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Chiral separations using dextran and bovine serum albumin as run buffer additives in affinity capillary electrophoresis

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ABSTRACT

A method utilizing dextran as a run buffer additive in addition to bovine serum albumin (BSA) for chiral separation by means of affinity capillary electrophoresis (ACE) has been developed. By adding different amounts of dextran to the run buffer, the net velocity of BSA can be adjusted to a desired rate. Enantiomers of some drugs [ibuprofen (IB) and leucovorin (LV)] and amino acids (dansyl-DL-leucine and dansyl-DL-norvaline) were separated on a capillary with 20 cm effective length by this method. The effect of dextran concentration on the retention of BSA and resolution of sample enantiomers was studied. Enantiomers of mandelic acid (MA), which have very weak affinity for BSA, were also resolved. Qualitative information pertaining to the binding interaction of the samples with BSA can be obtained by this method.

INTRODUCTION

Many pharmaceutical drugs have asymmetric centers with most of them being used clinically in the racemic form. However, drug enantiomers can have quantitative or even qualitatively different physiological actions. With the improved understanding of the biological action of pharmaceutical drugs with respect to their stereochemistry, investigations concerning the pharmacology and toxicology of individual drug enantiomers have become more and more important. The pharmaceutical industry has, therefore, a tremendous interest in techniques for enantioseparations to investigate the optical purity of drugs. Some proteins, *e.g.* bovine serum albumin (BSA) [1–3], α-acid glycoprotein (α-AGP) [4,5], and ovomucoid [6,7], have been successfully used in high-performance liquid chromatography (HPLC) as stationary phases. However, protein immobilized columns in HPLC often show poor efficiency [8].

Capillary electrophoresis (CE), which has developed considerably during the past ten years, represents an attractive alternative method to HPLC in chiral separation due to its high efficiency and selectivity [9]. Electrokinetic chromatography (EKC), an extension of CE which was introduced by Terabe *et al.* [10], is also very useful in chiral separations. Early work applying the principle of ligand-exchange electrophoresis to CE was done by Zare *et al.* [11]. Cyclodextrin and their derivatives [12,13] as well as chiral crown ethers [14] have been widely used to form inclusion complexes with enantiomers in CE. Chiral resolution has been demonstrated in micellar EKC which employs an ionic micelle as a run buffer modifier [15–17]. Recently, a new method for the determination of the (6*R*) and (6*S*) stereoisomers of leucovorin (LV) using EKC in the affinity mode has been developed in our laboratory [18]. In this method, BSA was used as a run buffer additive to incorporate

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enantiomeric selectivity into the system. Excellent separation of LV isomers was obtained by this method. A major drawback of this method occurs when the net mobility of the sample is similar to that of the run buffer additive, resulting in a loss of enantiomeric resolution unless very long capillaries are employed.

Recently, the dynamic polymer sieving matrices which are called polymer networks, such as low concentration linear polyacrylamide and dextran solution have become a popular technique for DNA and protein separation [19–22]. Karger *et al.* [23] recently used a UV-transparent polymer network of dextran to substitute polyacrylamide with successful molecular mass sieving of SDS–protein complexes. One of the major advantages of using dextran is that the UV-absorbance of dextran is much lower than that of polyacrylamide [23].

In this paper, dextran was employed as a run buffer additive in addition to BSA for chiral separation by means of CE. The addition of different amounts of dextran to the run buffer, allows the net velocity of BSA to be controlled to a desired rate. Enantiomers of some drugs and amino acids, which are difficult to separate using BSA solely as the only buffer additive in CE, can be separated by this method. The effect of dextran concentration on the retention of BSA and the resolution of sample enantiomers is also discussed.

EXPERIMENTAL

Apparatus

The experiments were performed on a laboratory-constructed instrument using a CZE 1000 PN 30 high-power supply (Spellman, Plainview, NY, USA) and a high-power supply local control (Chamonix Industries, Binghamtom, NY, USA). The detector was a Spectra Physics Model 100 and was connected to a Spectra Physics 4400 integrator (Spectra Physics, Reno, NV, USA). Fused-silica capillary (75 μm I.D. × 360 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) was coated with linear polyacrylamide by the method reported by Hjerten [24]. The effective length of capillaries was 20 or 60 cm. The high voltage applied was 300 V/cm. Cooling was achieved by fan.

Procedure

Stock solutions of 5% dextran were made by dissolving dextran in run buffer (10 m*M* phosphate at pH 7.12). Lower concentration solutions were obtained by dilution. Buffer containing BSA (1 mg/ml) was made by dissolving BSA in buffer.

Samples were electrically injected (5 kV × 4 s) from the negative side, and detected at 210 or 230 nm.

Chemicals

Calcium leucovorin (LV) was obtained from Lederle Laboratories, American Cyanamid (Pear River, NY, USA). Ibuprofen (IB), mandelic acid (MA), dansyl-DL-leucine, dansyl-DL-norvaline, BSA and dextran (M_r 2 000 000) were purchased from Sigma (St. Louis, MO, USA). Acrylamide, N,N,N',N'-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) were obtained from Bio-Rad Labs. (Richmond, CA, USA). 3-Methacryloxypropyltrimethoxysiline was obtained from Hüls America (Bristol, PA, USA) and all other chemicals from Fisher (Fair Lawn, NJ, USA).

RESULTS AND DISCUSSION

Effect of dextran concentration on the retention of BSA and samples

The experiments describing the effect of dextran concentration on the retention of BSA, the various drug samples, and amino acids were performed under the electrophoretic conditions stated in the Experimental section. Fig. 1 shows the results. It can be seen that before the dextran is added to the run buffer, BSA is detected at 6.31 min. After 5% dextran is added, the retention time increases to 10.83 min, whereas the changes in retention time of the drug and amino acid samples are much smaller than that of BSA. The molecular mass of BSA (*ca.* 65 000) is much higher than that of the samples. The polymer network, which is the dextran solution in this experiment, has a much larger effect on the mobility of the large species (BSA in this case) when compared to the samples. Therefore migration of BSA decreases considerably upon the addition of dextran to the buffer, while the retention time of the low molecular mass sam-

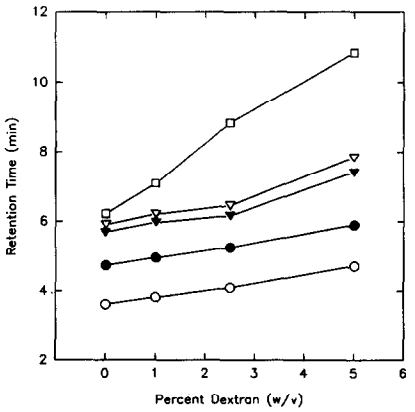

Fig. 1. Effect of dextran concentration on retention of BSA and samples. Conditions: linear polyacrylamide coated capillary with a 20 cm effective length; electric field strength, 300 V/cm; 10 m*M* phosphate buffer at pH 7.12; detection wavelength at 230 nm. ○ = leucovorin; ● = ibuprofen; ▼ = dansyl-DL-norvaline; ▽ = dansyl-DL-leucine; □ = BSA.

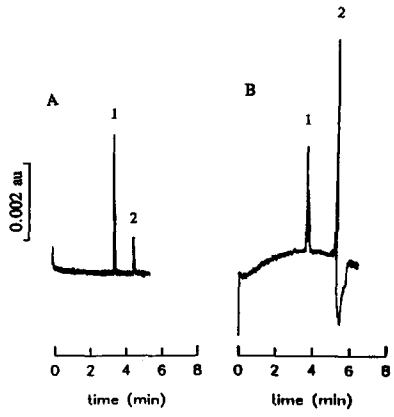

Fig. 2. Electropherograms of (1) LV and (2) IB mixture. CE conditions are the same as those of Fig. 1. (A) pH 7.12, 10 m*M* phosphate buffer; (B) pH 7.12, 10 m*M* phosphate buffer with BSA (1 mg/ml).

ples does not change much when compared to BSA.

By using the method developed in our laboratory recently [18], excellent chiral separation for LV was obtained by adding BSA to buffer. To obtain chiral resolution in a reasonable amount of time by this method, the difference between the velocities of BSA and the sample must be relatively large. For example, the velocity of dansyl-DL-leucine and dansyl-DL-norvaline are very close to that of BSA in the absence of dextran (Fig. 1), and chiral separation can not be achieved by this method even though these samples appear to have a strong affinity for BSA. By adding different percentages of dextran to the run buffer with BSA, one can slow down the velocity of BSA to the desired rate. In this way, the difference between the velocity of sample and BSA can be increased and chiral separation becomes feasible.

Chiral separation of drug and amino acid sample by using dextran and BSA as buffer additives

Fig. 2 shows the electropherograms for LV and IB. It can be seen that with the absence of BSA in the run buffer, both LV and IB elute as one peak. By using a run buffer modified with BSA (1 mg/ml), enantiomers of LV are separated to some degree while chiral separation of IB can not be obtained on a capillary with a relatively short effective length (20 cm). Interestingly the retention times of the isomers of LV do not change considerably when BSA is added to the run buffer. The retention time of IB experiences a change similar in magnitude to that of BSA, and the UV absorbance increases considerably for IB. This indicates that IB has a greater affinity for BSA than LV. After BSA has been added to the run buffer, an undefined negative peak appears following the peak of sample which has a strong affinity for BSA. The reason for this is not understood.

From Fig. 3 one can see that by adding dextran to the run buffer with BSA, optical enantiomers of both LV and IB can be resolved in 9 min on a capillary with an effective length of 20 cm. As the dextran concentration increases, the resolution of LV enantiomers increases and better chiral separation for IB is obtained. Again, it should be noted that the retention of IB changes considerably when the dextran polymer network is used because the velocity of BSA decreases largely when dextran is added to the run buffer and IB exhibits a strong interaction with BSA.

This new method can also be applied to the chiral separation of amino acids. Fig. 4 shows the chiral separation of dansyl-DL-leucine. With the addition of BSA alone to the run buffer, enantiomers of leucine cannot be resolved in the absence of dextran. A baseline separation of

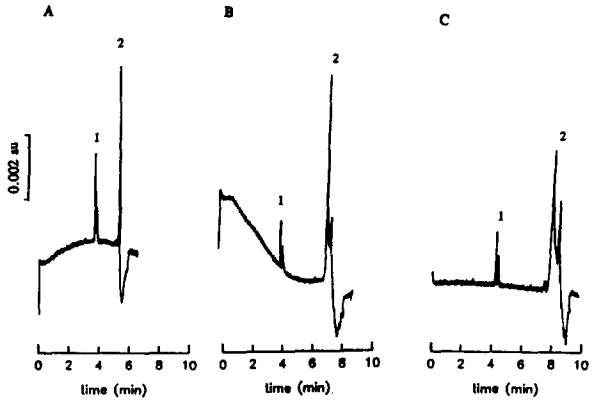

Fig. 3. Effect of dextran on chiral separation of (1) LV and (2) IB. Conditions are the same as those of Fig. 2B except different amount dextran was added to the buffer. (A) 0% Dextran, 1 mg BSA/ml; (B) 2.5% dextran, 1 mg BSA/ml; (C) 5% dextran, 1 mg BSA/ml.

leucine enantiomers is obtained when 5% dextran is added to the run buffer with BSA (1 mg/ml). In addition, it can be seen that dansyl-leucine has a strong affinity for BSA. Chiral separation of dansyl-DL-norvaline is also obtained by this method.

Fig. 5 illustrates the effect of dextran concentration of the run buffer on the retention of enantiomers for the different samples. In the absence of dextran in the run buffer, enantiomers of IB, leucine, and norvaline can not be separated using BSA (1 mg/ml in the run buffer). As the dextran concentration changes from 0 to 5% and the BSA concentration is kept constant, the difference between the retention of enantiomers for the samples increases. For LV the retention does not change much as the dextran concentration increases, since LV does not show a strong interaction with BSA. On the other hand, the retention times of IB, leucine, and norvaline evidently increase with the increase of dextran concentration because of their greater affinity for BSA. Therefore by using this method one can compare the binding ability of different drug samples qualitatively.

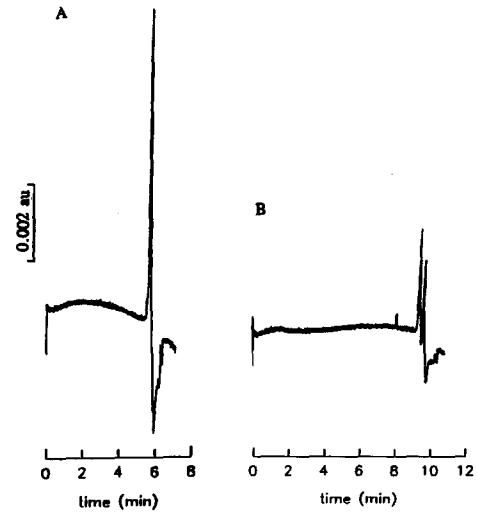

Fig. 4. Effect of dextran on the chiral separation of dansyl-DL-leucine. Conditions are the same as those of Fig. 2B except dextran was added to the buffer. (A) 0% Dextran, 1 mg BSA/ml; (B) 5% dextran, 1 mg BSA/ml.

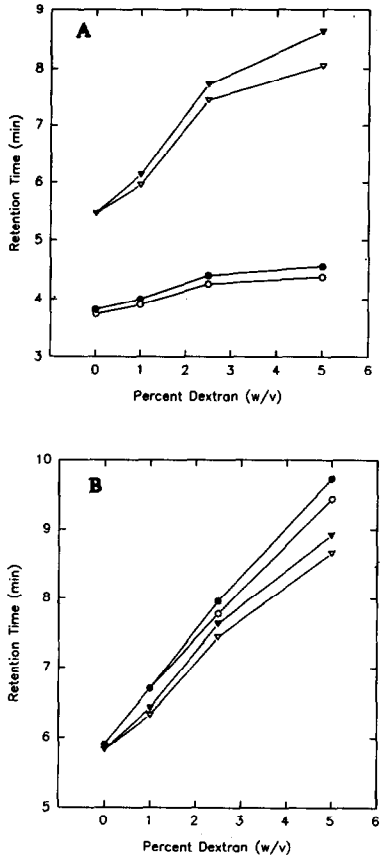

Fig. 5. Effect of dextran concentration on retention of enantiomers of drug and amino acid samples. (A) ○ and ●: enantiomers of LV; ▽ and ▼: enantiomers of IB. (B) ○ and ●: enantiomers of dansyl-DL-norvaline; ▽ and ▼: enantiomers of dansyl-DL-leucine.

Chiral separation of mandelic acid

When the sample, *e.g.* MA, has weak interaction with BSA, it is difficult to obtain chiral separation if only BSA is used as a run buffer additive, even in the case of using a capillary with a relatively long effective length of 60 cm (Fig. 6B). From Fig. 6 it can be seen that after BSA is added to a phosphate run buffer (1 mg BSA/ml), the retention of MA does not change considerably. This indicates that MA has a weak affinity for BSA at pH 7.12. After 5% dextran is added, the velocity of BSA decreases, the MA enantiomers interact with BSA more efficiently, and chiral separation of the MA isomers is achieved in 18 min.

Fig. 7 shows electropherograms of a LV and MA mixture. The mobilities of LV and MA are relatively close to each other in the free buffer system (Fig. 7A). If BSA is the only run buffer additive, the peaks of MA and LV overlap. Even though the enantiomers of LV can be separated

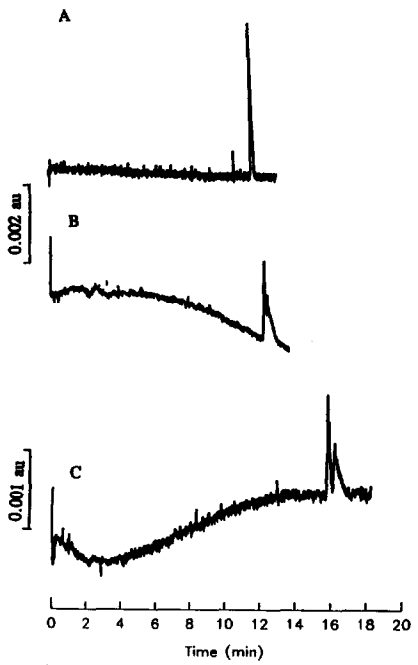

Fig. 6. Electropherograms of mandelic acid (MA). Conditions: linear polyacrylamide coated capillary with a 60 cm effective length; electric field strength, 300 V/cm; detection wavelength at 210 nm. (A) pH 7.12, 10 m*M* phosphate buffer; (B) pH 7.12, 10 m*M* phosphate buffer with BSA (1 mg/ml); (C) pH 7.12, 10 m*M* phosphate buffer with BSA (1 mg/ml) and 5% dextran.

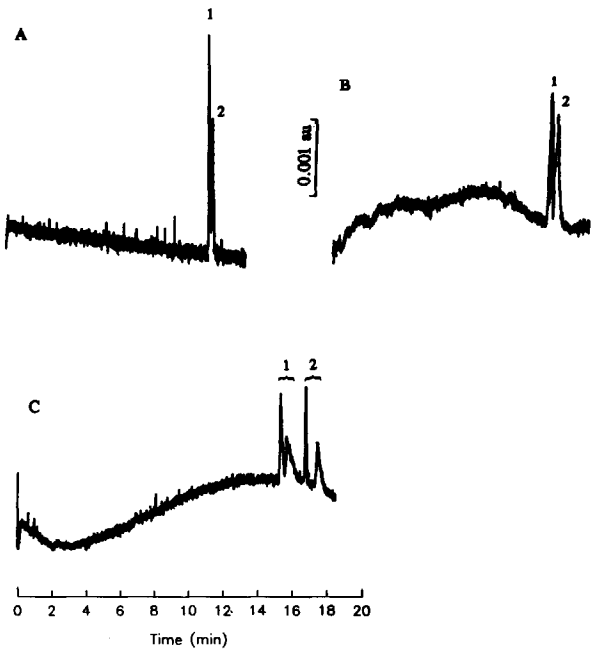

Fig. 7. Electropherograms of (1) MA and (2) LV mixture. Conditions are the same as those of Fig. 6. (A) pH 7.12, 10 m*M* phosphate buffer; (B) pH 7.12, 10 m*M* phosphate buffer with BSA (1 mg/ml); (C) pH 7.12, 10 m*M* phosphate buffer with BSA (1 mg/ml) and 5% dextran.

under these conditions, they elute with the MA sample. However, chiral separation of both MA and LV is obtained when 5% dextran is added to the run buffer with BSA. Since LV exhibits a stronger affinity for BSA than MA, the retention time of the LV enantiomers increases relative to that of the MA enantiomers when the velocity of BSA is lowered. The second peak in some of the enantiomeric pairs, such as in Fig. 7C, shows tailing. The second peak corresponds to the enantiomer with the stronger binding ability. The reason for this tailing is not well understood, but is currently under investigation in this lab.

CONCLUSION

The results show that a dextran polymer network is very useful for controlling the velocity of BSA for chiral separations. The enantiomers of samples which have mobilities similar to that of BSA or have very weak affinity for BSA are difficult to be separated by the method that

involves using only BSA as a run buffer additive. But the samples can be enantiomerically separated when dextran and BSA are used together as run buffer additives. The effect of dextran concentration on the velocity of BSA is much larger than the effect on the velocity of samples that have much lower molecular masses. The resolution of drug and amino acid enantiomers increases with the increase of dextran concentration in the run buffer when used in conjunction with BSA.

In this method the velocity of BSA can be controlled at the desired rate by adjusting the dextran concentration. The combination of this method with the method using BSA as a run buffer additive [18] and the protein immobilized method [25] recently developed in this lab should allow the technique using a biological species with high chiral selectivity (*e.g.* BSA) for chiral separation more general for CE.

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