Proc. Nat. Acad. Sci. USA
Vol. 70, No. 10, pp. 2850–2852, October 1973

Resolution of DL-Tryptophan by Affinity Chromatography on Bovine-Serum Albumin–Agarose Columns

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Communicated by Lyman C. Craig, June 27, 1973

ABSTRACT **Bovine-serum albumin, known to have antipodal specificity in the binding of tryptophan, was selected as the affinity chromatographic matrix for the attempted chromatographic resolution of DL-tryptophan. Complete resolution was accomplished when DL-tryptophan was chromatographed on bovine-serum albumin–succinoylaminoethyl–Sepharose.**

Resolution of optical isomers by conventional methods is frequently laborious and incomplete. The mechanical and chemical methods are empirical in nature, and in practice choosing the appropriate resolving agents and physical means of separation can be difficult. Resolutions based on highly specific biological properties have been limited to enzyme reactions, and these reactions always resulted in chemical modification of one of the isomers. Differential binding of some optical isomers by a few serum proteins has been reported. Examples include binding of DL-tryptophan to bovine-serum albumin (1) and *dl*-aldosterone to corticosteroid-binding globulin (2). It would seem that this property of steroselective binding of optical isomers by proteins could be used for resolution of some optical isomers. We have begun studies to investigate this possibility and report here the resolution of DL-tryptophan on affinity columns of bovine-serum albumin–succinoylaminoethyl–agarose.

EXPERIMENTAL MATERIALS AND PROCEDURES

L-Amino acid oxidase (Type 1) (EC 1.4.3.2) from *Crotalus adamanteus* venom, peroxidase (Type II) (horse radish; EC 1.11.1.7), Tris (free base), Sepharose 4B-200, and homovanillic acid were obtained from Sigma Chemical Co. Cyanogen bromide, ethylenediamine, succinic anhydride, and 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide were obtained from Eastman Chemical Co. Bovine-serum albumin (Cohn powder fraction V) was purchased from Schwarz–Mann and used either without further purification or after being defatted by the method of Chen (3).

The D, L, and the DL mixture of tryptophan were obtained from Merck and Co. L-Glutamic acid *N*-carboxyanhydride was a generous gift of Dr. J. M. Manning, who had obtained the reagent from Dr. D. F. Veber of Merck and Co.

Amino-acid analyses of the hydrolysates were performed by the method of Spackman, Stein, and Moore (4). D- and L-tryptophan contents were determined by the ion-exchange method of Manning and Moore (5), in which dipeptides are formed by reaction of the tryptophan with L-glutamic acid *N*-carboxyanhydride, the L-glutamyl-D-tryptophan is separated from the L-glutamyl-L-tryptophan by ion-exchange chromatography, and each diastereomer is quantitated. L-Tryptophan content was determined by the method of Guilbault and Hieserman (6) with L-amino-acid oxidase, and an Aminco fluoromicrophotometer with a CS 7–60 primary filter and a 47B, 2A combination secondary filter.

The substituted agarose supports were prepared according to Cuatrecasas (7) with cyanogen bromide. The extent of coupling was determined from amino-acid analyses of hydrolysates of the support material, and coupling efficiencies of 5–6% were normally obtained. Chromatographic columns were packed with either bovine-serum albumin linked directly to the agarose beads (bovine-serum albumin–agarose), bovine-serum albumin linked to the agarose beads by an ethylenediamine-succinic acid leash (bovine-serum albumin–succinoylaminoethyl–agarose), or defatted bovine-serum albumin linked to the agarose beads by the same leash (defatted bovine-serum albumin–succinoylaminoethyl–agarose). Control columns were packed with agarose or leashed agarose (no bovine-serum albumin) as appropriate.

RESULTS

Preliminary experiments in which bovine-serum albumin–agarose columns were used for chromatography of DL-tryptophan indicated that there was some resolution of the DL-tryptophan on these columns. However, these columns did not give reproducible results, due to the fact that the column material seemed to be altered during regeneration. In contrast, chromatography of DL-tryptophan on either of the two leashed column materials was reproducible, and hence these column materials were used in the remainder of the work.

The chromatographic pattern illustrated in Fig. 1 was observed when DL-tryptophan was applied to a column of defatted bovine-serum albumin–succinoylaminoethyl–agarose in pH 9.2 borate buffer containing 1% v/v $(CH_3)_2SO$, and the column was eluted with this buffer without $(CH_3)_2SO$ and then with 0.1 M acetic acid. Half the tryptophan was eluted with the borate buffer as one peak and the remainder was eluted with the breakthrough of the acetic acid. Assay of the material eluted with the buffer demonstrated that material in this peak contained only D-tryptophan. Only L-tryptophan was found when the material in the peak eluted with acetic acid was as-

Abbreviations: $(CH_3)_2SO$, dimethyl sulfoxide; Ve, elution volume of compound; Vo, void volume of column; Tris, tris-(hydroxymethyl)aminomethane.

TABLE 1. *Composition of material eluted from the defatted-bovine-serum albumin–succinoylaminoethyl–agarose columns*

	Borate peak		Acetic acid peak	
	Run 1	Run 2	Run 1	Run 2
Total tryptophan by A_{280nm} measurement (nmol)	200	227	262	236
Total L-tryptophan by L-amino-acid oxidase measurement (nmol)	0	0	248	208
% of recovered L-glutamyl-DL-tryptophan as L-glutamyl-L-tryptophan by Manning–Moore method*	0	0	100	100
% of recovered L-glutamyl-DL-tryptophan as L-glutamyl-D-tryptophan by Manning–Moore method*	100	100	0	0

* The average yield of L-glutamyl-tryptophan was 67% of total tryptophan applied to the column. Separate studies with L-, D-, and DL-tryptophan indicated that each isomer reacted with L-glutamic acid *N*-carboxyanhydride to the same extent under the conditions used.

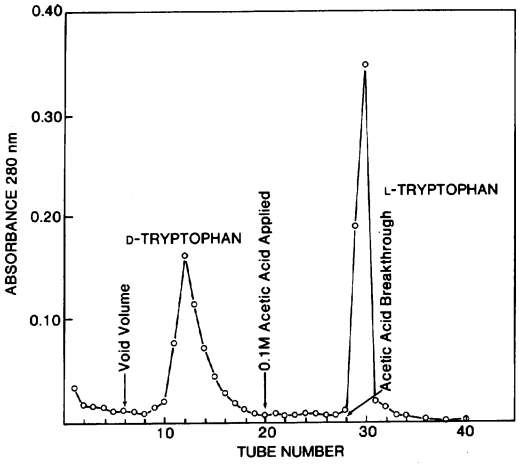

FIG. 1. Chromatography of DL-tryptophan on defatted bovine-serum albumin–succinoylaminoethyl–agarose. DL-tryptophan (500 nmol) dissolved in 0.1 ml of 0.1 M borate buffer (pH 9.2) containing 1% (v/v) $(CH_3)_2SO$, was applied to a 0.9 × 25-cm column of defatted bovine-serum albumin–succinoylaminoethyl–agarose. The column contained a total of 630 nmol of bovine-serum albumin. The column was eluted at 30 ml/hr with the borate buffer [no$(CH_3)_2SO$] for 20 tubes then with 0.1 N acetic acid. The void volume was determined from the elution volume of $(CH_3)_2SO$.

sayed (Table 1). Chromatography of DL-tryptophan on bovine-serum albumin–succinoylaminoethyl–agarose gave similar results, although the Ve/Vo for D-tryptophan was 1.6 compared with 1.8 on the defatted bovine-serum albumin–succinoylaminoethyl–agarose columns. These differences in the elution characteristics are compatible with the observed difference in the binding of tryptophan and tryptophan analogs to bovine-serum albumin and defatted bovine-serum albumin in solution (8). When DL-tryptophan was chromatographed on succinoylaminoethyl–agarose (no bovine-serum albumin) no resolution was observed, and the tryptophan eluted at the void volume.

DISCUSSION

Traditionally, affinity chromatography is considered to be an isolation technique for macromolecules such as enzymes, antibodies, and antigens. However, since the key concept in affinity chromatography is the use of specific biological interactions for separation of compounds (9), we feel that the term affinity chromatography should be extended to all chromatographic separations based on a specific biological interaction. Under this broader definition, resolution of DL-tryptophan on the bovine-serum albumin–succinoylaminoethyl–agarose columns is an example of affinity chromatography.

Recently there has been considerable interest in the use of chromatography for resolution of mixtures of chiral molecules. Studies of the use of optically active supports such as starch, wool, lactose, and cellulose (10–17) and, more recently, synthetic resin matrices (18–26) have yielded several successful resolution methods. Still, selection of the appropriate matrix required for resolution of a given pair of optical isomers is difficult and usually empirical. Selection of an appropriate matrix for resolution of *dl* pairs by affinity chromatography should be straightforward. The matrix should be formed from an immobilized biological material, which has been shown to have specific binding sites for the compound under study and which shows a differential binding constant for each optical antipode. The differential binding constants of bovine-serum albumin for D- and L-tryptophan have been reported (1) and, as shown here, these optical isomers were separated with ease. We believe that affinity chromatography will be an equally successful method for resolution of many other *dl* pairs.

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