Aggregation of Transcobalamin II (36619)

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Three cyanocobalamin binders have been isolated from normal serum by DEAE-cellulose column chromatography: transcobalamin II (TCII), main protein peak vitamin B_{12} binder (MPPB) and transcobalamin I (TCI) (1, 2). The molecular weight of TCII is approximately 35,000, TCI 120,000 and MPPB slightly higher than 120,000 (2, 3).

There is evidence that TCII has the tendency to aggregate. Hom (4) reported that TCI aggregated in solutions of low ionic strength as judged by gel filtration.

It has been demonstrated in this laboratory that TCII isolated from the serum of some pernicious anemia patients by DEAE-cellulose column chromatography and subjected to gel filtration disclosed the presence of two binders one with a molecular weight of about 120,000 (5). Cooper reported that heparin caused aggregation of TCII (6). Heating of serum at 56° for 30 min resulted in aggregation of TCII and liberation of bound ^{57}Co B_{12} (7). Grasbeck (8) observed that purification of TCII from Cohn fraction III by batchwise adsorptions to and elutions from ion exchange celluloses resulted in aggregation of the binder.

In this study preparative polyacrylamide gel electrophoresis was used for further purification of TCII prepared by DEAE-cellulose column chromatography. Polymerization of the binder was noted. The same phenomenon was observed when TCII was exposed to 8 *M* urea solution.

Materials and Methods. DEAE-cellulose column chromatography. The method has been previously described (2). DEAE-cellulose, Schleicher and Schuell, No. 70, of ion exchange capacity between 0.90 and 0.95 mEq/g, was packed after preparation, into 3 × 60 cm columns. The following buffers were used for elution at 4° and flow rate of 30 ml/hr: 0.0175 *M* sodium phosphate buffer, pH 6.3 (600 ml); 0.04 *M* sodium phosphate buffer, pH 5.9 (1000 ml); 0.1 *M* sodium phosphate buffer, pH 5.8 (500 ml); and 0.4 *M* sodium phosphate buffer, pH 5.2 (700 ml). The buffer solutions were prepared in distilled water containing 0.09% methylparaben and 0.01% propylparaben. Sera from three normal subjects previously stored at −20° were used. Three hundred picograms ^{57}Co B_{12}, (sp act between 100 and 200 $\mu Ci/\mu g$) purchased from Philips Duphar, Holland), were added per milliliter of thawed serum. The solution was allowed to stand at 37° for at least 15 min. After incubation the solution was dialyzed in the cold room against 0.0175 *M* sodium phosphate buffer, pH 6.3 for 24 hr. Following chromatography on DEAE-cellulose column, aliquots of 2 ml from each sample collected from the column (15 ml) were counted for radioactivity. Samples under the radioactive peaks were pooled, dialyzed and freeze-dried. Protein fractions were kept at −20° before subsequent use.

Preparative polyacrylamide gel electrophoresis. Preparations of TCII from normal serum prepared by DEAE-cellulose column chromatography were purified by preparative electrophoresis on a polyacrylamide column. A Buchler preparative gel electrophoresis apparatus was employed. Tris–HCl (0.1 *M*) (pH 8.1) was used as lower buffer and Tris–glycine (0.05 *M*), (pH 8.9) as upper buffer. The height of the column was 7.0 cm and the acrylamide concentration 7.5%. Electrophoresis was conducted at 50 mA for 20 hr at 0°. Voltage increased during the run from

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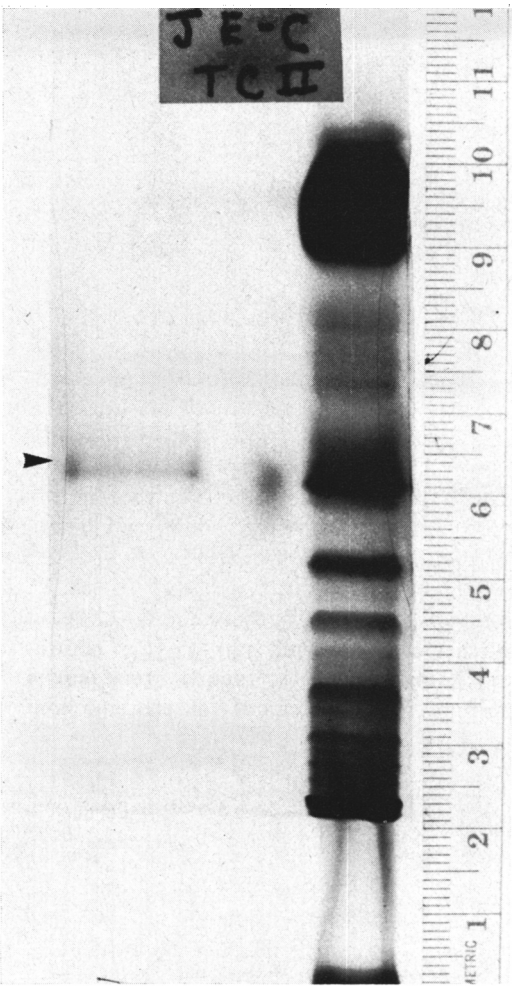

FIG. 1. (left) Zone (arrow) obtained by analytical polyacrylamide gel electrophoresis of TCII ^{57}Co B_{12} subjected to preparative gel electrophoresis. (right) Polyacrylamide gel electrophoresis of normal serum, for comparison. Both samples applied on one slab and run in parallel.

200 to 400 V. The eluate was monitored for radioactivity. The eluate under the radioactive peak was collected, dialyzed and freeze-dried. TCII prepared from 15 ml of serum was applied on the electrophoretic column in each run.

Analytical polyacrylamide gel electrophoresis. The method has already been described (2). Analytical polyacrylamide gel electrophoresis was carried out with an EC vertical gel electrophoresis cell. The running buffer was Tris–HCl (pH 8.9); the space buffer, Tris–HCl (pH 6.7); and the electrode buffer, Tris–glycine (pH 8.3). The acrylamide monomer concentration was 7.0%. One hundred milliliters of running gel solution was used. The remaining space in the cell was filled with spacer gel solution. Applied to each slot were 20 λ of 5% protein solution, leaving a space between samples. Two hundred volts were applied until the sample was stacked in the spacer gel. Electrophoresis was carried out at 400 V (starting current, 150 mA; final current, 125 mA), using running tap water for cooling. Electrophoresis was discontinued when the albumin zone had traversed 7 cm (approx 4 hr). At the end of this period the gel was stained with amido black (0.2% in 7% acetic acid).

Gel filtration. The method has already been described (2). Samples were chromatographed on Sephadex G-200 columns, 2 × 60 cm; void volume, 60 ml; prepared as suggested by the manufacturer (Pharmacia). Elution buffer was 0.005 *M* sodium phosphate (pH 7.4) containing 1.0 *M* NaCl. Two milliliter samples were collected at a flow rate of 12 ml/hr and counted for radioactivity in a Baird-Atomic well-type scintillation spectrometer.

Effect of urea. Transcobalamin II bound ^{57}Co B_{12}, prepared by DEAE-cellulose column chromatography (described above), was dissolved in 8 *M* urea solution. The solution was dialyzed against the same solvent for 48 hr. An aliquot of the dialyzed solution as described in the previous paragraph was further dialyzed against 0.005 *M* sodium phosphate buffer (pH 7.4) containing 1.0 *M* NaCl. Following dialysis an excess of ^{57}Co B_{12} was added and the solution filtered on a Sephadex G-200 column.

Results. Serum from three normal subjects was used for preparation of TCII by DEAE-cellulose column chromatography and preparative polyacrylamide gel electrophoresis. This preparation gave a single zone, with a beta-globulin mobility, by analytical polyacrylamide gel electrophoresis (Fig. 1). Gel filtration of the preparation obtained by

preparative electrophoresis gave a peak of radioactivity corresponding to a molecular weight slightly higher than 120,000 (Fig. 2).

Urea dissociated the ^{57}Co B_{12}-TCII complex. ^{57}Co B_{12} rebound to TCII which had been exposed to 8 *M* urea, was eluted from the Sephadex column with an elution volume corresponding to a molecular size higher than 120,000 (Fig. 3).

Discussion. Attempts at purification by preparative polyacrylamide gel electrophoresis of transcobalamin II prepared by DEAE-cellulose column chromatography resulted in a vitamin B_{12} binder preparation homogeneous enough to exhibit one zone by analytical polyacrylamide gel electrophoresis (Fig. 1).

Gel filtration of this purified preparation indicated that ^{57}Co B_{12} was associated with a binder of molecular weight slightly above 120,000 clearly higher than the accepted molecular weight (36,000) of TCII (2, 3). It appeared that TCII was aggregated during the polyacrylamide gel electrophoresis run.

Urea caused dissociation of ^{57}Co B_{12} from the complex with transcobalamin II. After removal of urea by dialysis, the binder retained the capacity to bind ^{57}Co B_{12} and exhibited a molecular weight slightly higher than 120,000 by gel filtration on Sephadex G-200 column.

In vitro aggregation of transcobalamin II has been reported from many laboratories

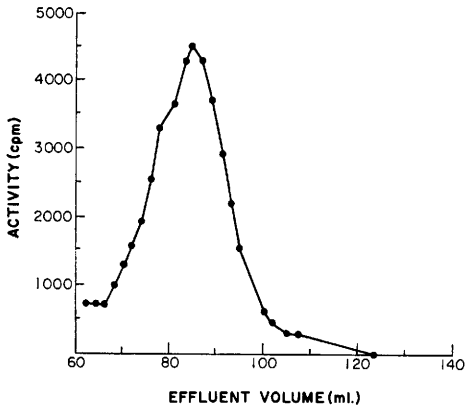

FIG. 2. Radioactive profile obtained by gel filtration on Sephadex G-200 column of ^{57}Co B_{12} binder purified by preparative gel electrophoresis. Elution volume of TCII, under the same experimental conditions is 124 ml.

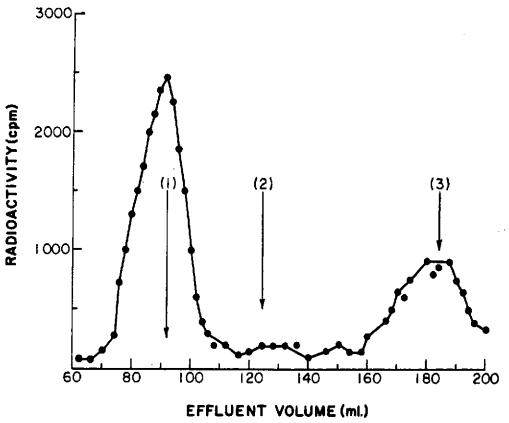

FIG. 3. Radioactive profile on Sephadex G-200 column of TCII ^{57}Co B_{12} treated as follows: TCII prepared by DEAE-cellulose column chromatography was dissolved in 8 *M* urea. Arrows: (1) the elution volume of MPPB; (2) of TCII; and (3) of free vitamin B_{12}.

(4–7). It is not known if this aggregation occurs *in vivo*. The molecular weight of the main protein peak vitamin B_{12} binder is close to that of aggregated transcobalamin II (2). Although plasma disappearance rate of MPPB and TCII in human subjects and uptake by perfused rat liver is similar, identity of aggregate transcobalamin II and main protein peak vitamin B_{12} binder has not been established (2, 9).

Summary. Purification of transcobalamin II prepared from normal serum by DEAE-cellulose column chromatography and polyacrylamide gel electrophoresis resulted in aggregation of this vitamin B_{12} binder. Exposure of transcobalamin II to 8 *M* urea resulted in liberation of the vitamin and aggregation of the binder.

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