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PARTIAL PURIFICATION OF A HIGH AFFINITY TAURINE BINDING PROTEIN BY AFFINITY CHROMATOGRAPHY*

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Taurine (2-aminoethanesulfonic acid) is a ubiquitous amino acid found in very high concentrations in mammalian tissues (1). The content of this β-amino acid appears to be altered in such disease states as heart failure, epilepsey and retinal degeneration (2-4). It has been proposed that taurine may function as a neuromodulator and regulator of ion transport by interacting at low affinity binding sites (5,6). The brain, retina, kidney, platelets and heart also possess high affinity taurine transport systems (7-11). Recently, high affinity taurine binding sites were identified, detergent solubilized, and shown to have affinities similar to the β-amino acid transport system (12-14).

In this communication, we present for the first time an affinity chromatographic method for the purification of a high affinity taurine transport protein from cardiac sarcolemma. This procedure may be applicable not only to the purification of taurine binding sites from brain, retina and platelets, but may also aid in the characterization of the biochemical and pharmacological actions of taurine in these tissues.

METHODS: Guanidinoethyl sulfonate agarose was prepared by incubating agarose hexanoic acid (P-L Biochemicals, Inc., Milwaukee, WI) with a 100-fold molar excess of 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide-metho-p-toluenesulfonate (Aldrich Chemical Co., Milwaukee, WI) and a 30-fold molar excess of guanidinoethyl sulfonate, the latter prepared according to the procedure of Morrison and coworkers (15). The solution was maintained at pH 4.5 throughout the procedure and was gently stirred for 24 hr at room temperature. Upon completion of the reaction, the affinity resin was thoroughly washed with distilled water and then equilibrated in 10 mM Tris-HCl, pH 8.0, containing 0.02% Ammonyx 4002 at 4°.

Solubilized porcine heart membrane was prepared according to the procedure of Kulakowski and coworkers (14). In this study, 2.0 ml of the Ammonyx 4002 solubilized membrane (2.0 mg protein/ml buffer) was applied to a column containing 1.0 ml of the affinity resin. The column was washed with 12.0 ml of 10 mM Tris-HCl, pH 8.0, containing 0.02% Ammonyx 4002 to remove any loosely associated proteins. The high affinity taurine binding protein was eluted from the column with buffer supplemented with 0.4 M taurine. Fractions (1.0 ml) were collected and assayed for taurine binding and protein content (A_{280}). Both high and low

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affinity taurine binding were determined using the standard polyethylene glycol method at [^{3}H]- and [^{14}C]taurine (New England Nuclear Corp., Boston, MA) concentrations of 0.12 and 3.5 mM taurine, respectively (14,16). Briefly, after 1 hr incubation polyethylene glycol and fraction 4 γ-globulin are added to precipitate the soluble proteins. Following 10 minutes on ice, the denatured protein is collected by vacuum filtration and counted for radioactivity. Estimation of protein content was determined according to the method of Lowry et al. (17) with bovine serum albumin as the standard.

Samples of the detergent solubilized protein and the extract from the guanidinoethyl sulfonate affinity column were chromatographed on Sepharose 6B. All samples were preincubated with 0.12 mM [^{3}H]taurine at 24^{o} for 1 hr and then were placed on ice for 10 min. Prior to preincubation, the taurine extract from the affinity column was dialyzed against buffer to remove taurine. The column was eluted with 10 mM Tris-HCl, pH 8.0, containing 0.02% Ammonyx 4002.

Porcine hearts were a gift of the A&B Meat Packers, Allentown, PA, while the Ammonyx 4002 was donated by the Onyx Chemical Co., Jersey City, NJ.

RESULTS AND DISCUSSION: Figure 1 shows the elution pattern obtained following application of the detergent solubilized cardiac sarcolemma protein to the affinity resin. Two peaks of taurine binding activity were obtained. Low affinity taurine binding was eluted in the early wash fractions and was associated with the major protein peak, while high affinity taurine binding could only be displaced from the column with buffer containing 0.4 M taurine. Since the high affinity sites (K_d=150 μM) have a greater affinity for taurine than the low affinity protein (K_d=3.5 mM) (14), it is not unreasonable to expect that the high affinity taurine binding sites would stick to the column preferentially.

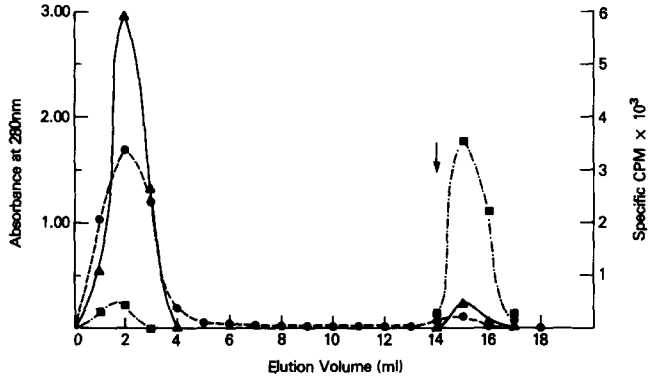

Fig. 1. Elution profile of solubilized porcine cardiac sarcolemma on guanidinoethyl sulfonate agarose. Key: (●---●) A_{280} nm; (▲——▲) low affinity taurine binding; and (■-·-■) high affinity taurine binding. In this experiment, 2.0 ml of solubilized membrane was added to a 1.0 ml column of affinity resin and allowed to react for 1 hr. The column was washed with 10 mM Tris-HCl, pH 8.0, containing 0.02% Ammonyx 4002. Taurine (0.4 M) in buffer was utilized to displace the high affinity binding sites. The eluted fractions were first dialyzed to remove taurine and then assayed using the polyethylene glycol method (14). Both high and low affinity taurine binding were determined at 0.12 and 3.5 mM radiolabeled taurine respectively.

As seen in Fig. 2, gel permeation of the solubilized heart cell membrane extract on Sepharose 6B leads to two peaks of taurine binding activity. The elution of the free taurine is represented by the third peak. The high molecular weight fraction was reduced when pooled samples eluted from the affinity column were applied to the Sepharose 6B column. Previous studies have shown that the low and high affinity taurine binding sites correspond to the high and low molecular weight fractions respectively (14).

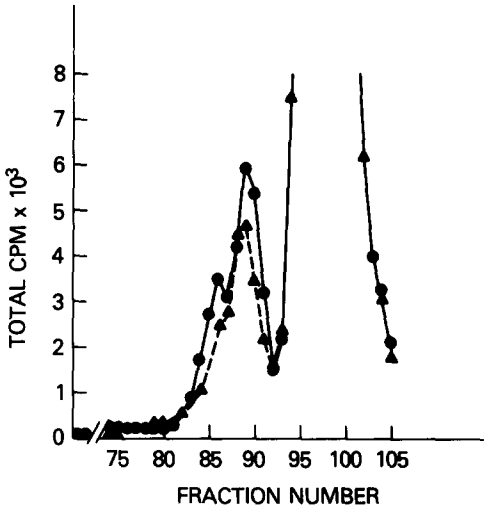

Fig. 2. Sepharose 6B chromatography of the solubilized cardiac sarcolemma (●——●) and the post-affinity column extract (▲---▲). The samples, prepared as described in Methods, were applied to the Sepharose 6B column (113 cm x 1.5 cm, bed volume 68 ml) and eluted with 10 mM Tris-HCl, pH 8.0, containing 0.02% Ammonyx 4002 at 4°. Fractions (2.2 ml) were collected, and aliquots were counted in Bray's solution for radioactivity. The remainder was concentrated, dialyzed to remove taurine, and assayed for taurine binding.

The degree of purification achieved for each procedure is shown in Table 1. Use of the guanidinoethyl sulfonate affinity chromatography method resulted in an overall purification of the high affinity protein of 44-fold. When Fraction 89 was assayed for taurine binding, 3.2 nmoles of taurine were bound per mg protein. This increased activity is 66 times greater than that of the crude homogenate.

This is the first reported attempt to purify a taurine binding protein. Taurine has been shown to interact non-cooperatively with a high affinity binding site (14). The proposed bimolecular reaction for taurine assumes that one molecule of taurine binds per mg binding protein (molecular weight 110,000) (14). Theoretically, we calculate that a purified binding protein would bind 9 nmoles taurine/mg binding protein. Chromatographic separation of the affinity purified binding protein on Sepharose 6B yielded a fraction which bound 3 nmoles taurine/mg binding protein. Therefore, less than a 3-fold purification is necessary

to yield a purified membrane bound taurine binding protein. Complete purification, subunit structure, reconstitution of purified taurine transport system, and physical and chemical characterization of the taurine binding protein await large scale purification.

Table 1. Purification of high affinity taurine binding sites by affinity chromatography*

Fraction	Specific binding (nmoles bound/mg protein)	Degree of purification
Crude Membrane	0.050	1.0
Solubilized Extract	0.085	1.7
Post Affinity	2.2	44.0
Post Sepharose 6B	3.2	64.0

* The crude membrane preparation was assayed for taurine binding by the method of Kulakowski et al. (13) with other fractions determined by the polyethylene glycol method (14,16). Assay conditions are described in the legend of Fig. 1.

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