JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE

VOLUME 52, NUMBER 1, JANUARY, 1977

ASSAY FOR THE DETECTION OF A PROTEIN COMPONENT IN THE SERUM OF INDIVIDUALS WITH CANCER

EDSEL T. BUCOVAZ, JOHN C. MORRISON, WILLIAM C. MORRISON, AND WALTER D. WHYBREW

University of Tennessee Center for the Health Sciences

Memphis, Tennessee 38163

ABSTRACT

A specific type of protein, which has been designated B-protein (Bucovaz-protein), is present in serum of most individuals with cancer. The B-protein interacts with a low molecular weight protein component, which is released by the coenzyme A-synthesizing protein complex (CoA-SPC) of Bakers' yeast during the course of CoA synthesis. This low molecular weight protein, for purposes of identification, is referred to as binding protein. The binding protein has a molecular weight of 10,000 to 15,000. Interaction of radiocatively labeled binding protein of CoA-SPC with the B-protein of serum provides a marker for the detection of cancer. Of 1000 serums assayed, which included 322 patients diagnosed as having cancer, the B-protein assay agreed with the clinical diagnosis in more than 88% of the cases.

INTRODUCTION

During the past several years, numerous methods have been reported for the detection of cancer. Some of these have been physical, while others have been chemical in nature. Most of the physical methods, such as mammography and x-ray, have had reasonable success in the detection of cancer, but the hazards of radiation may be greater than generally believed, and may outweigh its value.

Of the chemical assays, the carcinoembryonic antigen (CEA) assay (Gold and Freedman, 1965a; Gold and Freedman, 1965b; and Thomas et al., 1969) has been most extensively publicized, and has been the subject of extensive investigation. The CEA assay appears to have its greatest potential value in the area of cancer management. There are mixed opinions in regard to the usefulness of the CEA assay for the routine diagnosis of cancer in the clinic and in population screening. (Stevens et al., 1975).

Since 1973, a test for the detection of cancer has been under investigation and development in our laboratory.

A specific type of protein normally present in human serum was observed to interact with a low molecular weight protein component released from the coenzyme A-synthesizing protein complex (CoA-SPC) of Bakers' yeast. In cancer patients the serum protein is modified, or a protein with somewhat similar characteristics is produced and released into the blood, which also interacts with the low molecular weight protein of yeast.

To provide a means of reference, this protein found in the serum of individuals with cancer has tentatively been designated the Bucovaz-protein (B-protein), and the assay procedure used to detect the presence of this protein is referred to as the B-protein assay (Bucovaz et al., 1976a and Bucovaz et al., 1976b). The source of B-protein is not known. It may be produced by the cancer cells, or possibly the presence of cancer cells triggers a particular immune system or some other system in the body which produces this protein component.

The CoA-SPC used in this assay was described by us (Sobhy et al., 1975; Morrison and Morrison, et al., 1975; Morrison and Whybrew, et al., 1975; and Bucovaz et al., 1976) as a protein complex which synthesizes CoA. This coenzyme A-synthesizing protein complex utilizes L-cysteine, D-pantothenic acid and ATP as substrates (Sobhy et al., 1975 and Morrison and Morrison, et al., 1975).

The initial reaction catalyzed by the CoA-SPC is between the β-phosphorous group of ATP and the 4'-hydroxyl group of pantothenic acid resulting in the formation of CoA-SPC bound ADP-4-pantothenic acid. The α-amino group of cysteine then reacts with the carboxyl group of the pantothenic acid moiety. At the time of reaction, cysteine is decarboxylated forming CoA-SPC bound dephospho-CoA. Dephospho-CoA is either phosphorylated and released as CoA, or is hydrolyzed to yield what appears to be 4'-phosphopantetheine bound to a low molecular weight protein component of the CoA-SPC (10,000-15,000), which then detaches from the complex (Sobhy et al., 1975; Morrison and Morrison, et al., 1975; Morrison and Whybrew, et al., 1975; and Bucovaz et al., 1976).

One function of the CoA-SPC apparently is the synthesis of CoA; the other may be associated with the synthesis of acyl-carrier protein. The low molecular weight protein is suspected of being related to the latter of these two functions of CoA-SPC. In regard to the B-protein assay, the low molecular weight protein is referred to as the binding protein.

The binding protein released from the CoA-SPC can readily be radioactively labeled by using [^{14}C]-D-pantothenic acid or [^{35}S]- or [^{14}C-U]-L-cysteine as radioactive markers in the reaction medium.

Although the B-protein of cancer patients is similar to a serum protein present in individuals who do not

have cancer in that both proteins interact with radioactively labeled binding protein, differences in the properties between the two are sufficient for separation and identification of the B-protein.

This paper describes the B-protein assay, and presents data obtained using this assay in the assessment of 1000 serum samples of which 322 were from individuals diagnosed as having cancer.

MATERIALS AND METHODS

Patient Population—One thousand patients both male and female, of all economic levels, are included in this report. Most of the patients were in the age group between 30 and 70. Some patients, however, were as young as 13 and others were as old as 92.

Serum for Assay—The blood was collected in 10 ml red stoppered, silicone coated Vacutainer tubes. No anticoagulant was added. The clot that formed was removed, and the blood was centrifuged. Following centrifugation, a Seraclear plug (Technicon) was inserted to separate the packed cells from the serum. Serum which was extensively hemolyzed was discarded and a new sample collected. The red color caused by hemolysis interferes with the assay by producing quenching of the radioactivity measurement. Lipemic serum, however, does not appear to have a significant effect on the results of the assay. Serum can be stored at —20°C for several weeks or stored for longer periods of time at cryogenic temperature of —100°C. Normal and cancer controls were chosen by screening serum from a number of individuals with and without cancer. These controls were used to provide the range of values for both normal and cancer.

Preparation of the Coenzyme A-Synthesizing Protein Complex (CoA-SPC) of Bakers' Yeast—The CoA-SPC was prepared by an extensive modification of the method previously reported (Morrison and Morrison, et al., 1975). Three pounds of Bakers' yeast were crumbled and then frozen for four to six hours in an ether-CO_2 mixture. The frozen yeast was allowed to thaw, and residual ether and CO_2 were removed by vacuum. Fifteen g of KCl were added to the homogenate, and the mixture was stirred at 0°C to 4°C for 17 h. Following the stirring step, the homogenate was centrifuged for 20 min at 7700 x g, and the supernatant layer was decanted through several folds of cheesecloth. The volume of the crude extract varied between 350 and 375 ml, depending upon the water content of the yeast. The crude extract was stored at cryogenic temperatures of —100°C. The CoA-SPC prepared as described retained capacity for synthesis of CoA and for the release of the binding protein for several months.

B-Protein of Cancer Serum—Individuals with and without cancer have a specific protein present in their blood serum which interacts with the radioactively labeled binding protein of CoA-SPC. The B-protein present in the serum of cancer patients may or may not be a modification of this serum protein. The B-protein, however, also interacts with the binding protein. Moreover, the B-protein of cancer patients and the specific protein present in the serum of all individuals migrates in an electrical field in the same general area as the γ-globulin fraction of human serum. Both patients have a molecular weight of approximately 140,000-160,000, and have the same pattern of separation in a 3% to 10% continuous sucrose gradient. Also both proteins are precipitated by 50% saturation of the serum with $(NH_4)_2SO_4$. Furthermore, as the titer of B-protein in the serum of cancer patients increases, the amount of its normal counterpart appears to decrease in a proportional manner. Although present information is not adequate to make a definite statement, we are encouraged to speculate that the B-protein represents a modification of the yet unidentified protein of normal serum which interacts with the binding protein of CoA-SPC. The characteristics of the binding protein—B-protein complex on exposure to heat and TCA provide a means for the separation by filtration of the B-protein from the normal serum protein. This difference in solubility could be due to subtle differences in configuration and charge of the B-protein, which may be influenced by the location, type and extent of the malignancy. It appears, however, that if the progression of the malignancy is directly related to charge and configurational changes in this protein, these alterations are relatively small, because 89% of the 322 cancer patients studied had a detectable level of serum B-protein with approximately the same diminished solubility regardless of extent, type or location of the malignancy.

B-Protein Assay Procedure—The reaction mixture described is one in which [^{35}S]-L-cysteine was used as the radioactive tracer (substrate). [^{14}C-U]-L-cysteine or [^{14}C]-D-pantothenic acid can be used in place of [^{35}S]-L-cysteine.

A reaction mixture containing the following components was used: 2mM disodium ATP, pH 7.2; 0.5 ml buffer A, pH 7.2 (containing 0.05 mM Tris-acetate, pH 7.2; 0.01 mM magnesium acetate; 0.02 mM KCl); 0.5 mM dicalcium D-pantothenic acid; 0.02 mM [^{35}S]-L-cysteine (60,000 cpm); 0.05 ml of the yeast extract containing CoA-SPC and water to a total volume of 1 ml. To this reaction mixture was added 0.05 ml of the serum being tested, and the total mixture was incubated at 36°C for 2 h. The reaction was terminated by heating the tubes in a H_2O-bath at 68°C to 70°C for 5 min. The tubes were then cooled to room temperature followed by centrifugation at approximately 2,200 rpm for 5 min in a Model CL International Clinical Centrifuge. This procedure removes most of the yeast protein as a precipitate. The remaining supernatant liquid of the reaction mixtures in which normal serum was added contains the [^{35}S]-binding protein-normal protein complex; whereas, the supernatant liquid of the reaction mixtures in which serum from cancer patients was added contains both [^{35}S]-binding protein-normal protein and [^{35}S]-binding protein-B-protein. Two milliliters of 10% TCA were added to each tube containing the supernatant liquid, and the tubes were shaken to assure proper mixing of the TCA. The resulting protein precipitates, which were primarily serum protein, were recovered by filtration using a Millipore filtering apparatus and Whatman No. 3 MM paper discs. The precipitates collected on the discs were washed 4 times with approximately 2 ml of water per wash. The discs containing the serum protein precipitates were dried in an oven at <100°C. Precautions were taken to prevent scorching the discs. The dried discs were then transferred to scintillation vials containing scintillation liquid described by Hoskinson and Khorana (1965), and the radioactivity levels were measured in a Nuclear Chicago liquid scintillation counter. When this method of assay is followed, less radioactivity and protein are trapped on the discs if B-protein is not present. Approximately the same quantity of the binding protein interacts with serum of individuals whether they do or do not have cancer. This interaction may or may not be enzyme catalyzed. Experimental evidence would indicate that the specific protein of normal serum, which interacts with the binding protein, is slightly more resistant to denaturation by heat at 68°C to 70°C and TCA than is the B-protein of serum of individuals with cancer. Thus, a greater quantity of the normal serum protein passes through the filter; whereas, B-protein present in the serum of cancer patients is less soluble after the described treatment, and more of this protein is trapped on the filter. Therefore, a higher level of radioactivity is detectable if B-protein is present in the serum being tested.

RESULTS AND DISCUSSION

As shown in Table 1, 322 of the patients studied were diagnosed as having cancer, whereas the patients listed in Table 2 were hospitalized for various other reasons. Of the cancer patients included in this study, the B-protein assay gave a positive reaction for 287 of the 322, or agreed with the clinical diagnosis in 89% of the cases. Approximately 11% of the patients diagnosed as having cancer in the clinic did not show a detectable level of B-protein in their serum. This raised a point of interest, particularly since the absence of B-protein could not be related to type, origin or stage of progression of the cancer. This discrepancy may be explained on the basis of preliminary data which indicate

that in some instances the B-protein is destroyed during collection or storage of the serum sample.

The data of Table 2 show that 597 of the 678 patients in the control group gave a negative B-protein value, and 81 patients from this group had positive values. Thus, 88% of this test group gave results indicating the absence of cancer. These patients, however, were being treated for other reasons, as indicated in Table 2. The diagnoses of 149 patients included in Table 2 were classified as unknown, but not suspected of having cancer. Some of the patients in this unknown group may have cancer which was not detected because of diagnostic limitations. The category labeled miscellaneous included five patients with severe burns over 20% of their body area. All of the burn patients had a positive B-protein reading.

Overall, the test results were not in accordance with the clinical information provided in 116 of the 1000 patients, but were in agreement in the assessment of 884 cases or 88.4% of those tested.

One might rationalize that age should be a prime factor, because the older an individual becomes, the more likely that individual is to have other diseases and physical problems which might influence the assay. This did not prove to be the case. As indicated (Table 2), other diseases and other physical problems do not have a profound influence on the result of the assay.

At present we do not have information concerning the stage of cancer development which results in formation of the B-protein. Some cancer patients appear to have a higher ratio of B-protein to its normal protein counterpart in their serum than other patients. There are some indications that B-protein production may take place during the early stage of cancer development. Of the patients studied, some had very early signs of abnormal changes in cell structure; whereas, in other patients, the cancer was in a more advanced stage of development. In most of the patients studied, however, morphological changes in the tissues were apparent to the extent that cancer had been diagnosed or suspected prior to assay of the serum. It is unlikely that cancer must develop to the initial stage of metastasis before detection is possible by this assay procedure. If the initial stage of metastasis must be reached, then in some cases initiation of the metastatic event must take place in cancerous tissue much earlier than presently believed.

Further investigation is needed to determine which stage of cancer development is required before a detectable level of B-protein appears in the serum. Based on available scientific information, the earlier cancer is detected and treated, the better the individual's chances of recovery. It is well established that early cancer, in many instances, is not detected, particularly if the lesion is not on the body surface. The B-protein assay most certainly appears to have the potential to detect most cancers of internal origin much earlier than they would presently be recognized.

Many facets of the B-protein assay remain to be resolved before its ultimate value can be determined. For example, what is B-protein? What is its origin? At what stage of cancer development does this protein first appear in the serum? Answers to these and to other questions may provide a common denominator between various types of cancer as well as differences between normal and cancer cells.

TABLE 1: *B-protein assay results of patients with cancer.*

Cancer Location or System[a]	Number in Category	Test Results[b] Positive	Negative	Positive %
Head & Neck	27	25	2	92.59
Gastro-intestinal	37	33	4	89.19
Genito-urinary	107	96	11	89.72
Respiratory	26	25	1	96.15
Reticulo-endothelium	29	26	3	89.66
Integument	9	7	2	77.78
Breast	45	35	10	77.78
Other	8	7	1	87.50
Unknown	34	33	1	97.06
Total	322	287	35	89.13

[a] Information provided by the physician or obtained from medical records.
[b] A positive test result indicates the presence of cancer; a negative test result indicates the absence of cancer.

TABLE 2: *B-protein assay results of patients in control group.*

Patient Type[a]	Number in Category	Test Results[b] Positive	Negative	Negative %
Cardiovascular	28	1	27	96.43
Orthopedic	81	6	75	92.59
Gynecological	19	1	18	94.74
Obstetrical	246	32	214	86.99
Surgical	50	3	47	94.00
Medical	42	2	40	95.24
Neurological	32	4	28	87.75
Miscellaneous	31	9	22	70.97
Unknown	149	23	126	84.56
Total	678	81	597	88.05

[a] Information was provided by the physician or obtained from medical records.
[b] A positive test result indicates the presence of cancer; a negative test result indicates the absence of cancer.

Literature Cited

Bucovaz, E. T., Morrison, J. C., Morrison, W. C., and Whybrew, W. D. (1976a). An Abnormal Protein Present in Human Serum of Patients with Cancer can be Detected by Protein-Protein Interactions. 10th International Congress of Biochemistry, in press.

Bucovaz, E. T., Morrison, J. C., Morrison, W. C., and Whybrew, W. D. (1976b). Protein-Protein Interaction used as Assay for Detection of Abnormal Serum Protein of Patients with Cancer. Third International Symposium on Detection and Prevention of Cancer, in press.

Bucovaz, E. T., Morrison, J. C., Rhoades, J. L., Morrison, W. C., Fryer, J. E., and Whybrew, W. D. (1976). Purification of the Coenzyme A-Synthesizing Protein Complex of Bakers' Yeast. Amer. Soc. Biol. Chem., in press.

Gold, P., and Freedman, S. O. (1965a). Demonstration of Tumor-Specific Antigens in Human Colonic Carcinomata by Immunological Tolerance and Absorption Techniques. J. Exp. Med., *121*, 439-462.

Gold, P., and Freedman, S. O. (1965b). Specific Carcinoembryonic Antigens of the Human Digestive System. J. Exp. Med., *122*, 467-481.

Hoskinson, R. M., and Khorana, G. H. (1965). Studies on Polynucleotides SLI. Purification of Phenylalanine-Specific Transfer