Binding Studies of Boron Hydride Derivatives to Proteins for Neutron Capture Therapy

Eugene I. Tolpin, Hou S. Wong, and William N. Lipscomb*

Gibbs Chemical Laboratory, Harvard University, Cambridge, Massachusetts 02138. *Received November 21, 1973*

Ionically solubilized boron hydride derivatives previously reported by us, including diazonium ions, imido esters, aldehyde, and amines, are used to bind boron to human γ-globulin and bovine serum albumin. The results indicate that an average of 0.6% boron by weight can be attached to the antibody at 50% precipitation of the original protein and a maximum of 0.8% boron by weight near quantitative precipitation as determined by extrapolation. An isocyanate derivative of the $B_{10}H_{10}^{2-}$ anion gives up to 1.77% by weight of boron bound without significant loss of protein solubility.

Preliminary investigations[1,2] into the preparation of boron-containing immunoglobulins for neutron capture therapy of brain tumors[3] have given low percentage weight values of boron (about 0.2%). Although the exact quantity of ^{10}B needed per antibody to produce tumor destruction is unknown, it has been estimated to be at least of the order of several hundred ^{10}B atoms per antibody.† In order to increase substantially the binding of boron atoms to human γ-globulin, we have synthesized a variety of boron cage compounds having high boron contents and good immunoglobulin binding potential (Table I).[4] These compounds are used here to modify γ-globulin and bovine serum albumin (BSA). Our results indicate improved binding (0.7–1.7% boron by weight) to γ-globulin. We also show that unless the boron cage is adequately solvated, only a limited number of boron moieties can be chemically attached to an immunoglobulin before precipitation of the modified protein becomes quantitative.

Experimental Section

The syntheses of all borane derivatives have been described.[4] Human γ-globulin (Cohn fraction II) was purchased from Miles Laboratories. Bovine Serum Albumin (BSA) was purchased from Nutritional Biochemicals Corp. Analyses of the boron and protein contents of the products were made by methods in the literature and were repeated to give average results.[5] A solution of each reagent with no protein was dialyzed and then analyzed as a check of dialysis efficiency. Analyses were made 1–2 weeks after reaction. The procedures we used to modify the proteins with the reagents are similar to those in the literature for diazonium,[6] imido ester,[7,8] aldehyde,[9] isocyanate,[10] and carbodiimide reagents.[11,12] Variations in the general procedure for specific compounds are indicated.

Diazonium Modification. The diazonium salts were prepared from their respective amines and reacted with protein. That of 1-(4-aminophenyl)-1',2'-dicarba-*closo*-dodecaborane (reagent 1) was prepared according to literature.[1] The procedure involving *N*-[4,5-(1,2-orthocarboranyl)pentyl]-*N*-(2-*p*-aminophenylethyl)-*N,N*-dimethylammonium iodide (reagent **2**) is given below. The preparation of the diazonium salt of *p*-azodecaborate–aniline (reagent **3**) was similar to **2**, except that acid was added to the amine solution only until the pH was 1.8. Nitrite was added at this pH.

By passage through an anion exchange column (Mallinckrodt IR-400) in the chloride form, 500 mg of **2** was converted to its chloride salt, and the aqueous solution adjusted to 25 ml. To a mixture of 1.0 ml of this amine salt solution (4.11×10^{-2} mmol/ml) and 0.1 ml of concentrated HCl at 0° was added 0.1 ml of sodium nitrite solution (4.11×10^{-1} mmol/ml). After stirring for 15 min, sulfamic acid was added until the starch iodide test was negative with the solution. Then, NaOH was added until the pH was about 5, and the solution was diluted to 1.4 ml. The diazonium salt (2.94×10^{-2} mmol/ml) was used immediately. Then 15 mg of protein was dissolved in 3 ml of 0.1 *M* phosphate buffer and 0.05 *M* NaCl at pH 10.0. The diazonium reagent was added at 0° and the pH was maintained by a pH-stat. Assuming an average molecular weight of 160,000 for γ-globulin, and 69,000 for BSA, the reagent was added in molar ratios (reagent to protein) of from 5:1 to 200:1. After 1 hr, the solution was diluted with buffer to 10 ml and extensively dialyzed (seven changes) over 4 days. After centrifuging, the supernatants were analyzed for boron and protein contents.

Imido Ester Modification (Reagents 4, 5, and 6). To 40 ml of phosphate buffer containing 0.05 *M* NaCl and 1.5 ml of dimethylformamide at pH 9.0 (controlled by pH-stat) was added 30 mg of protein. To this solution was added gradually weighed amounts of the imido ester dissolved in 0.2 ml of MeOH containing 25 μl of 1 *N* NaOH per 15 mg. The pH of the protein solution was maintained with 1 *N* NaOH. After stirring for 2 hr, the solution was dialyzed nine times over 5 days and then centrifuged and analyzed. In other runs we used 5.0 ml of buffer and no dimethylformamide.

For the reaction of **4** with BSA, the reaction time was 24 hr. The reaction time of **6** was 4 hr.

Carbodiimide Reactions. To 4 ml of water containing 0.05 *M* NaCl and 1 ml of dimethylformamide at pH 7.9 (controlled with a pH-stat) was added 15 mg of protein and 15 mg of **8**. Then 5, 15, or 50 mg of 1-ethyl-3-dimethylaminopropylcarbodiimide was added. After stirring for 3 hr, the solution was dialyzed and analyzed.

A pH of 4.5 was used for the carbodiimide-catalyzed reaction of the anions decaborate and *p*-azodecaborate–aniline (reagent **10**) with the proteins.

Aldehyde Modification. An amount of 170 mg (0.35 mmol) of the acetal of **7** was converted into the aldehyde[4] and dissolved in 4 ml of water, and appropriate volumes were added to 200 mg of protein in 2 ml of 0.1 *M* phosphate buffer at pH 9.0. Calculated amounts of $NaBH_4$ were then added to each sample. After 4 hr the samples were dialyzed and analyzed as before.

Isocyanate Modification. An amount of 294 mg (1 mmol) of the tetramethylammonium salt of **9** was ion exchanged into the sodium form. This was adjusted to 10 ml and calculated volumes were added to 2 ml of a protein solution (carbonate buffer, pH 9.0, 10 mg of protein per milliliter). The samples were stored overnight and then treated as above.

Results. The boron contents and amounts of γ-globulin and BSA remaining in solution after modifications are given in Figure 1 and Tables II and III.

Discussion

Previously, we synthesized several borane derivatives (Table I) that are potentially useful in protein binding and that are solubilized by ionic centers.[4] Since ionic binding of these borane cages might lead to precipitation of the protein, we first studied the effects of mixing some model ionic compounds with γ-globulin.‡ γ-Globulin is the immunoglobulin serum fraction composed 90% of molecules having a molecular weight of 160,000, having a sedimentation coefficient of 7S.[13,14] γ-Globulin is composed of three heterogeneous classes of proteins: IgA, IgG, and IgE.[13] The ability of γ-globulin to bind borane cages and the solubility of such modified γ-globulins should be a reasonable model for the behavior of tumor directed, 7S fraction immunoglobulins.

In the ionic binding study, the tertiary amine salt and

†C. Dohen, Massachusetts General Hospital, Boston, private communication.

‡Miles Laboratories, γ-globulin, fraction II.

Table I. Borane Reagents for Protein Modification

No.	Compound	Amino acid residues to be modified
1	$HCB_{10}H_{10}C\text{-}C_6H_4N_2^+\ Cl^-$	Tyrosines, lysines, histidines, guanidines, indoles
2	$HCB_{10}H_{10}C(CH_2)_3\overset{+}{N}Me_2(CH_2)_2C_6H_4N_2^+\ 2Cl^-$	
3	$^-B_{10}H_{10}NNC_6H_4N_2^+$	
4	$HCB_{10}H_{10}C(CH_2)_3\overset{+}{N}Me_2CH_2C_6H_4C(\overset{+}{N}H_2)OCH_3\ 2Br^-$	Lysines
5	$HCB_{10}H_{10}C\text{-}(CH_2)_3C(NH\cdot HCl)OCH_3$	Lysines
6	$^-C_2B_9H_{11}(CH_2)_3C(\overset{+}{N}H_2)OCH_3$	Lysines
7	$HCB_{10}H_{10}C(CH_2)_3\overset{+}{N}Me_2(CH_2)_2CHO\ Cl^-$	Lysines
8	$^-C_2B_9H_{11}(CH_2)_3\overset{+}{N}H_3$	Aspartates, glutamates
9	$Me_2SB_{10}H_8NCO^-\ Na^+$	Lysines
10	$B_{10}H_{10}NNC_6H_4NH_2^-\ Na^+$	Aspartates, glutamates

Table II. Human γ-Globulin Modification with Borane Derivative **9**

Reagent/protein	% protein remaining in soln[a]	Boron content, γ/ml[b]	% boron bound by wt
20	98	9.34	0.32
60	99	18.95	0.64
160	88	29.50	1.12
1200	93	49.60	1.77

[a]Protein concentration is to 5% accuracy ($\gamma = 10^{-3}$ mg). [b]Boron concentration is corrected for background and is to about 15% accuracy.

Table III. Bovine Serum Albumin Modification with Borane Derivatives

Reagent	Reagent/protein molar ratio	% protein remaining in soln[a]	Boron content, γ/ml[b]	% boron bound by wt
1	25	64	52.7	2.2
	75	47	143.6	8.1
2	30	60	55.0	2.47
	40	50	61.5	2.92
4	5	85	6.71	0.31
	10	66	10.5	0.49
	15	75	30.3	1.35
	35	27	17.9	2.22
5	100	12.5	13.85	6.66
7	60	91	3.01	0.20
	180	85	10.19	0.72
	600	40	14.30	2.15
8	40	100	77.9	0.61
	80	67	104	1.20
9	80	98	23.9	1.60
	160	89	29.7	1.98
	400	83	34.4	2.48
$K_2B_{10}H_{10}$	80	100	2.2	0.02

[a]Protein concentration accurate to 5%. [b]Boron concentration accuracy is about 10%.

the aminocarbollide were synthesized as reported.[4] The other compounds were prepared by methods in the literature.[15,16]

Experimentally the salts were mixed with a solution of human γ-globulin. Visual estimates of protein precipitation were then taken 12 hr later. Results are as given in Table IV. Experimental conditions are given in footnotes to this table.

The results of these studies indicate that some ionic borane compounds do cause precipitation. In particular, carbollide derivatives at pH <6 precipitate most of the protein. Perhaps this effect is due to the proximity of the lysine ε-amino sites (and to arginine sites) thus reducing the protein-solubilizing, cationic interactions with water. Fortunately, however, most of the other ionic compounds do not cause γ-globulin precipitation when mixed in high concentration with the protein.

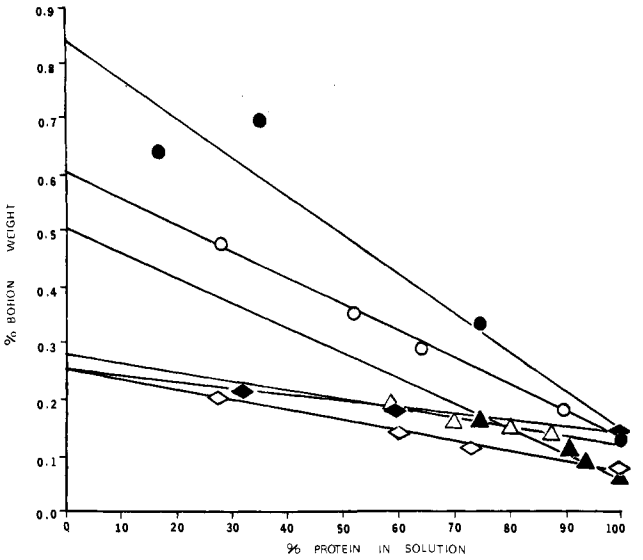

Figure 1. The percentage by weight of boron bound to protein in solution after the reaction of carborane reagents with human γ-globulin. The abscissa measures the per cent of original protein still in solution. Key: (1) ◇, $HCB_{10}H_{10}CC_6H_4N_2^+\ Cl^-$; (2) ◆, $HCB_{10}H_{10}C(CH_2)_3N^+Me_2(CH_2)_2C_6H_4N_2^+\ 2Cl^-$; (4) ●, $HCB_{10}H_{10}C(CH_2)_3N^+Me_2CH_2C_6H_4C(NH_2^+)OCH_3\ 2Br^-$; (5) △, $HCB_{10}H_{10}C(CH_2)_3C(NH\cdot HCl)OCH_3$; (6) ▲, $^-C_2B_9H_{11}(CH_2)_3C(NH_2^+)OCH_3$; (7) ○, $HCB_{10}H_{10}C(CH_2)_3N^+Me_2(CH_2)_2CHO\ Cl^-$.

The results for binding of the carborane derivatives to human γ-globulin are shown in Figure 1. The abscissa indicates the percentage of original protein in solution after completion of reaction, rigorous dialysis, and centrifugation. The ordinate measures the percentage of weight of boron bound to the soluble protein. Increasing the molar ratio of the reagent to protein generally increased the amount of precipitation of protein, while also increasing the per cent of boron by weight bound to the remaining protein.

The diazonium salts **1** and **2** (lines 1 and 2 in Figure 1) show nearly linear response with a slight negative slope. The linearity of amount of precipitation *vs.* added reagent suggests that the protein can bind only a small number of diazonium derivatives and still retain solubility. Analysis of the yellow-orange precipitate using molar reagent **2**: protein ratios of 300:1 and 600:1 give a large percentage by weight of boron, 1.7 and 3.2%, respectively. These precipitates are partially soluble in 0.01 *N* NaOH. Thus, the extra ionic center of the tetraalkylammonium reagent (line 2) apparently increased only slightly the capacity of the protein to bind a diazonium reagent containing carbo-

Table IV. Effects of Adding Ionic Boranes to Human γ-Globulin[a]

Compound	pH	Result
$HCB_{10}H_{10}C\text{-}(CH_2)_3\overset{+}{N}(CH_3)_2H\ Br^-$	7.0[a]	0
$HCCB_{10}H_{10}CCOOH$	7.0[a]	0
$HOOCCB_{10}H_{10}CCOOH$	7.0[a]	X
$C_2B_9H_{12}^-\ K^+$	5.0[a,b]	XX
$B_{10}H_{10}^{2-}\ 2K^+$	5.0[b]	0
$^-HCB_9H_{10}C(CH_2)_3NH_3^+$	5.0[a,b]	XX
$Me_2SB_{10}H_9^-\ Na^+$	7.0[a]	0
$Me_2SB_{10}H_8NCO^-\ Na^+$	7.0[a]	0

[a] To 2 ml of protein (15 mg/2 ml) was added 2 ml of borane in water [total of 15 mg of compound except for $KC_2B_9H_{12}$, where 6 mg is used, and a saturated solution of $C_2B_9H_{11}(CH_2)_3NH_3$ of about 3 mg/2 ml]. Visual estimates on precipitation after 12 hr are given. Key: 0, no precipitation; X, slight precipitation; XX, gross precipitation. [b] The pH was adjusted to 5.0 to simulate carbodiimide reaction conditions.

rane. However, the boron content of the protein in solution in either case is relatively low (less than 0.3%). Under the conditions of this reaction the boron bound to γ-globulin was about 50% less than that reported earlier for binding to the 7S fraction of rabbit antibovine serum albumin.[1] In Figure 2 we show the uv spectra at pH 7 of γ-globulin (0.61 mg/ml) bound to 0.21% boron from the tetraalkylammonium-diazonium reagent. These spectra are similar to those reported for the azo-γ-globulin derived from diazotized *p*-arsanilic acid.[17]

Solubilizing the carborane diazonium reagent 2 with a tetraalkylammonium center does not significantly alter its binding ability to γ-globulin as compared to the nonsolubilized diazonium reagent 1. The slight negative slope in Figure 1 indicates that the number of nonprecipitating reaction sites are few and may be reasonably similar in all of the γ-globulin molecules. Thus, the possible number of diazonium-bound carboranes to the hydrophobic groups of γ-globulin seems very limited, and increasing the hydrophylicity of these reagents may not significantly alter this number.

The diazotization reaction of the *p*-azodecaborate–aniline (3) with $NaNO_2$ under acidic conditions (pH 1.8) yielded a purple solution which did not pass through a dialysis bag. Under more acidic diazotization conditions (1 *N* HCl), the purple product precipitated out of solution in 0.5 hr. These results and the synthesis of bis(azodecaborate) compounds[18] suggest that the arylamine is probably polymerizing under reaction conditions.

In Figure 1, the imido esters 4–6 generally show higher negative slope and greater overall binding than the diazonium compounds. Compound 5 is extremely sensitive to moisture and use of up to 800:1 reagent to protein ratio gave only up to 0.18% boron (by weight) binding, under conditions that denatured 50% of the protein. Imido ester 6, solvated by the carbollide anion, shows better binding (0.3% for 50% recovery of protein) and imido ester 4, solvated by a tetraalkylammonium center, shows an even more significant improvement in boron binding (0.48% for 50% recovery of protein).

The aldehyde 7 attached up to 0.37% boron with loss of half of the original protein concentration. Control experiments with the acetal of 7 under identical reaction conditions failed to show any significant boron binding. Hence, the aldehyde functional group is essential to this modification reaction. Another control run with the aldehyde 7 but omitting the subsequent addition of $NaBH_4$ resulted in extensive precipitation of the protein. We suggest that the intermediate Schiff base complex formed between the aldehyde and the protein amino group renders the protein

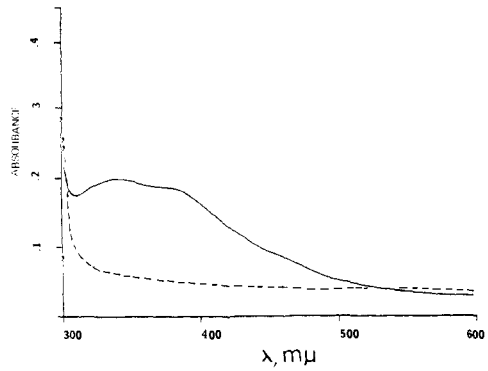

Figure 2. Uv spectra of human γ-globulin (1.86 mg/ml) at pH 7 (dashed line) and of human γ-globulin (0.61 mg/ml) bound with 0.21% boron from the reagent $HCB_{10}H_{10}C(CH_2)_3N^+(CH_3)_2\text{-}CH_2CH_2C_6H_4NN^+\ 2Cl^-$ at pH 7 (normal line).

insoluble, while subsequent reduction with borohydride to give the secondary amine (which can protonate at pH 7) enables the modified γ-globulin to remain in solution.

It is thus likely that the interaction of the protonated lysine ε-amino groups with water appears to help solubilize γ-globulin. X-Ray structural studies of several enzymes indicate that lysine residues are generally found on the surface of proteins.[19,20] Their side chains thus project into the surrounding water medium. This suggests that extensive modification of the ε-amino groups with retention of charge would be least likely to affect essential protein conformation when compared to that on the more hydrophobic amino acid residues. Some evidence of the validity of this argument as pertaining to γ-globulin is seen in the almost quantitative modification by molecular reagents which are smaller than carborane cages.[7,8]

Figure 1 indicates that the specific ε-amino modifying reagents 6, an imido ester, and 7, an aldehyde, show much better binding than the diazonium reagent 2 which may have attacked less hydrophilic groups such as tyrosines and histidines (see the uv spectra of Figure 2). All three of these reagents are solubilized by tetraalkylammonium centers.

The greater negative slope for the imido ester 4 and the aldehyde 7 in Figure 1 relative to the diazonium reagent 2 can be rationalized in two ways. The first is that binding can either take place randomly on acceptable ε-amino sites (soluble protein) or on unacceptable ε-amino sites (insoluble protein). As more reagent is bound, more protein precipitates due to binding onto unacceptable sites. At the same time, the boron content of the soluble protein increases from improved binding to these acceptable sites. A second explanation is that there are two or more types of protein in solution. One or more may have a lower binding limit compared to the others. As more reagent is added, the lower binding protein precipitates linearly. Further binding studies with a more homogeneous group of immunoglobulin may clarify this situation.

Our best results are as follows (Table II). Extensive modification of up to 25% of the γ-globulin's free amino groups was achieved with the isocyanate 9 with little loss by precipitation. Binding of up to 1.77% boron is obtained by using a 1300:1 ratio of reagent to protein. Control runs with sodium 1-dimethylsulfoniumdecaborate ($NaMe_2SB_{10}H_9$) under similar conditions failed to show any binding. Thus, the participation of the isocyanate functional group is essential to the boron binding in this example. Partial destruction of the NCO group in 9 by previous reaction with 0.5 equiv of propylamine also gave a corresponding decrease in binding. The excellent binding ability of 9 is another example of the advantage of a solu-

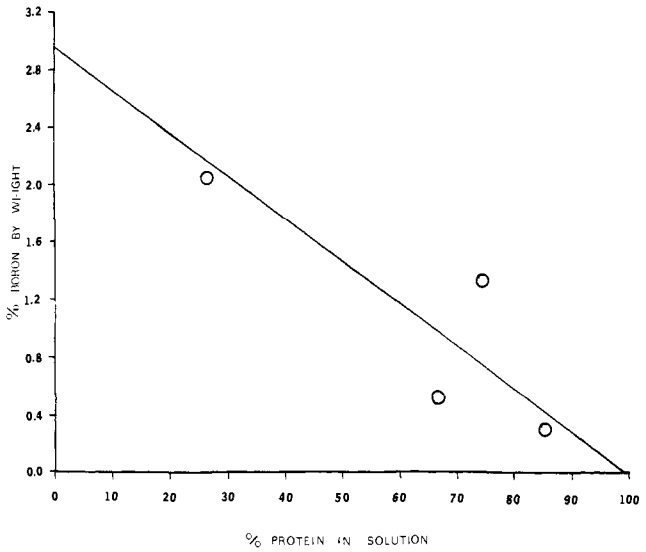

Figure 3. The percentage by weight of boron bound to protein in solution after the reaction of 3 with BSA. The absissa measures the per cent of original protein still in solution.

bilized borane cage when attached to specific amino groups.

Several nucleophilic borane derivatives were used with 1-ethyl-3-dimethylaminopropylcarbodiimide (EDC) in attempts to modify the free carboxyl groups in the protein.[11,12] The aminocarbollide (reagent 8) reacted in a DMF–salt water mixture to yield 86% soluble protein binding from 0.18 to 0.38% by weight boron. However, there is some possibility that this boron is not covalently bound. The ionic binding study suggests that the carbollide may strongly bind ionically to γ-globulin. In either case, the binding capacity of 8 is low. The reaction was run at pH 7.9 since the borane reagent precipitated protein at lower pH in the absence of EDC.§

When added to EDC and γ-globulin at pH 4.5, the anion decaborate ($B_{10}H_{10}^{2-}$) did not show significant binding. Neither this decaborate anion nor the aminoazoborane (reagent **10**) seems to be a good nucleophile for the carbodiimide activated additions to carboxyl groups.

Modification reactions on BSA gave generally improved results for all reagents. The diazonium ion **1** especially can give up to 8.1% boron binding. The imido ester **4** results are plotted in Figure 3. Thus, up to 1.5% of boron can be bound for 50% recovery of BSA. Assuming one type of reaction, we find that this percentage corresponds to some 20% modification of BSA's 61 free amino groups,[21] whereas the same reagent can only modify 10% of γ-globulin's 90 free amino groups.[21] The linear relationship of increased precipitation of BSA with increasing boron binding is similar to that found for γ-globulin.

Conclusion

For a recovery of 50% of the γ-globulin, up to 0.5% of boron by weight can be chemically bound with certain carborane derivatives. This averages to about eight carborane moieties per γ-globulin molecule. This result occurs in spite of the fact that both the localities and character of charges on the protein residues are preserved in both the imido ester and reductive alkylation modifications. As mentioned above, solubilization of the carborane cage by introduction of ionic centers does show improved binding results in γ-globulin compared to the strictly hydrophobic imido ester **5**. Thus, the attachment of over ten carborane moieties either disrupts the protein's overall conformation causing denaturation or decreases solvent interactions of the antibody, causing precipitation. Since γ-globulin has very little helical structure and has also a fairly uniform distribution of ionic groups on its surface,[22,23] it is possible that the binding of carboranes on the surface ionic centers results in a drastic decrease of protein–solvent interaction due to the inherent hydrophobic nature of the carborane nucleus. The more soluble BSA gives improved binding for all reagents, further illustrating the solubility factor. Hence the significant enhancement of binding using the decaborate derivative **9** is probably due to the inherent water solubility of the anion, allowing more extensive modification without causing loss of solubility. We note that extensive ε-amino modifications of ε-amino groups on immunoglobulins have produced little change in conformations or antigen activities.[7,8,24-27] It is hoped that further studies of γ-globulins as modified here will similarly show the retention of antibody–antigen activities.

Thus, we have been able to bind increased amounts of boron to γ-globulin while maintaining its solubility at the physiological pH. Evaluation of more homogeneous antibodies when modified by these reagents will aid in furthering their potential use in the ^{10}B neutron capture therapy of neoplasm. This work is now in progress.

Acknowledgment. We thank Dr. W. Sweet of Massachusetts General Hospital, Boston, Mass., for encouragement of this project and J. Messer and C. Pierce at that hospital for generously performing the protein and boron analyses. The National Institutes of Health supported a predoctoral fellowship for E. I. T. This study was supported by the Office of Naval Research and the National Institutes of Health.

References

(1) M. F. Hawthorne, R. J. Wiersema, and M. Takasagi, *J. Med. Chem.*, **15**, 449 (1972).
(2) A. G. Mallinger, E. L. Jozwiak, Jr., and J. C. Carter, *Cancer Res.*, **32**, 1947 (1972).
(3) A. H. Soloway, "Progress in Boron Chemistry," Vol. 1, H. Steinberg and A. L. McCloskey, Ed., Macmillan, New York, N. Y., 1964, Chapter 4.
(4) H. S. Wong, E. I. Tolpin, and W. N. Lipscomb, *J. Med. Chem.*, **17**, 785 (1974).
(5) (a) D. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951); (b) A. Kaczmarczyk, J. R. Messer, and C. E. Pierce, *Anal. Chem.*, **43**, 271 (1971).
(6) K. H. Saunders, "Aromatic Diazo Compounds," E. Arnold and Co., London, 1947.
(7) S. Fuchs and M. Sela, *J. Biol. Chem.*, **240**, 3558 (1965).
(8) L. A. Wofsy and S. J. Singer, *Biochemistry*, **2**, 104 (1963).
(9) G. E. Means and R. E. Feeney, *Biochemistry*, **7**, 2192 (1968).
(10) S. J. Hopkins and A. Wormall, *Biochem. J.*, **27**, 838 (1933).
(11) D. G. Hoare and D. E. Koshland, *J. Amer. Chem. Soc.*, **88**, 2057 (1966).
(12) D. G. Hoare and D. E. Koshland, *J. Biol. Chem.*, **242**, 2447 (1967).
(13) J. L. Fahey, *Advan. Immunol.*, **2**, 000 (1962).
(14) P. L. Carpenter, "Immunology and Serology," W. B. Saunders, London, 1968, p 92.
(15) R. A. Wiesboech and M. F. Hawthorne, *J. Amer. Chem. Soc.*, **86**, 1642 (1964).
(16) S. Y. Tyree, Jr., *Inorg. Syn.*, **9**, 16 (1967).
(17) G. F. Fairclough, Jr., and B. L. Vallee, *Biochemistry*, **9**, 4097 (1970).
(18) M. F. Hawthorne and F. P. Olsen, *J. Amer. Chem. Soc.*, **87**, 2366 (1965).
(19) T. Amoto, L. N. Johnson, A. C. J. North, D. C. Phillips, and J. A. Rupley in "The Enzymes," P. D. Boyer, Ed., Academic Press, New York, N. Y., 1972, Chapter 21.
(20) F. A. Quiocho and W. N. Lipscomb, *Advan. Protein Chem.*, **25**, 1 (1971).
(21) G. R. Tristram, "The Proteins," Vol. 1, H. Neurath and K.

§At pH less than 7.5, the γ-globulin was grossly precipitated in the presence of the aminocarbollide.

Bailes, Ed., Academic Press, New York, N. Y., 1900, p 181.
(22) H. J. Gould, T. J. Gill, and P. Doty, *J. Biol. Chem.*, **239**, 2842 (1964).
(23) J. K. Weltman and M. Sela, *Biochim. Biophys. Acta*, **92**, 553 (1964).
(24) F. Karush and M. Sela, *Immunochemistry*, **4**, 259 (1967).
(25) M. H. Freedman, A. L. Grassberg, and D. Pressman, *Immunochemistry*, **5**, 6367 (1968).
(26) A. F. S. A. Habeeb, *Arch. Biochem. Biophys.*, **121**, 652 (1967).
(27) C. C. Chen, A. L. Grossberg, and D. Pressman, *Biochemistry*, **1**, 1025 (1962).

Protein-Binding Polyhedral Boranes. 1

R. L. Sneath, Jr., A. H. Soloway,* and A. S. Dey

Department of Medicinal Chemistry, College of Pharmacy, Northeastern University, Boston, Massachusetts 02115.
Received January 21, 1974

The development of neutral polyhedral boranes with protein-incorporating functions offers the opportunity of binding these structures to tumor antibodies. This research has led to the synthesis of such boron compounds. Their low incorporation level into proteins indicates the need for water-solubilizing groups in order to increase the number of boron atoms which may be attached to the antibody carrier. This work is a prelude to any clinical trials of this chemoradiotherapeutic procedure.

Clinical failures in the treatment of malignant tumors by neutron capture therapy have stemmed largely from an inability to localize a neutron absorber, such as boron-10, uniformly throughout the neoplasm.[1-3] This major deficiency results in part from the inadequate biochemical information regarding differences between normal and malignant tissues and the inability to utilize the available knowledge to fabricate tumor-seeking boron compounds. However, there is evolving information in the field of tumor immunology and immunochemistry that has a direct bearing on this problem. The capacity of the host, both animal and man, to recognize the malignant process by antibody elaboration against such cells presages a capability to deliver specific nuclides selectively to the tumor. A key requirement is an ability to incorporate such substances into proteins as models for tumor antibodies.

The possible use of ^{10}B antibodies for neutron capture therapy has been suggested[4] and more recently preliminary steps in this direction have been undertaken.[5,6] The types of boron compounds which would be suitable are those which contain a high percentage of boron since it is this boron percentage which will determine the effectiveness of this chemoradiotherapeutic procedure. Two basic types of boron compounds meet this requirement: (1) carboranes and (2) polyhedral boranes. Since ^{10}B is the only isotope of boron which is useful for neutron capture, a major consideration when approaching this research problem must be the ease of synthesizing these boron hydride species from ^{10}B-enriched starting materials. Since ^{10}B-enriched polyhedral boranes can be more easily synthesized and in higher yields from $^{10}BF_3$ etherate than the corresponding carboranes, most of this research effort has involved the readily synthesizable $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$ anions. These anions per se have been shown to be strongly bound by ionic forces to proteins. Such a property might readily obscure and prevent selective tumor localization *via* the boron-bound antibody, since ionically bound compounds may be readily exchanged with other blood proteins. In order to obviate the protein binding by ionic species, the synthesis of neutral polyhedral boranes containing protein-incorporating functions was undertaken. These functional groups would permit covalent incorporation into proteins. An important binding requirement is that this must occur without conformational alterations since such changes may render the antibody unsuitable as a carrier entity. For these reasons mild conditions for the incorporation of these boron compounds into proteins are essential. Such procedures are well established in protein chemistry utilizing certain functional moieties. Among these are carboxyl groups, aromatic amines, and isothiocyanates. Such entities were chosen for the studies reported here.

Results and Discussion

Synthetic Chemistry. Bis(dimethylsulfido)octahydrodecarborane, $B_{10}H_8[S(CH_3)_2]_2$,[7] and the corresponding dodecaborane, $B_{12}H_{10}[S(CH_3)_2]_2$,[8] can be readily synthesized in suitable yield from their respective anions. Reaction of either 1,10- or 1,6-$B_{10}H_8[S(CH_3)_2]_2$ with potassium phthalimide at elevated temperatures results in the removal of one methyl group to form the corresponding anionic species **1**.[7] This anion has been shown to be quite

$$(CH_3)_2SB_{10}H_8S(CH_3)_2 \xrightarrow[\text{phthalimide}]{\text{potassium}} K^+[(CH_3)_2SB_{10}H_6SCH_3]^- \quad \mathbf{1}$$

nucleophilic, displacing both alkyl and benzylic halides and tosylates. This displacement reaction has led to the synthesis of type **2** compounds containing various functional groups.

$$(CH_3)_4N[(CH_3)_2SB_{10}H_8SCH_3] \xrightarrow[\text{where X = halogen or tosylate}]{RX} (CH_3)_2SB_{10}H_8S(CH_3)R \quad \mathbf{2}$$

a, R = $-CH_2CH_2COOH$
b, R = p-$CH_2C_6H_4NO_2$
c, R = $-(CH_2)_5COOH$
d, R = m-$CH_2C_6H_4COOH$
e, R = p-$CH_2C_6H_4NH_2$
f, R = p-$CH_2C_6H_4NCS$
g, R = $-CH_2CH{=}CH_2$

A similar reaction scheme has been applied to the synthesis of functionally substituted $B_{12}H_{10}R_2$ species.[8] In this case, separation of the isomeric dimethylsulfidodecahydrododecaboranes was not carried out as in the B_{10} species. The entire isomeric mixture was used in the synthesis of compounds **3** and **4**.

$$B_{12}H_{10}[S(CH_3)_2]_2 \xrightarrow[\text{phthalimide}]{\text{potassium}} K^+[(CH_3)_2SB_{12}H_{10}SCH_3]^- \quad \mathbf{3}$$