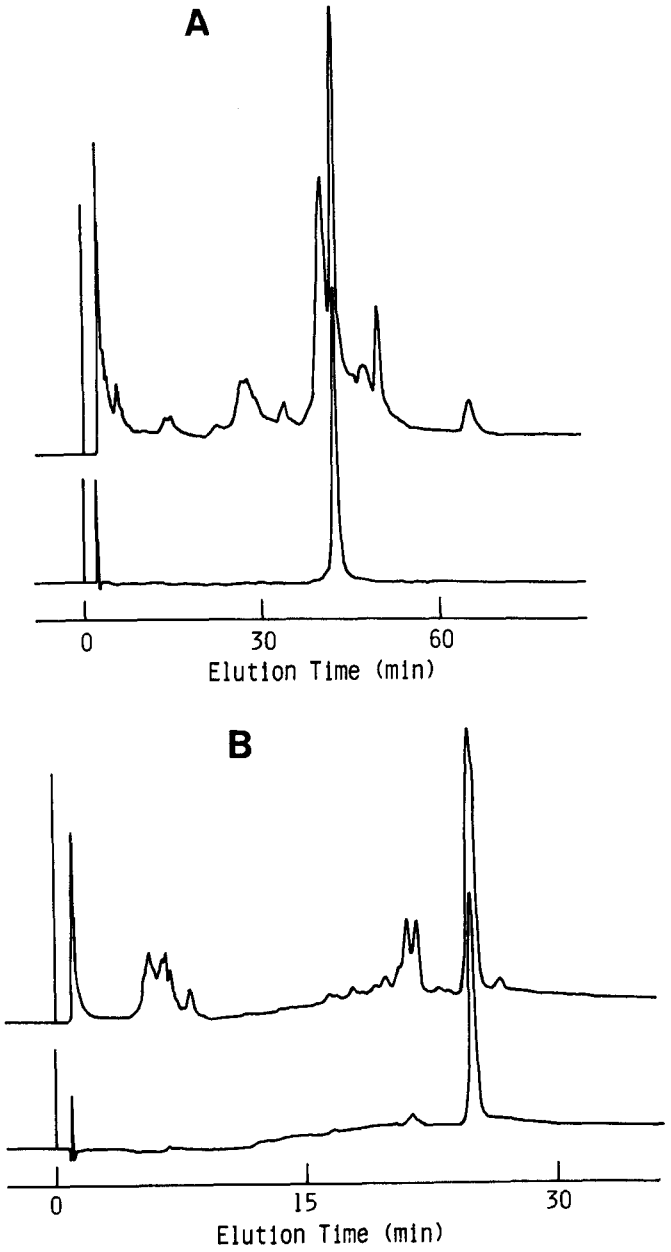

FIGURE 4 Chromatograms of crude lipoxidase sample (upper curves) and the fraction in Fig. 3 (lower curves) obtained by hydrophobic interaction chromatography (A), reversed-phase chromatography (B), gel filtration (C) and ion-exchange chromatography (D).

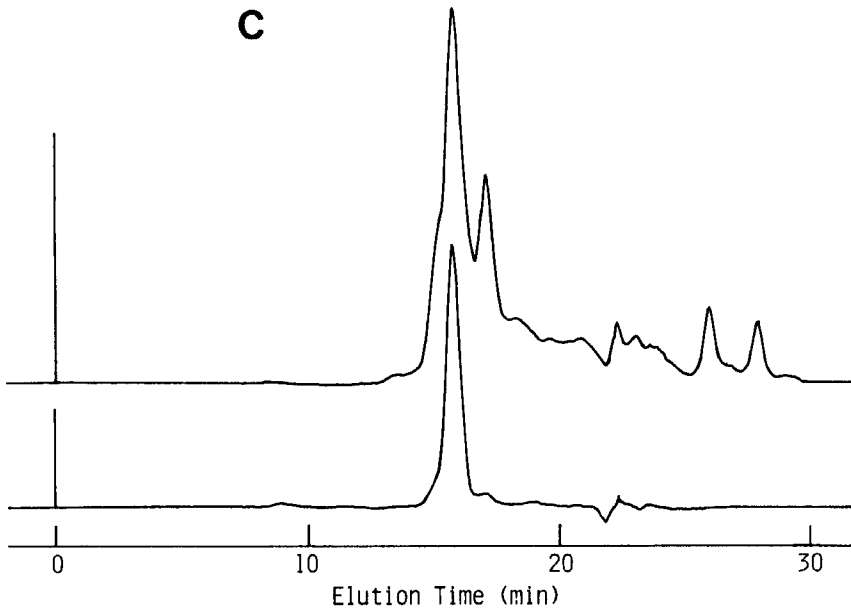

FIGURE 4C

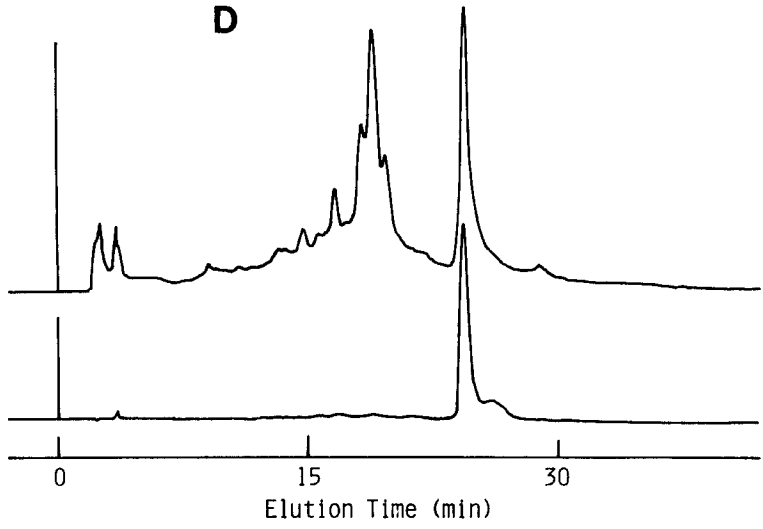

FIGURE 4D

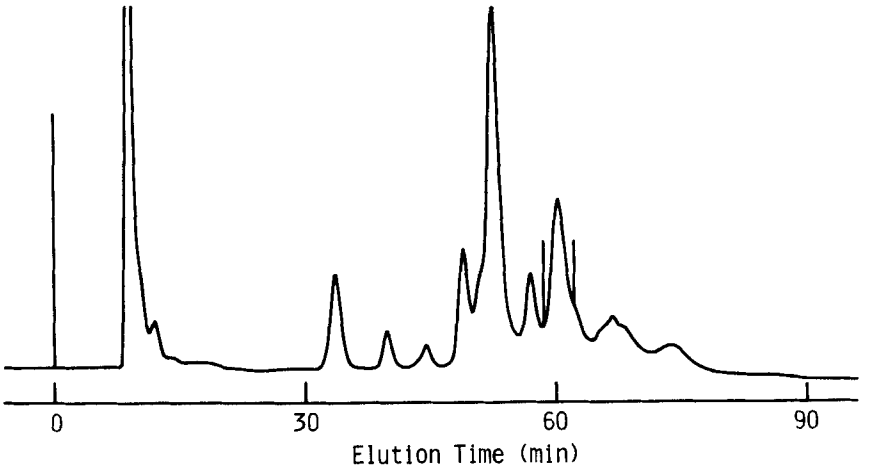

FIGURE 5 Separation of 500 mg phosphoglucose isomerase on the Phenyl-5PW preparative column of 200 x 55 mm I.D..

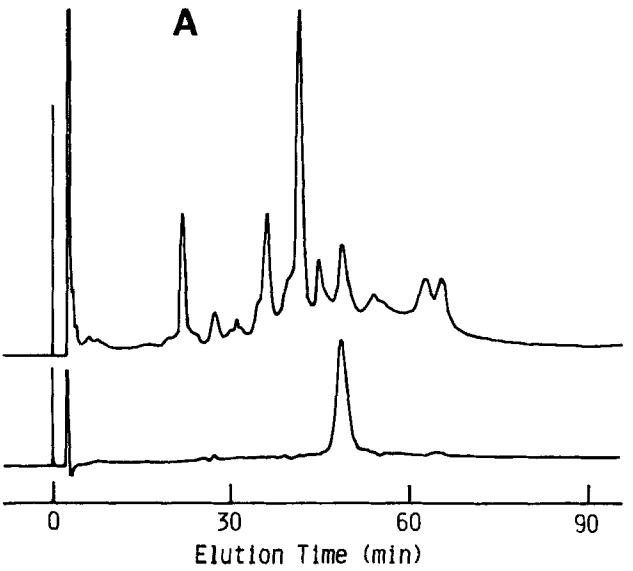

FIGURE 6 Chromatograms of crude phosphoglucose isomerase sample (upper curves) and the fraction in Fig. 5 (lower curves) obtained by hydrophobic interaction chromatography (A), reversed-phase chromatography (B) and gel filtration (C)

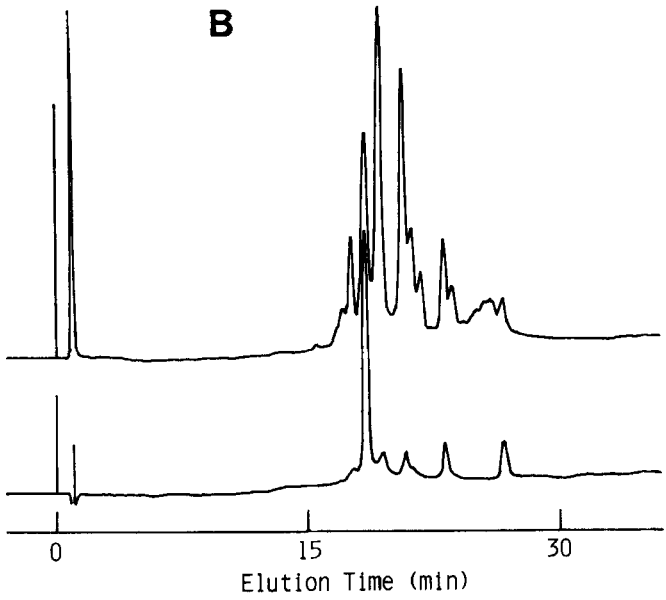

FIGURE 6B

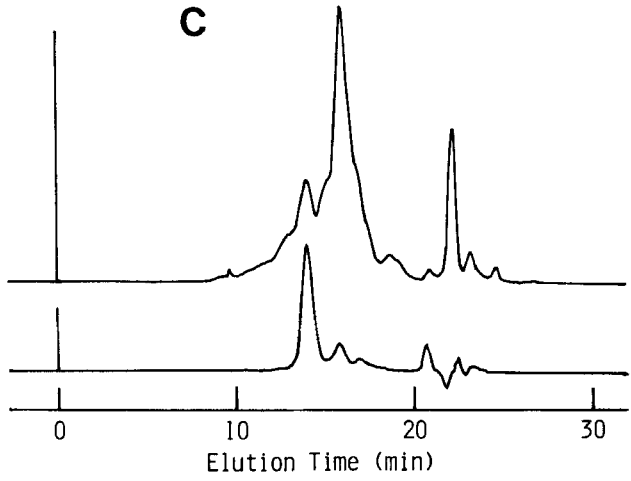

FIGURE 6C

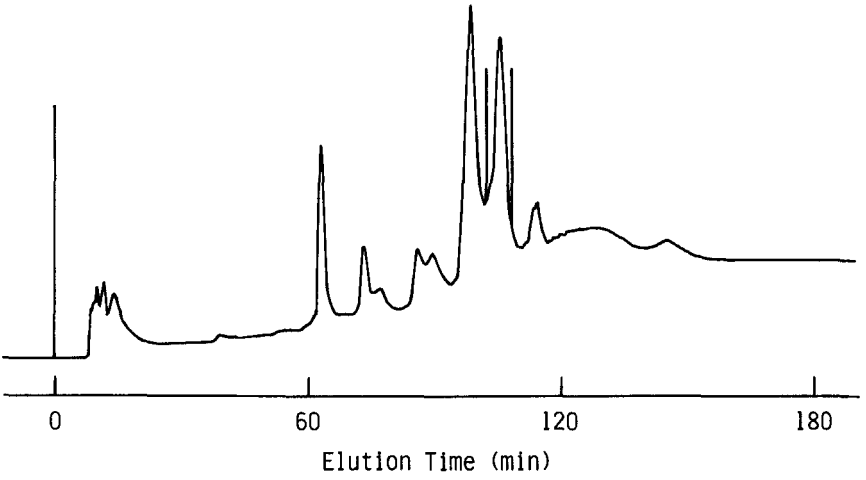

FIGURE 7 Separation of 200 mg lactate dehydrogenase on the Phenyl-5PW preparative column of 200 x 55 mm I.D..

recovery of enzymatic activity was almost quantitative. Fig. 6 shows the results of purity tests of this fraction. The pain peaks were confirmed to correspond to phosphoglucose isomerase by enzymatic activity test. This indicates that rather pure phosphoglucose isomerase was obtained.

Fig. 7 shows a separation of 200 mg crude lactate dehydrogenase. The peak corresponding to lactate dehydrogenase between the two vertical lines was fractioned. The recovery of enzymatic activity in the fraction was 82 %. The results of purity tests of the fraction are shown in Fig. 8. Lactate dehydrogenase was purified to a great extent.

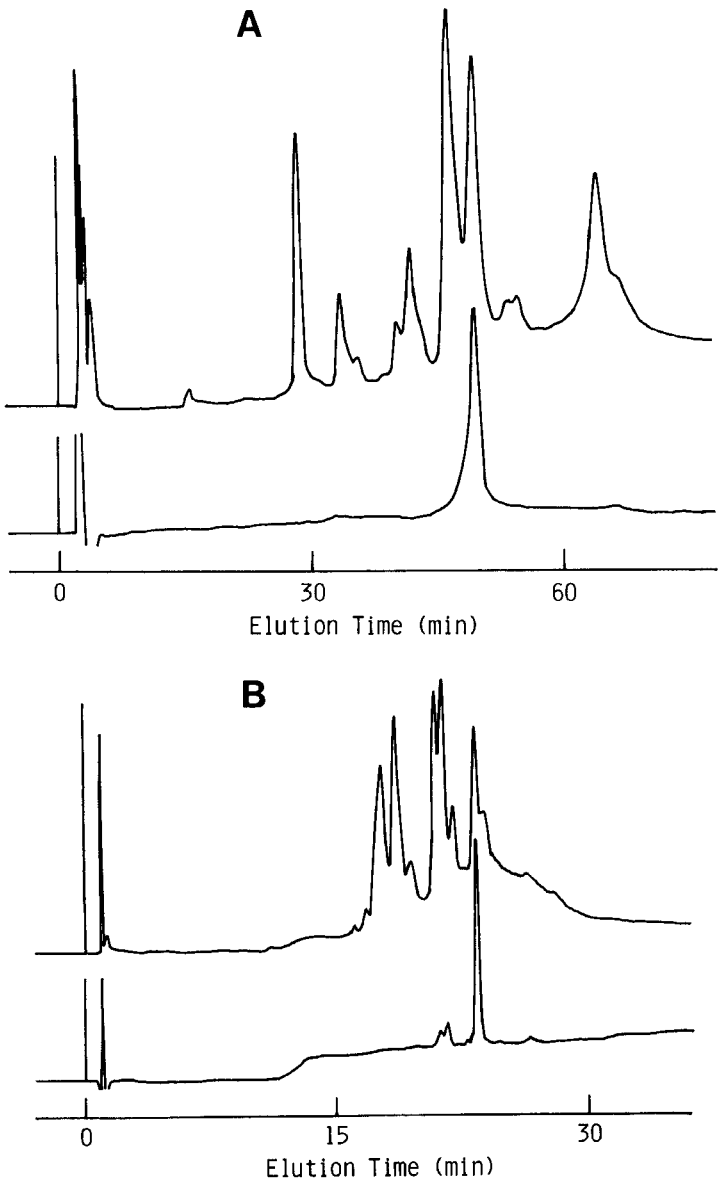

FIGURE 8 Chromatograms of crude lactate dehydrogenase sample (upper curves) and the fraction in Fig. 7 (lower curves) obtained by hydrophobic interaction chromatography (A), reversed-phase chromatography(B) and gel filtration (C).

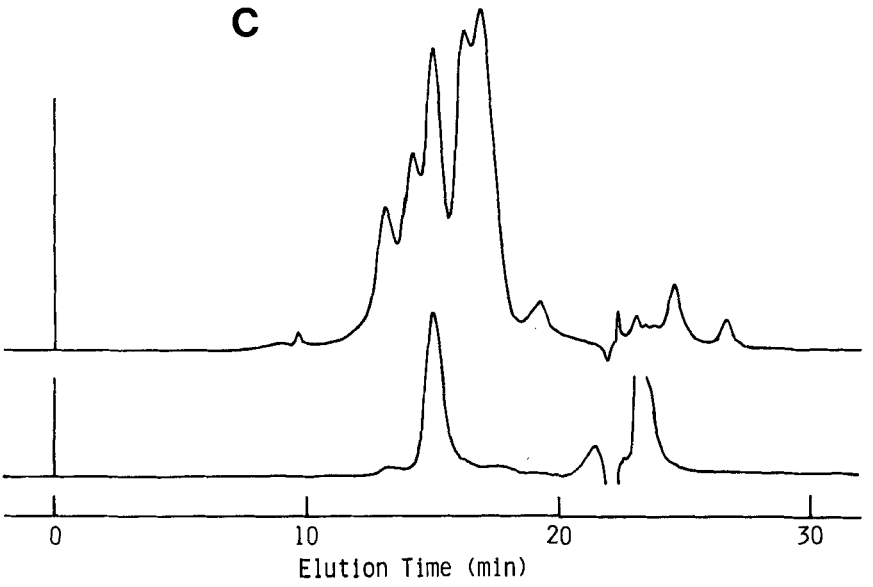

FIGURE 8C

As demonstrated above, a fairly high degree of purification was achieved with high yield in one step of hydrophobic interaction chromatography on the Phenyl-5PW preparative column. The sample loading capacity was 200 - 1000 mg per injection. Besides, substantially large amounts of samples can be treated by repeated injections. Typically, 2 - 20 g of crude sample can be treated in a day. Consequently, this hydrophobic interaction chromatography should be very useful as a technique for large scale purification of proteins.

REFERENCES

1. Kato, Y., Kitamura, T., and Hashimoto, T., High Performance Hydrophobic Interaction Chromatography of Proteins, J. Chromatogr., 266, 49, 1983.

2. Regnier, F. E., and Fausnaugh, J., High Performance Hydrophobic Interaction Chromatography of Proteins and Polynucleotides, LC Magazine, 1, 402, 1983.

3. Kato, Y., Kitamura, T., and Hashimoto, T., New Support for Hydrophobic Interaction Chromatography of Proteins, J. Chromatogr., 292, 418, 1984.

4. Gooding, D. L., Schmuck, M. N., and Gooding, K. M., Analysis of Proteins with New, Mildly Hydrophobic High Performance Liquid Chromatography Packing Materials, J. Chromatogr., 296, 107, 1984.

5. Kato, Y., Kitamura, T., and Hashimoto, T., Operational Variables in High-Performance Hydrophobic Interaction Chromatography of Proteins on TSKgel Phenyl-5PW, J. Chromatogr., 298, 407, 1984.

6. Goheen, S. C., and Engelhorn, S. C., Hydrophobic Interaction High-Performance Liquid Chromatography of Proteins, 317, 55, 1984.

7. Melander, W. R., Corradini, D., and Horváth, Cs., Salt-Mediated Retention of Proteins in Hydrophobic Interaction Chromatography, Application of Solvophobic Theory, J. Chromatogr., 317, 67, 1984.

8. Fausnaugh, L. A., Kennedy, L. A., and Regnier, F. E., Comparison of Hydrophobic Interaction and Reversed Phase Chromatography of Proteins, J. Chromatogr., 317, 141, 1984.

9. Fausnaugh, J. L., Pfannkoch, E., Gupta, S., and Regnier, F. E., High-Performance Hydrophobic Interaction Chromatography of Proteins, Anal. Biochem., 137, 464, 1984.

10. Miller, N. T., Feibush, B., and Karger, B. L., Wide Pore Silica-Based Ether-Bonded Phases for Separation of Proteins by High-Performance Hydrophobic Interaction and Size-Exclusion Chromatography, J. Chromatogr., 316, 519, 1985.

11. Chang, J.-P., Rassi, Z. E., and Horvath, Cs., Silica-Bound Polyethylene Glycol as Stationary Phase for Separation of Proteins by High-Performance Liquid Chromatography, J. Chromatogr., 319, 396, 1985.

12. Kato, Y., Kitamura, T., and Hashimoto, T., Preparative High-Performance Hydrophobic Interaction Chromatography of Proteins on TSKgel Phenyl-5PW, J. Chromatogr., 333, 202, 1985.

13. Miller, N. T., Feibush, B., Corina, K., Lee, S. P., and Karger, B. L., High-Performance Hydrophobic Interaction Chromatography: Purification of Rat Liver Carbamoylphosphate Synthetase I and Ornithine Transcarbamoylase, Anal. Biochem., 148, 510, 1985.

14. Tan, L., and Muto, N., HPLC of NADPH Cytochrome P-450 Reductase, LC Magazine, 3, 522, 1985.

15. Schmuck, M. N., Gooding, K. M., and Gooding, D. L., Hydrophobic Interaction Chromatography for Preparative Protein Purification, LC Magazine, 3, 814, 1985.

16. Bergmeyer, H. U., Grassl, M., and Walter, H.-E., Methods of Enzymatic Analysis, Vol. II, Bergmeyer, H. U., eds., 3rd ed., Verlag. Chemie, Weinheim, 1983, p. 126.