Identification, purification,and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol

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Abstract Tocopherol binding activity accompanying a rat liver cytosolic protein with molecular weight of 30–36 kDa has been demonstrated previously, although the isolation of the protein has not been reported. We now report the purification of an α-tocopherol-binding protein (TBP) from rat liver cytosol utilizing three chromatographic procedures: gel filtration, Affi-Gel Blue affinity chromatography, and chromatofocusing. Three peaks of specific α-tocopherol-binding activity were resolved on Affi-Gel Blue, referred to as AFB-1A, 1B, and 2. A 32-kDa homogeneous form was obtained after chromatofocusing of AFB-1B. D-α-[^{3}H]tocopherol was displaced from homogeneous TBP in the presence of 500-fold excess of nonlabeled α-tocopherol, indicating the specificity of the binding. Anti-TBP rabbit antisera identified only one protein in rat hepatic cytosol on Western blotting. TBP immunoreactivity was found in the cytosol of rat liver and the lysate of fractionated hepatocytes, but not in the cytosol of other organs (including the heart, spleen, testes, and lung) nor in the lysate of fractioned Ito cells, endothelial cells, or Kupffer cells isolated from rat liver. Semi-quantitative ELISA demonstrated that rat liver cytosol contained approximately 2 mg TBP/g of cytosol protein. This immunoreactivity was associated with only the 30–36 kDa gel filtration fractions of rat liver cytosol and with both AFB-1A and -1B but not with AFB-2.—**Yoshida, H., M. Yusin, I. Ren, J. Kuhlenkamp, T. Hirano, A. Stolz, and N. Kaplowitz.** Identification, purification, and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol. *J. Lipid Res.* 1992. **33:** 343–350.

Supplementary key words gel filtration • Affi-Gel blue • affinity chromatography • chromatofocusing • vitamin E

Tocopherol (vitamin E) plays an important role in the scavenging of radicals and the preservation of membrane integrity (1). It is a highly lipophilic substance which travels in the circulation in association with lipoproteins (2). Binding of α-tocopherol with lipoprotein in rat liver cytosol was reported by Rajaram, Fatterpaker, and Sreenivasan in 1973 (3). In 1975, Catignani (4) and Catignani and Bieri (5) reported that a 31-kDa gel filtration fraction from rat liver cytosol, which was different from lipoproteins, specifically bound α-tocopherol with high affinity. Murphy and Mavis (6) demonstrated that a gel filtration fraction of 34 kDa from rat liver cytosol was able to transfer α-tocopherol from liposomes to microsomes and that this activity was present only in the liver. Mowri et al. (7) also reported that a pooled liver cytosolic protein of 30 kDa was capable of transferring the vitamin from liposomes to mitochondria, although they claimed that the activity was present in liver, heart, spleen, and lung. Behrens and Madere (8) demonstrated that [^{3}H]tocopherol administered in vivo bound to a liver cytosolic protein of 32 kDa; they partially purified it using ammonium sulfate fractionation, gel filtration, and ion exchange chromatography (9). Transfer of α-tocopherol between membranes mediated by this fraction was further investigated by Verdon and Blumberg (10). We have previously reported a preliminary approach to the purification of TBP (11), which produced insufficient material for either immunization or further characterization. In the present report we have purified sufficient protein to establish an immunoassay and have performed immunochemical characterization of the forms, localization, and quantity of TBP.

MATERIALS AND METHODS

D-α-[5-methyl-^{3}H]tocopherol (8.3 Ci/mmol, Amersham, Arlington Heights, IL) was purified on an Ultrasil-ODS HPLC column using methanol–water 98:2(v/v) as the mobile phase. HPLC fractions with

Abbreviations: TBP, α-tocopherol-binding protein; HPLC, high performance liquid chromatography; PBS, phosphate-buffered saline; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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the most radioactivity (10^4 cpm/μl) were stored at –80°C before use, and are referred to as [^{3}H]tocopherol solution. Radioactivity was measured by a Beckman LS-3150T liquid scintillation counter using Aquasol-2 (New England Nuclear, Boston, MA). Quantitative assay of protein concentration was performed with the Bio-Rad Protein Assay Kit (Bio-Rad) with appropriate dilution of the sample, and standardized against bovine serum albumin. All chemicals used were of analytical grade.

Purification of tocopherol binding protein (TBP)

Preparation of 30–36 kDa fraction. Liver cytosol was obtained from 33% (w/v) homogenate of pre-perfused male Sprague-Dawley rats (300–400 g) with 0.25 M sucrose 0.01 M sodium phosphate (pH 7.4) by ultracentrifugation at 100,000 *g* for 60 min. Eighty ml of cytosol from five rats (per run) was mixed with 80 μl of [^{3}H]tocopherol solution, incubated with stirring for 60 min at 4°C, and eluted on a Sephacryl S200 (Pharmacia, Uppsala, Sweden) gel column (6 × 90 cm) with 0.01 M sodium phosphate (pH 7.4). Ten-ml fractions were collected and radioactivity and A_{280} were determined. The fractions in the second elution peak of radioactivity, corresponding to proteins with molecular weights of 30,000 to 36,000, were pooled (90 ml/run) and designated as the Y′ fraction (11). The first peak, corresponding to the void volume, did not bind tocopherol specifically (11) and was discarded.

Affi-Gel Blue affinity chromatography. One hundred eighty ml of pooled Y′ fraction from 10 male rat livers was mixed with 100 μl of [^{3}H]tocopherol solution, incubated for 1 h, and eluted on an Affi-Gel Blue (Bio-Rad, Richmond, CA) column (3 × 40 cm) with 0.01 M sodium phosphate (pH 7.4) at 4°C. The addition of radioactivity was necessary due to dilution from the preceding step. The column was eluted with a linear NaCl gradient by mixing 400 ml of the initial buffer and the same volume of 0.55 M sodium chloride, 0.01 M sodium phosphate containing 2 mM EDTA (pH 7.4). After completion of the gradient, the column was eluted with 5 M sodium chloride, 0.01 M sodium phosphate (pH 7.4). Four-ml fractions were collected and monitored for radioactivity, A_{280}, and conductivity. The fractions corresponding to each peak of radioactivity were pooled. Each pool was separately dialyzed in Spectropor membrane (cut-off molecular weight 12,000) against 2 l 0.05 M Tris-HC1 (pH 7.5) and concentrated to about 5 ml by ultrafiltration using Diaflo YM-10 membrane (Amicon, Danvers, MA). Liver cytosol from confirmed tocopherol-deficient rats was kindly provided by Dr. R. Sokol, University of Colorado, and examined for TBP activity by elution of ^{3}H-tocopherol from gel filtration and Affi-Gel Blue chromatography as described above.

Chromatofocusing. Preliminary experiments revealed that under acidic conditions below pH 6.5, TBP irreversibly lost tocopherol-binding capacity but this could be prevented by the presence of 0.02% (v/v) Triton X-100. A Mono-P HR 5/20 chromatofocusing FPLC column (Pharmacia) was equilibrated with 0.025 M bis Tris-HC1 containing 0.02% Triton X-100, pH 6.4, at 4°C. Pooled peaks from Affi-Gel Blue chromatography were applied to the column, and eluted with the same buffer followed by Polybuffer 74 (diluted 1:10 with distilled water) containing 0.02% Triton-X-100, pH 4.0, using an Altex single pump HPLC. The eluate was collected in 1.0-ml fractions, immediately monitored for pH, and then neutralized with 0.5 M sodium phosphate, pH 7.8.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional (2-D) gel electrophoresis

The homogeneity of protein was examined by SDS-PAGE performed on vertical slab gels according to Laemmli (12), using 3.0% and 12.5% polyacrylamide for the stacking and resolving gels, respectively. The gels were silver-stained according to Wray et al. (13). Two-dimensional gel electrophoresis was performed according to the manufacturers instructions (Bio-Rad, Richmond, CA). The first dimension consisted of isoelectric focusing with a pH 3–10 gradient followed by SDS-PAGE in the second dimension.

Specific binding of tocopherol

Samples of cytosol or homogeneous TBP were incubated with [^{3}H]tocopherol for 2 h at room temperature, and then fractionated by gel filtration on Superose 12 HR 10/30 FPLC column (Pharmacia) eluted with 0.01 mM sodium phosphate, 0.15 M sodium chloride (flow rate 0.5 ml/min) at pH 7.4 (PBS) at 4°C. When [^{3}H]tocopherol was detected in the 30–36 kDa gel filtration fractions, specific tocopherol binding was examined under the same conditions after incubation for 2 h with a 500-fold excess of nonlabeled D-α-tocopherol. Displacement of the label was considered specific binding.

Immunochemical studies

Immunization. The TBP fraction on chromatofocusing, when proven to be homogeneous on SDS-PAGE, was injected subcutaneously into female New Zealand White rabbits. Since the purified TBP had a tendency to be unstable after prolonged storage, TBP was repurified on SDS-PAGE prior to immunization. After Coomassie-Blue staining, the portion of the gel containing protein identical to that of fresh TBP was cut out. The gel containing about 20 μg of TBP was homogenized in PBS (1:1 vol) and mixed with Freund's ad-

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juvant (1:1 v/v). The injection procedure was repeated four times at 2-week intervals and the animals were bled before each immunization and 2 weeks after the last immunization.

Western blotting. The titration of anti-TBP antisera and the detection of TBP immunoreactivity were performed on nitrocellulose membrane after SDS-PAGE or 2-D electrophoresis and electroblotting (14). The membrane was incubated with diluted (1:400 to 1:1000) anti-TBP antisera, followed by anti-rabbit IgG antibody-alkaline phosphatase conjugate and its substrate according to manufacturers recommendation (Promega, Madison, WI).

Titration of TBP on microtiter plates. Fifty-μl samples, adequately diluted with PBS, were placed in polystyrene microtiter plate wells. Nonspecific binding sites were blocked by 0.1% bovine serum albumin–PBS solution. The plated antigen was incubated subsequently with anti-TBP antisera, anti-rabbit IgG antibody-horse radish peroxidase conjugate, and peroxidase substrates (Bio-Rad), and washed between incubations with PBS; A_{415} was measured with Bio-Rad Model 3550 microplate reader. The concentration of TBP was calculated compared to TBP standards using the linear portion of the standard curve.

TBP in other samples

Cytosol was prepared from the kidney, spleen, adrenal gland, testis, lung, stomach, intestines, heart, skeletal muscle, and brain of three rats, according to the method described above except that these organs were not perfused prior to the homogenization. Serum was obtained from blood drawn from the aorta. Plasma obtained from rats after collecting blood in EDTA (1.0 mg/ml final concentration) was also examined along with VLDL, HDL, and LDL prepared by the density gradient method of Hatch (15). Fractionated rat liver cells, namely hepatocytes, Ito cells, endothelial cells, and Kupffer cells, were kindly supplied by Drs. Joe Roll and Scott Freidman, Liver Center Core Laboratory for Cell Fractionation and Culture, University of California San Francisco, and were lysed with 10% Triton X-100. Human liver cytosol was obtained from three histologically normal human livers of organ donors, which were stored at −70°C prior to use. The specific binding of α-tocopherol in the 30–36 kDa gel filtration fraction of human hepatic cytosol prepared from these samples and the immunoreactivity with anti-rat TBP antibody were examined as described above.

Determination of amino acid composition

Two hundred fifty μl of pooled tocopherol binding protein after chromatofocusing was isolated from the polybuffer by reverse phase chromatography on a microbore Vydac C 18 column (2.1 mm × 15 cm) eluted over 60 min with a linear gradient from buffer A (0.1% trifluoroacetic acid) to buffer B (0.1% trifluoroacetic acid–90% acetonitrile). Thirty two kDa protein was lyophilized to dryness and amino acid hydrolysis was performed as described by Ronk et al. (16). The amino acid analysis was performed twice with 1 μg of protein and the composition was calculated assuming a molecular weight of 32,000. The amino acid analysis was performed in the laboratory of John E. Shively, Beckman Research Institute of the City of Hope, Duarte, CA, as previously described (16).

Metabolism of tocopherol

Cytosol (12 mg protein) containing 0.5 μCi [^{3}H]tocopherol was incubated in 0.5 ml phosphate buffer for 1 h at 37°C without or with NAD^+ 0.8 mM plus NADH 0.2 mM, ATP 1 mM, NADP 0.02 mM, NADPH 0.4 mM, PAPS 2.25 mM, and 5 mM GSH either individually or all together. The mixtures were extracted with an equal volume of butanol (≥ 98% label extracted) and analyzed by reverse phase HPLC as described above; fractions were collected for radioactivity and compared to the elution of [^{3}H]tocopherol standard.

RESULTS

Purification of TBP

When rat liver cytosol incubated with [^{3}H]tocopherol was eluted on a gel filtration column, there were two separate peaks of radioactivity, one corresponding to the molecular mass of 30–40 kDa (Y′ fraction) and the other eluting in the void volume as previously reported (4–11). When aliquots of both peaks were eluted on Superose 12 gel filtration column, only the 30-36 kDa fraction showed specific binding of [^{3}H]tocopherol as determined by displacement by excess nonlabeled α-tocopherol (not shown).

Affi-Gel Blue affinity chromatography of the 30–36 kDa fraction from gel filtration revealed three [^{3}H]tocopherol peaks in the conductivity range from 3 to 16 μS/cm (**Fig. 1**). They were designated AFB-1A, AFB-1B, and AFB-2 in order of increasing elution conductivity. The AFB-1A and 1B peaks were not resolved in our previous study which used a steeper elution gradient. Thus, we first identified AFB-1 and subsequently resolved this into AFB-1A and AFB-1B. Although radioactivity also eluted in fractions with much higher conductivity in the high salt wash (AFB-3), this radioactivity was not displaceable and was not pursued further. However, AFB-1A, 1B, and 2, when individually examined on Superose 12, each exhibited specific, displaceable tocopherol binding.

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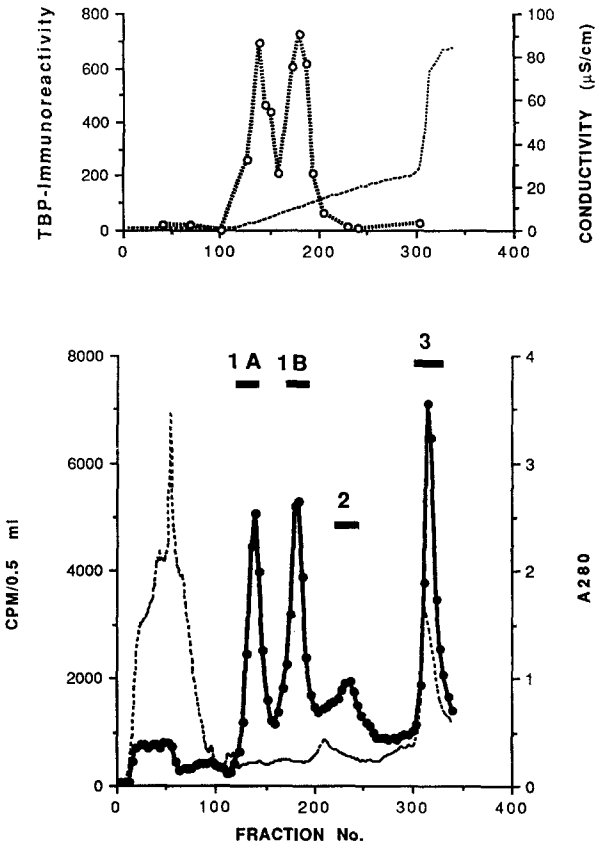

Fig. 1. Affi-Gel Blue affinity chromatography of the Y′ fraction of rat liver cytosol. The lower panel shows elution of [^{3}H]α-tocopherol (closed circles) and absorbance at 280 nm (dotted line). The upper panel shows elution of immunoreactivity with anti-TBP antisera (open circles, arbitrary unit) and conductivity (dotted line). The fractions pooled for further purification are indicated by horizontal bars and are designated AFB-1A, 1B, 2, and 3. Using steeper conductivity gradients AFB-1A and AFB-1B appeared as a single peak (AFB-1), accounting for the nomenclature that was used.

Chromatofocusing of separately pooled AFB-1A and AFB-1B each showed a peak of radioactivity in fractions eluting around pH 5.3 (**Fig. 2**). In each case, two peaks of radioactivity were observed. The first was not associated with protein and appeared only when Triton X-100 was used; the second corresponded to TBP. [^{3}H]tocopherol also eluted from the column with high salt wash (not shown). The fraction with peak radioactivity from the chromatofocusing of AFB-1B (Fig. 2B) revealed a single band on SDS-PAGE with silver staining, corresponding to molecular mass of 32 Kda (**Fig. 3**). This fraction, when applied to a Superose 12 gel filtration column, eluted in the 30–36 kDa fraction and tocopherol-binding was displaced with 500-fold excess of nonlabeled α-tocopherol (**Fig. 4**). This homogeneous fraction was used to immunize rabbits as described in Materials and Methods and to determine amino acid composition (**Table 1**). The peak fraction from the chromatofocusing of AFB-1A (Fig. 2A) also revealed a dominant protein with identical molecular weight along with other impurities (not shown).

The chromatofocusing of AFB-2 is shown in Fig. 2C. Two radioactivity peaks were eluted. The first was not associated with protein as above and the second eluted at a pH slightly higher than 5.3. The SDS-PAGE of this peak fraction revealed a major band with molecular mass of 40 kDa and no band at 32 kDa (not shown). However, tocopherol-binding activity of this fraction was found to be very unstable and was thus not further purified.

Affi-Gel Blue chromatography of Y′ fraction of hepatic cytosol from tocopherol-deficient rats revealed a nearly identical pattern of elution of [^{3}H]tocopherol-binding peaks (not shown).

We attempted to determine whether [^{3}H]tocopherol's retention time on reverse phase HPLC might change to reflect metabolism to a more polar product when incubated with cytosol containing a variety of natural cofactors. However, no detectable metabolism of tocopherol was observed under these conditions. Therefore, we have no evidence that TBP is involved in the biotransformation of tocopherol.

Immunochemical studies

The reactivity of the antisera with both rat liver cytosol and the purified TBP (after chromatofocusing of AFB-1B) was examined by Western blotting. The antisera, diluted 1:1000, identified only a single protein of molecular mass 32 kDa in both samples (**Fig. 5**), indicating the specificity of the antisera. Western blot of 2-D electrophoresis of rat liver cytosol revealed two positive staining bands of the same molecular weight (not shown).

The TBP immunoreactivity of individual fractions from gel filtration, Affi-Gel Blue chromatography, and chromatofocusing was semi-quantitatively examined using the microtiter plate method described above. Fractions on Superose 12 gel filtration of rat liver cytosol revealed one peak of TBP immunoreactivity in the 30–36 kDa fractions corresponding to the elution peak of [^{3}H]tocopherol binding (**Fig. 6**). Although there was another peak of radioactivity in the void volume fractions, it contained no immunoreactivity.

Of the four radioactivity peaks on the Affi-Gel Blue chromatogram, only AFB-1A and AFB-1B contained TBP immunoreactivity. The elution of TBP immunoreactivity corresponded to elution of [^{3}H]tocopherol binding (Fig. 1). Neither AFB-2 nor AFB-3 demonstrated TBP immunoreactivity. In chromatofocusing of AFB-1A and AFB-1B, the elution of immunoreactivity corresponded with a single peak of [^{3}H]tocopherol binding activity (Figs. 2A and 2B).

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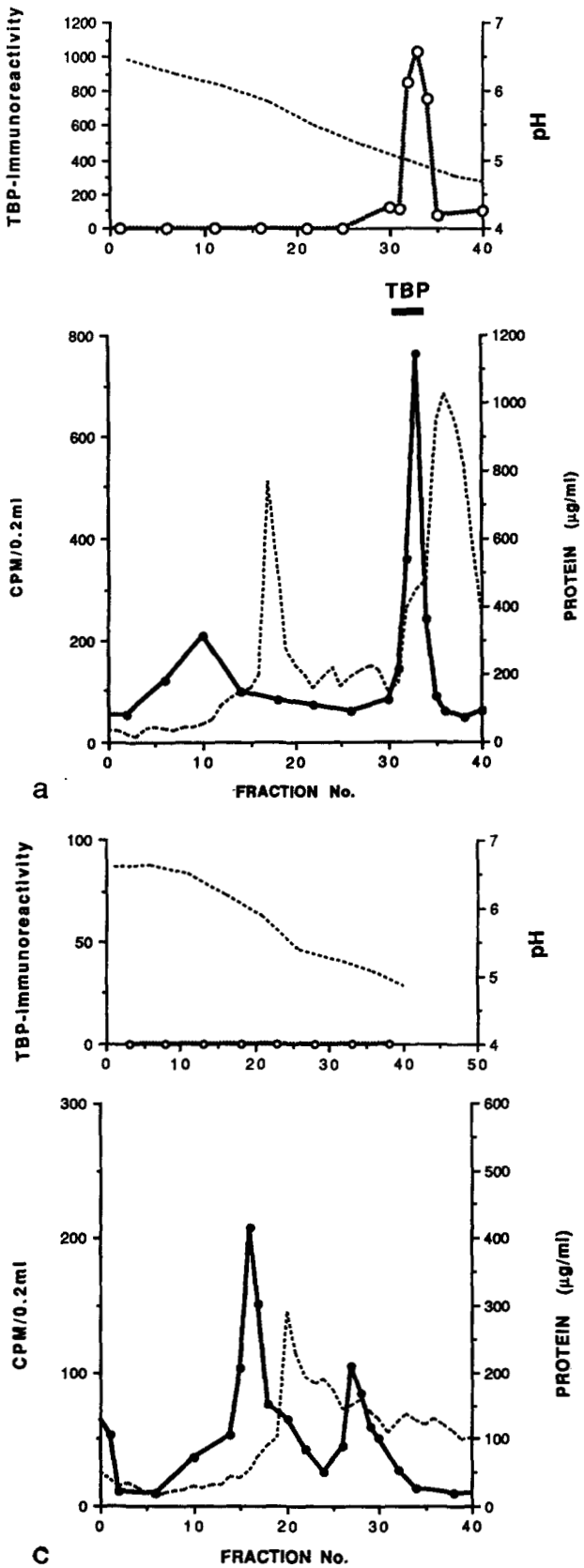

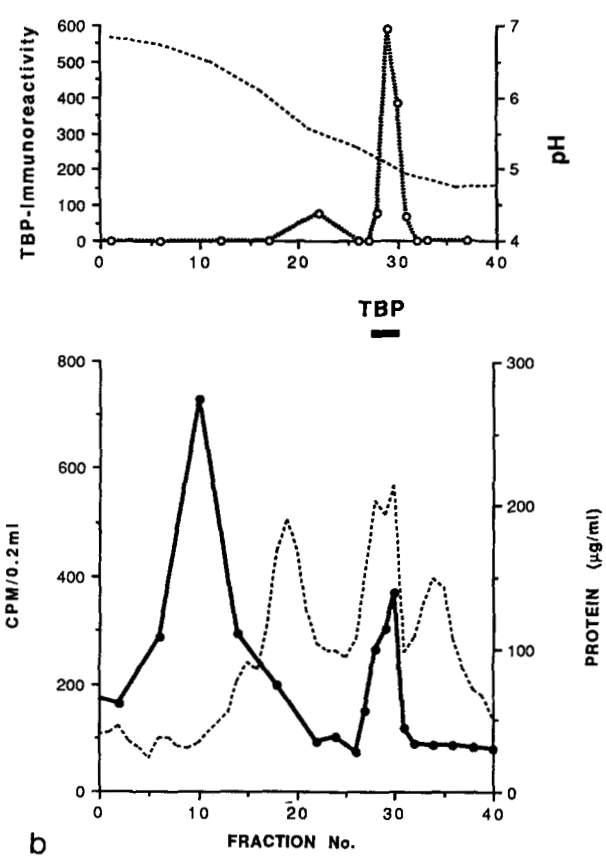

Fig. 2. Chromatofocusing of AFB-1A (a), 1B (b), and 2 (c). The lower panels show elution of [^{3}H]α-tocopherol (closed circles) and protein (dotted line). The upper panels show pH gradient (dotted line) and elution of immunoreactivity (open circles).

Table 2 summarizes the purification and yield of TBP. The TBP immunoreactivity represents 0.23% of the total cytosol protein. It is apparent that significant losses are due to instability of the protein during the purification procedures as well as loss from the selective pooling of only peak fractions.

Localization of TBP

On Superose 12 gel filtration, rat serum showed elution of tocopherol in the range of 60–80 kDa. Cytosol of organs other than the liver, including kidney, spleen, adrenals, testes, lung, stomach, intestines, heart, skeletal muscle, and brain, revealed radioactivity eluting only in the void volume fractions. None of these organs, as well as plasma and VLDL, HDL, and LDL fractions, reacted with anti-TBP antisera when electroblotted on nitrocellulose membranes (not shown). Of lysates of fractionated liver cells, only that of hepatocytes contained the 32-kDa immunoreactivity on Western blotting. Cytosols from three normal human livers were also tested for the tocopherol binding activity and immunoreactivity with the antisera to rat TBP. Human hepatic cytosol samples exhibited specific binding of tocopherol in 30–36 kDa gel filtration fractions (11), but none reacted with anti-(rat) TBP antisera on Western blotting.

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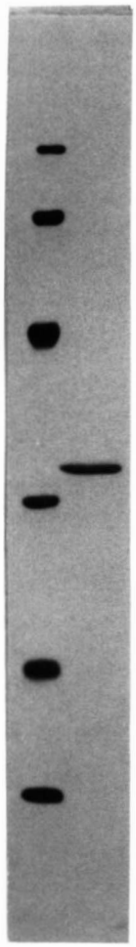

Fig. 3. Purified TBP on SDS-PAGE with silver staining. Molecular masses of marker proteins in left lane are 14.4, 21.5, 31.0, 42.7, 66.2, and 97.4 kDa from bottom to top.

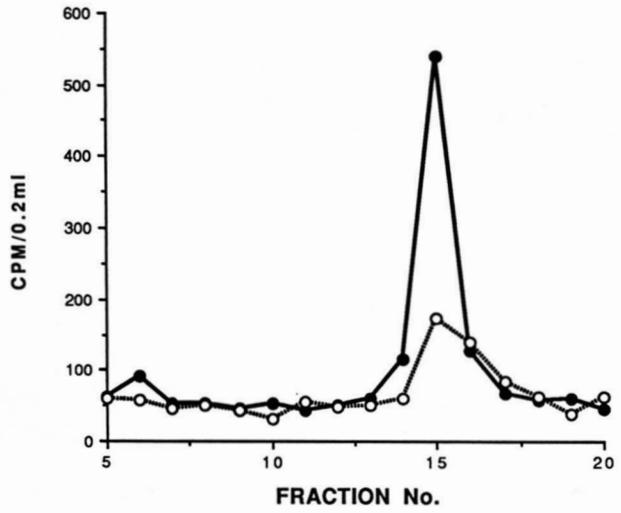

Fig. 4. Displacement of [^{3}H]tocopherol from purified TBP (10 μg) on Superose 12. Closed circles show elution with tracer [^{3}H]α-tocopherol and open circles with 500-fold excess of nonlabeled α-tocopherol.

TABLE 1. Amino acid composition of TBP from rat liver

Amino Acid	Residues/Mole	
	A	B
Asx	27	29
Thr	15	16
Ser	23	22
Glx	36	36
Pro	18	19
Gly	29	27
Ala	20	20
Val	20	20
Met	4	4
Ileu	18	17
Leu	30	32
Tyr	10	9
Phe	14	12
His	9	9
Lys	17	17
Arg	16	16

Amino acid analysis was performed twice (A and B) with 0.09 and 0.17 μg, respectively, of TBP hydrolysate.

DISCUSSION

We and others (4–11) have previously described a tocopherol-binding activity in rat liver cytosol in the molecular weight range of 30,000–36,000, which specifically binds α-tocopherol as opposed to other forms of tocopherol or other lipophilic substances.

In the present report, we have resolved this binding activity into three fractions on Affi-Gel Blue affinity chromatography, each of which specifically binds α-tocopherol. We purified the TBP to homogeneity in one of these fractions and developed a polyclonal monospecific antibody to it that identified a 32-kDa protein exclusively in the cytosol of hepatocytes. The TBP immunoreactivity co-purified with D-α-[^{3}H]tocopherol-binding activity during the purification of TBP. TBP immunoreactivity was identified in only two of the three peaks of specific tocopherol-binding activity eluting from Affi-Gel Blue. The third peak (Affi-Gel-2), corresponding to an apparent 40-kDa protein, represents a distinct form of binding activity.

AFB-1A and AFB-1B appear to have the same molecular weight and are both identified by the AFB-1B antiserum. These results suggest that they are highly related proteins, probably charge isomers. The possibility that the amount of bound tocopherol determines the separation of AFB-1A and AFB-1B seems unlikely since AFB chromatography of Y′ fractions from tocopherol-deficient cytosol yielded a nearly identical pattern of binding. Furthermore, the identification of two isoforms, on Western blot, of 2-D gel electrophoresis of whole liver cytosol further supports the likelihood that AFB-1A and AFB-1B represent two naturally occurring isoforms of TBP and are not ar-

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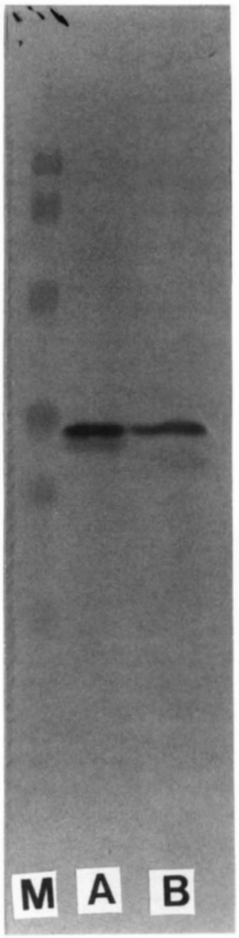

Fig. 5. Immunoreactivity with anti-TBP-antisera in rat liver cytosol (A) and purified TBP (B) by Western blotting. Molecular masses of marker proteins (M) are 17, 27, 39, 50, 75, and 130 kDa from bottom to top. The faint band appearing below the TBP represents nonspecific reaction which was also seen with pre-immune serum (not shown).

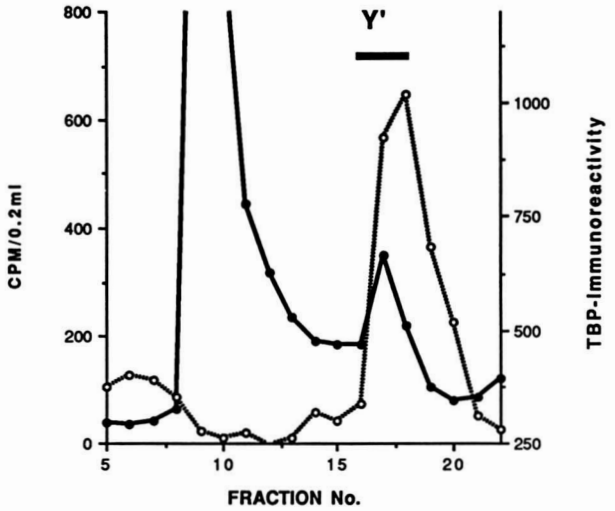

Fig. 6. Superose 12 gel filtration of liver cytosol. Closed circles represent elution of [^{3}H]α-tocopherol and open circles represent immunoreactivity with anti-TBP antiserum (arbitrary units). The Y′ fraction, which contains TBP, is indicated by the horizontal bar.

tifacts of protein purification. Recently, Sato et al. (17) have confirmed our previous and present purification of two forms of TBP using a strategy similar to the one we previously reported (14).

In conclusion, two distinct proteins that specifically bind tocopherol were identified in rat liver cytosol. A 32-kDa protein (existing in two closely related forms) was found to represent 0.2% of the total cytosol protein and to reside exclusively in hepatocytes and was absent from other tissues. Another distinct 40-kDa TBP requires further study. The localization of TBP immunoreactivity and α-[^{3}H]tocopherol-binding activity only in the liver suggests that these proteins are involved in an aspect of α-tocopherol metabolism specific to the liver. Since TBP immunoreactivity was not detected in Ito cells, it is unlikely that it is involved in storage of tocopherol analogous to vitamin A storage in lipocytes. Since α-tocopherol is ubiquitously distributed in organelles of various organs but TBP resides only in hepatocytes, a general intracellular transfer function seems unlikely. However, the liver may have a more selective role in retaining α-tocopherol compared to other forms of tocopherol (18, 19). Chylomicrons contain all forms of tocopherol in proportion to their content in the diet indicating a lack of intestinal discrimination during the absorption process, whereas VLDL, derived from the liver, contains mainly the α-tocopherol form (*RRR*-α-tocopherol) (18, 19). This study strongly suggests that a mechanism is present in the liver to preferentially export α-tocopherol with VLDL, after uptake of various forms of tocopherol with chylomicron remnants (18, 19). Recently, a syndrome of familial isolated vitamin E deficiency has been described which appears to be due to a failure of the liver to release α-tocopherol with VLDL (20). It has been speculated that this familial condition is due to a genetic defect in the

TABLE 2. Summary of purification of TBP

Method	Total Protein	TBP Immunoreactivity	
	mg	μg	*% total protein*
Cytosol	4800	10800	0.23
Y′	360	4320	1.2
AFB-1A	3.2	101	3.2
AFB-1B	2.4	154	6.4
CF/AFB-1A	0.06	54	90
CF/AFB-1B	0.08	80	100

The poor yield reflects both selection of only peak fractions of [^{3}H]tocopherol binding at each step as well as instability of the proteins. AFB, Affi-Gel Blue; CF, chromatofocusing; Y′ refers to peak in gel filtration.

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function of TBP (20). Thus, TBP may be critical in the hepatic handling of α-tocopherol by specifically binding and retaining α-tocopherol as well as possibly transferring α-tocopherol from an endocytic to a secretory compartment.

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