QUANTITATIVE SEPARATION OF SERUM TRANSCOBALAMINS ON CHARGED CELLULOSE FILTERS

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Received 8 May 1974

1. Introduction

In recent years there has been a growing interest in the serum transcobalamins, i.e. vitamin-B_{12} binding proteins [1,2]. This interest was augmented by findings that certain diseases are associated with specific changes in the concentration of either of the two major transcobalamins (TC I and TC II). Thus, in diseases such as chronic myelogenous leukemia (CML) and acute promyelocytic leukemia (APL) increased levels of both bound and unsaturated TC I have been found [3–8]. In acute hepatitis, on the other hand, the TC II concentration is markedly increased while TC I is unchanged [9]. Congenital disorders in which TC I or TC II are lacking were also reported [10–12].

Thus, estimation of the various transcobalamins may be of value in the diagnosis of specific disorders. However, such a possibility has not gained wide acceptance, mainly because of difficulties encountered in the quantitative separation between these proteins; TC I, a protein of acidic nature, is retained by anion exchange columns, while TC II, a basic protein, is eluted from the column at low salt concentrations [2,13]. A rapid and simple method for the determination of the major transcobalamins, making use of their electrical charges, is described in the present communication.

2. Materials and methods

Radioactive vitamin-B_{12} ($^{57}CoB_{12}$, 135 μCi/μg) was purchased from the Radiochemical Centre (Amersham, Bucks, England). Circular filter papers (diameter 25 mm) were used. DE-81 discs were obtained from Whatman Biochemicals Ltd. (Maidstone, Kent, England) and cellulose-nitrate discs from Sartorius Membranfilter Co. (Göttingen, Germany). Norit A charcoal, used for adsorption of the unbound vitamin-B_{12}, was a product of Nutritional Biochemicals (Cleveland, Ohio, USA). Radioactivity was determined with a Packard Autogamma Spectrometer.

Transcobalamin I (TC I) was released by sonication of leukocytes obtained from the blood of a patient with CML [14] and was used as such, TC II was partially purified from normal human serum by chromatography on DEAE-cellulose [2].

2.1. *Determination of vitamin-B_{12} binding capacity*

To determine the unsaturated vitamin-B_{12} binding capacity (UBBC) of the respective transcobalamins, the sample (serum or other biological material) was incubated for 15 min at room temperature with an excess of $^{57}CoB_{12}$ at pH 7.0. Further details are given in the tables. The unbound radioactive-B_{12} was re-

Table 1
Properties of transcobalamins, TC I and TC II*

Property	TC I	TC II
Molecular weight	38,000	115,000-120,000
Mobility on paper electrophoresis (pH 8.6)	α	β
Mobility on starch and geon block electrophoresis (pH 4.5)	Anodal	Cathodal
DEAE-cellulose column	Eluted after albumin	Eluted before albumin
CM-cellulose column	Eluted early	Retained

* Ref. [13]

moved either by polyvinylpyrrolidone-coated charcoal [15] or by filtration through a three-layered stack of charged cellulose filters as follows: two DE-81 discs were placed on the bottom of a Millipore-type filter holder and one cellulose-nitrate disc was placed on the top; the entire stack was moistened with water prior to use. The incubation mixture was diluted with 10 to 12 ml of 0.1 M sodium borate buffer (pH 8.5) and passed through the filter stack by applying vacuum. To remove completely the unbound radiovitamin, three 10 ml aliquots of the same buffer were passed through the filter stack. The two DE-81 filters were separated from the single cellulose-nitrate disc and were inserted into separate test tubes and their radioactivity counted. The sum of radioactivities on both filter types represents the UBBC of the sample.

3. Results

Some of the physical and chemical properties of the two transcobalamins are summarized in table 1. At intermediate pH values the charges of the two transcobalamins are opposite each other; separation between these two should, therefore, be feasible by passing them through cellulose filters of opposite charges, like cellulose-nitrate and DEAE-cellulose.

Table 2 shows that TC I is adsorbed only to the DEAE filters, while TC II is retained by cellulose-nitrate discs. The B_{12}-binding protein of chicken serum showed a filtration pattern similar to that of human TC I. This finding is consistent with previous reports on the nature of chicken serum transcobalamin [15–17].

Although binding of the radiovitamin to the respec-

Table 2
Trapping of $^{57}CoB_{12}$-transcobalamin complex by cellulose-nitrate and DE-81 filter discs

Sample	Transcobalamin type	Net c.p.m. on filters/0.4 ml incubation mixture	
		Cellulose-nitrate	DE-81
Serum fraction not retained by DEAE-cellulose	TC II	1420	160
Sonicate of leukocytes from a patient with CML	TC I	0	5930
Chicken serum	TC I	0	5600

The various samples (not exceeding 300 μg protein) were incubated for 15 min at room temperature with $^{57}CoB_{12}$ (14 000 cpm) in potassium phosphate buffer (0.02 M, pH 7.0). Total vol. 1 ml; 0.4 ml aliquots were filtered through the filter stack as described under Materials and methods.

Table 3
Effect of pH on retention of transcobalamin I by cellulose-nitrate filters

Buffer solution (10 ml) added to reaction mixture after incubation	Percent of bound $^{57}CoB_{12}$ retained on the cellulose-nitrate filter (TC I)
0.1 M Sodium borate, pH 8.5	1.5
0.1 M Potassium phosphate, pH 8.2	0
0.1 M Potassium phosphate, pH 7.5	53
0.25 M Acetic acid, pH 2.6	100

The TC I preparation was incubated with $^{57}CoB_{12}$ as described in table 2. Bound radioactivity was determined by using the coated charcoal method [15]. Aliquots of the same incubation mixture were added to 10 ml of the buffer solutions mentioned and filtered through cellulose-nitrate filters. Traces of unbound $^{57}CoB_{12}$ were removed by washing the filters thrice with 10 ml of the same buffer. The radioactivity was counted directly on the filter.

tive transcobalamins is equally effective at relatively wide pH ranges [17], the filtration through charged cellulose discs is very much pH-dependent. The filtration of the incubation mixtures described in table 2 was carried out at pH 8.5. At pH values lower than 8.2 there was a retention of TC I to the cellulose-nitrate filter which increased with the decrease in pH (table 3).

Separation of the two transcobalamins of various human sera using the filtration method is shown in table 4. The data obtained are in agreement with previous reports concerning the levels of the transcobalamins in human serum of normal individuals and patients with various pathological conditions. The bulk of the UBBC of serum from healthy individuals is due to TC II [3–9]. Similar results were obtained in patients with acute hepatitis. On the other hand, sera from patients with CML have high TC I as found by using other procedures [3–8].

4. Discussion

Although both transcobalamins (TC I and TC II) bind vitamin-B_{12}, they differ from each other by their

Table 4
Determination of the B_{12}-binding capacity of TC I and TC II in human sera

Patient	Disease	pg of $^{57}CoB_{12}$ retained *		TC II	UBBC*	
		Cellulose nitrate (TC II)	DE-81 (TC I)	%	Filters**	Charcoal
A.I.	Normal	1900	950	67	2850	
B.D.	Normal	715	180	80	895	
T.H.	Hepatitis	1900	165	92	2065	
B.E.	Hepatitis	1030	355	74	1385	
B.A.	CML	1250	4300	22.5	5550	5370
B.S.	CML	1920	3500	35.6	5420	5250
G.G.	CML	1530	3370	31.2	4900	
Pooled serum	Normal	1190	485	71	1675	1260

The sera (0.01 ml) were incubated with $^{57}CoB_{12}$ (80 pg) as outlined in table 2; 0.4 ml aliquots were filtered and counted as described. The UBBC of some samples were also determined by the charcoal adsorption method.
* Calculated per 1 ml serum.
** Sum of the radioactivity values of the cellulose-nitrate and DE-81 filters.

molecular weight as well as their primary structure. TC II is a basic protein with a lower molecular weight (M.W. 38 000) than TC I and other 'R' proteins (M.W. 120 000) which are of acidic nature [1,2]. At pH values higher than 8.0 the two transcobalamins acquire opposite charges and adhere, therefore, to differently charged filters, i.e. TC I to DE-81 and TC II to cellulose-nitrate. The separation of a third binder (TC III), whose existence is still in controversy [18–20], was not thoroughly studied; however, preliminary findings show that its filtration properties are similar to that of TC I.

The capacity of the filter discs used by us is small. Thus, in order to obtain quantitative retention of the respective transcobalamins, the amount of protein applied on the filter pad should not exceed 300 μg and the vitamin should be of high specific radioactivity (100–300 μCi/μg).

To determine the total B_{12} binding capacity the transcobalamins retained on the respective filters should be removed by extraction and the unlabeled B_{12} thus released could be determined. This possibility is being investigated by us at present. A radioassay utilizing the filtration principle outlined in this paper is also being applied in this laboratory.

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