The experimental intercept obtained by extrapolation of the k_2 slope may be equated to the last two terms at the right in equation 18. Evaluation of the intercepts for runs I-23 and I-12 gives values of 0.9 and 1.3×10^{-3} for α.

In the partially swept reactions, where initially reaction 8 prevails but the oxygen is not removed and may react with any alkyl radicals formed in reaction 9 by reactions 5 and 6 to form a hydroperoxide, SOOH, $-\mathrm{d}\ \ln\{[\mathrm{H}] + [\mathrm{SOOH}]\}/\mathrm{d}t = k_a$. (It was assumed that SOOH may be treated as ROOH and that $\mathrm{d}[\mathrm{O}_2]/\mathrm{d}t = 0$.) Thus the proposed mechanism and the assumptions above can account for the fact that the observed rates in the partially swept system were about half of those in the swept system.

The extremely slow decomposition in sealed ampoules can be explained in terms of the postulated mechanism with the additional assumptions that the evolution of oxygen is prohibited in a closed system and instead reaction 8 leads to a dimer, D, which effectively lowers the hydroperoxide concentration available for reaction with any alkyl radicals. The dimer would still have peroxidic properties toward iodide ion and would be so determined throughout the observation period. The rate under these circumstances would be a function of the total peroxide concentration, [P], where $[\mathrm{P}] = [\mathrm{H}] + 2[\mathrm{D}]$, and $-\mathrm{d}\ \ln[\mathrm{H}]/\mathrm{d}t = 2k_a$ and $\mathrm{d}[\mathrm{D}]/\mathrm{d}t = k_a k_b[\mathrm{H}]^2/\{[\mathrm{H}] + \alpha[\mathrm{HS}]^2\}$. These equations may be solved to give the relationship

$$[\mathrm{P}] = \alpha[\mathrm{HS}]^2 \ln\left\{\frac{[\mathrm{H}_0]e^{-2k_a t} + \alpha[\mathrm{HS}]^2}{[\mathrm{H}_0] + \alpha[\mathrm{HS}]^2}\right\} + [\mathrm{H}_0] \quad (19)$$

which cannot be conveniently used. The pseudo first-order rates in Tables IB are of value only for orientation. That the formation of a dimer is not unreasonable was demonstrated by one of the experiments, run I-9, which was initially heated in a sealed tube for 404 hours before being transferred to a swept system in which there was a trace of oxygen being added systematically. The remaining hydroperoxide still had an absorption band appearing at 12 μ, and decomposed at a rate equal to that of the anaerobic system. This observation (similar results were noted qualitatively on dilute solutions) would only be possible if conversion to the dimer occurred in the sealed tube and it could decompose unaffected by the trace of added oxygen. (Run I-10 affords a comparison for the aerobic system.)

An approximate E_a may be calculated for the reaction from data obtained at 86.1° in the helium-swept apparatus: $k_1 = 1.32 \times 10^{-6}$, $k_2 = 3.88 \times 10^{-6}$ sec.$^{-1}$. From the respective values of k_1 at the two temperatures one obtains a value of 30.7 kcal./mole for the E_a of reaction 7. Using the absolute rate equation

$$k = (kT/h)e^{-\Delta H^*/RT}e^{\Delta S^*/R}$$

and assuming that Δv^* is equal to zero for reaction 7 in the expression, $\Delta H^* = E_a - RT + p\Delta v^*$, one obtains the following values: $\Delta H^* = 30.0$, $\Delta F^* = 30.9$ kcal./mole, and $\Delta S^{\ddagger} = -2.3$ cal./deg./mole.

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[CONTRIBUTION FROM THE DEPARTMENT OF PEDIATRICS, UNIVERSITY OF PENNSYLVANIA AND THE CHILDREN'S HOSPITAL OF PHILADELPHIA]

The Interaction of Purified Anti-β-lactoside Antibody with Haptens[1]

BY FRED KARUSH

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A study has been made of the combination of purified anti-β-lactoside antibody with the uncharged azo dye *p*-(*p*-dimethylaminobenzeneazo)-phenyl β-lactoside by the method of equilibrium dialysis. The extrapolation of the binding curves yields a value of 2.0 for the valence of the antibody. The values of ΔF^0 fall within the same range previously observed for another system involving an anionic haptenic group. The temperature dependence of the binding curves reveals, however, a substantial difference in the values of ΔS^0 and ΔH^0 for the two systems. These differences are related to the absence of a charge in the β-lactoside haptenic group. The heterogeneity of the antibody as shown by the non-linearity of the binding curves can, except in one case, be described by a Gauss error function with a value of 1.5 for the heterogeneity index. The large value of ΔH^0, about −10 kcal./mole of hapten, is interpreted to mean that the R groups of the antibody can form strong hydrogen bonds with the disaccharide portion of the hapten, sufficient to withstand the disruptive effects of competing water molecules. The association constants of a number of sugar derivatives have been evaluated by their inhibition of the dye binding. These results demonstrate in quantitative energetic terms the selective character of the combining region of the antibody with respect to the configuration of the terminal hexoside and the geometry of the glycosidic linkage (α or β), as well as the participation of the non-terminal hexoside in the specific interaction.

In a previous communication[2] we have presented the results of a study of the structural and energetic aspects of the combination of haptens with a purified antibody specific for the haptenic group phenyl-(*p*-azobenzoylamino)-acetate (I_p). In order to extend our understanding of these aspects of antibody–hapten interaction, it appeared desirable to obtain similar results with an antibody homologous to a different type of haptenic group. The particular differences that were of greatest interest were the absence of a charged group in the new haptenic group and a strong affinity for water in contrast to the uncharged form of the earlier haptenic group.

The choice of *p*-azophenyl β-lactoside as the haptenic group to be studied was based largely on the pioneering investigations of Goebel, Avery and

(1) This investigation was supported by a research grant (H-869) from the National Heart Institute of the National Institutes of Health, Public Health Service.

(2) F. Karush, THIS JOURNAL, **78**, 5519 (1956).

Babers.[3] Following the demonstration by Avery and Goebel[4] that antibodies were formed against the azophenyl β-glycosides of glucose and galactose which could distinguish between these sugars, these authors investigated the properties of antisera specific for the azophenyl β-glycosides of several disaccharides, maltose, cellobiose, gentiobiose and lactose. This work showed that the homologous antibody was complementary to both hexose units of the disaccharide and sufficiently closely fitted to the contour of the latter to be able to distinguish between a galactose unit and a glucose unit as the terminal hexoside and between the 1,4-α- and 1,4-β-linkages. The use of a disaccharide as the main portion of the haptenic group was also of interest because of its close relationship to the large number of naturally occurring polysaccharide antigens.

To secure the information desired we have studied the combination of purified antibody specific for the *p*-azophenyl β-lactoside group (Lac group) with the uncharged azo dye *p*-(*p*-dimethylaminobenzeneazo)-phenyl β-lactoside (Lac dye) and quantitatively evaluated the competitive effect on this combination of structurally related haptens.

Experimental

Materials.—The compound which was required for the preparation of the immunizing and precipitating antigens and for the synthesis of Lac dye was *p*-aminophenyl β-lactoside. The first step in its synthesis was the preparation of acetobromolactose by the adaptation of a method described for acetobromoglucose.[5] The main difference in the procedures is that the reaction product in our case was suspended in isopropyl ether rather than recrystallized from the solvent because of its relative insolubility. This crude preparation of acetobromolactose was then employed for the synthesis of *p*-aminophenyl β-lactoside in accordance with the procedure of Babers and Goebel.[6] The acetobromolactose was converted to heptaacetyl *p*-nitrophenyl β-lactoside according to the method of Glaser and Wulwek[7] as described by Babers and Goebel. It was found that an improvement in yield was obtained if the reaction was carried out at *p*H 9 for three hours and the *p*H maintained constant by addition of 50% NaOH solution. After one recrystallization from methanol the product melted at 131–133°. It was then deacetylated with barium methylate to yield *p*-nitrophenyl β-lactoside which melted at 259° with decomposition as reported by Babers and Goebel. The reduction of this material was effected by hydrogenation in aqueous solution at a pressure of 45 p.s.i. with 5% Pd on alumina as the catalyst. The product was crystallized from 70% ethyl alcohol and melted at 240°. Recrystallization of the material left the melting point unchanged. The value of $[\alpha]_D$ at room temperature was −35° (*c* 2.5) as compared with $[\alpha]^{27}_D$ −36° reported by Babers and Goebel.[6]

The antigens used in this study were prepared by diazotizing *p*-aminophenyl β-lactoside and coupling to bovine γ-globulin and human fibrinogen to yield Lac-BγG and Lac-HF. The former was employed as the immunizing antigen and was obtained by the method previously described.[2] The human fibrinogen used to prepare the precipitating antigen, Lac-HF, was contributed by the American Red Cross and was further purified by the method of Laki.[8] Some modification of the previously reported procedure[9] for the preparation of azofibrinogen was necessary. The coupling was done at *p*H 10 to keep the product in solution and the reaction mixture was dialyzed *vs.* twenty volumes of 0.01 *M* borate buffer, *p*H 10 for several days in the cold room with daily changes of the outside solution. Prior to its use as a precipitating agent the Lac-HF was diluted in 0.15 *M* NaCl to a concentration of 400 μg. N/ml. and the solution adjusted to *p*H 7.4. This was done at least 24 hours preceding its addition to antiserum. The original solution at *p*H 10 was stored in the frozen state.

The binding experiments were carried out with the Lac dye prepared by coupling diazotized *p*-aminophenyl β-lactoside with dimethylaniline at *p*H 4.1 to give *p*-(*p*-dimethylaminobenzeneazo)-phenyl β-lactoside. The product was separated from the reaction solution by virtue of its low solubility at *p*H 7 and recrystallized twice from 95% ethanol; m.p. 238°. On drying to constant weight *in vacuo* over P_2O_5 at 80° the melting point remained unchanged. However, when the product was subjected to prolonged heating (44 hr.) at 170° there was a weight loss of 2.8%, before evidence of decomposition was observed, compared to a calculated value of 3.1% for a monohydrate. On the basis of this information and its elementary composition, the compound obtained after drying at 80° was considered to be a monohydrate.

Anal. Calcd. for $C_{26}H_{35}O_{11}N_3.H_2O$: C, 53.51; H, 6.40. Found: C, 53.44; H, 6.32.

The absorption spectrum of Lac dye in 0.15 *M* NaCl, 0.02 *M* phosphate, *p*H 7.4 is shown in Fig. 3. The molar extinction coefficient, ε, at the wave length of maximum absorption λ 455 mμ is 2.48×10^4. A minimum occurs at λ 340 mμ with a value of ε of 0.52×10^4.

Of the nine compounds used in the inhibition experiments lactose, galactose, cellobiose and maltose were commercial products of which all but cellobiose were reagent grade. Methyl α-D-galactoside, methyl β-D-galactoside and methyl β-lactoside were generously provided by Dr. N. K. Richtmyer and methyl β-D-glucoside by Dr. H. B. Wood, Jr. The remaining compound, *p*-nitrophenyl β-lactoside, was the intermediate used in the preparation of the corresponding amino compound.

Preparation of Antisera.—The same procedures for injection, bleeding and pooling of sera were employed in the preparation of anti-Lac antisera as were described for the anti-I_p system.[2]

Purification of Anti-Lac Antibody.—The purification of anti-Lac antibody follows the same general procedure which has been presented for anti-I_p antibody.[9] After removal of complement by the BγG–anti-BγG precipitate the anti-Lac antibody was precipitated by the addition of Lac-HF in the amount of 10 μg. N/ml. of serum. The washed specific precipitate was extracted with 25 ml. of a solution containing 0.1 *M* lactose in 0.05 *M* phosphate, *p*H 7.4. The extraction was carried out for one hour at 37° and provided a solution which contained almost all of the originally precipitated antibody. The amount of antigen which was solubilized could be judged from the color of the solution and was quite variable. When the quantity was more than negligible it was removed from the extract by precipitation with 0.8 *M* phosphate, *p*H 7.4, with some loss of antibody. The antibody was then precipitated in an ice-bath by the addition of the quantity of 4 *M* phosphate solution required to give a final concentration of 1.6 *M*. The 4 *M* phosphate solution was a mixture of K_2HPO_4 and KH_2PO_4 whose *p*H was adjusted so that on dilution to 1.6 *M* a *p*H of 7.4 was obtained. The precipitated antibody was dissolved with 24 ml. of 0.05 *M* phosphate and reprecipitated in an ice-bath by the addition of 16 ml. of the 4 *M* phosphate solution. This step was repeated two times and the antibody was then dissolved in 25 ml. of 0.15 *M* NaCl. The solution was dialyzed against 2 liters of 0.15 *M* NaCl in the cold for several days with daily changes of the outside solution. The small amount of precipitate which was formed during the dialysis was removed by centrifugation. The N content of the supernatant was determined by micro-Kjeldahl analysis. Finally, 1% of its volume of 2 *M* phosphate was added to give 0.02 *M* phosphate, *p*H 7.4. The yield of antibody appears to be about 50% of that initially precipitated. A quantitative precipitin analysis with one preparation of purified antibody showed that 144 μg. N/ml. of antibody could be precipitated with Lac-HF from a solution containing 150 μg. N/ml.

Binding Experiments.—The binding of Lac dye by the purified antibody was measured in the specially designed cells and under the same conditions as described for our previous investigation.[2] One ml. of 2.5×10^{-5} *M* antibody,

(3) W. F. Goebel, O. T. Avery and F. H. Babers, *J. Exp. Med.*, **60**, 599 (1934).

(4) O. T. Avery and W. F. Goebel, *ibid.*, **50**, 533 (1929).

(5) "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., p. 81.

(6) F. H. Babers and W. F. Goebel, *J. Biol. Chem.*, **105**, 473 (1934).

(7) F. Glaser and W. Wulwek, *Biochem. Z.*, **145**, 514 (1924).

(8) K. Laki, *Arch. Biochem. Biophys.*, **32**, 317 (1951).

(9) P. Karush and R. Marks, *J. Immun.*, in press.

based on a molecular weight of 156,000, in 0.15 M NaCl, 0.02 M phosphate, pH 7.4, was used in one compartment of the cell and one ml. of dye in the same solvent in the other. In the experiments involving inhibitors these were included in the dye solutions. The dye concentrations measured after equilibration ranged from 0.2×10^{-5} M to 5×10^{-5} M. In calculating the amount of dye bound to antibody correction was made for the dye adsorbed to the cellulose casing. The amount of adsorbed dye relative to the amount bound to antibody ranged from 11 to 22% at 25° and from 6 to 19% at 7°.

Absorption Spectrum.—The absorption spectrum of the Lac dye bound to antibody was determined as previously described.[2] The total concentration of the dye in the solution measured was 1.72×10^{-5} M of which 93% was bound to the antibody present at a concentration of 2.64×10^{-5} M.

Results and Discussion

The Binding of Lac Dye by Antibody.—The binding curves for the combination of Lac dye with its homologous antibody are shown in Figs. 1 and 2 for two different pools of purified antibody. Data

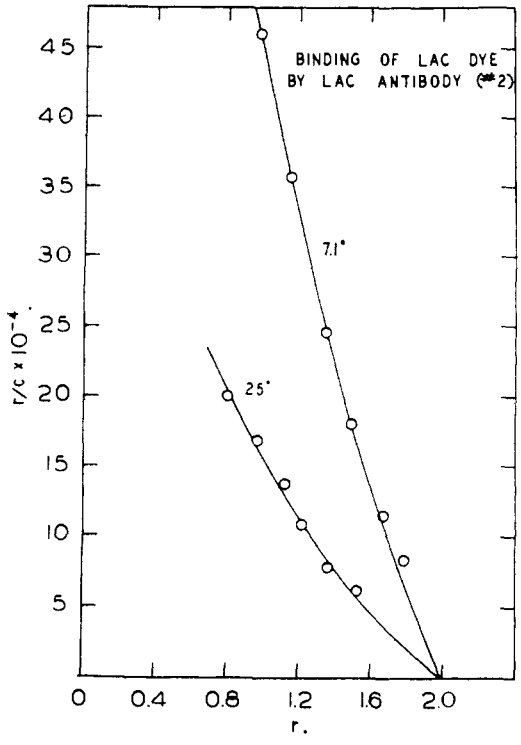

Fig. 1.—Binding results at 25 and 7.1° for the reaction between Lac dye and purified anti-Lac antibody (#2). The points are experimental and the curves are theoretical.

were obtained at two temperatures and the results were plotted in the form of r/c *vs.* r, for reasons previously given,[2] where r is the average number of dye molecules bound per antibody molecule at the equilibrium concentration of free dye c. In agreement with the results of the earlier study the present data allow the unambiguous extrapolation of the binding curves to yield a value of 2.0 for the binding capacity (n) or valence of the antibody. The same result was found with binding curves obtained at 25° with two other pools of purified antibody.

The deviation of the binding curves from linearity reflects the heterogeneity of the purified antibody with respect to its affinity for the Lac dye. It was found previously that such heterogeneity could

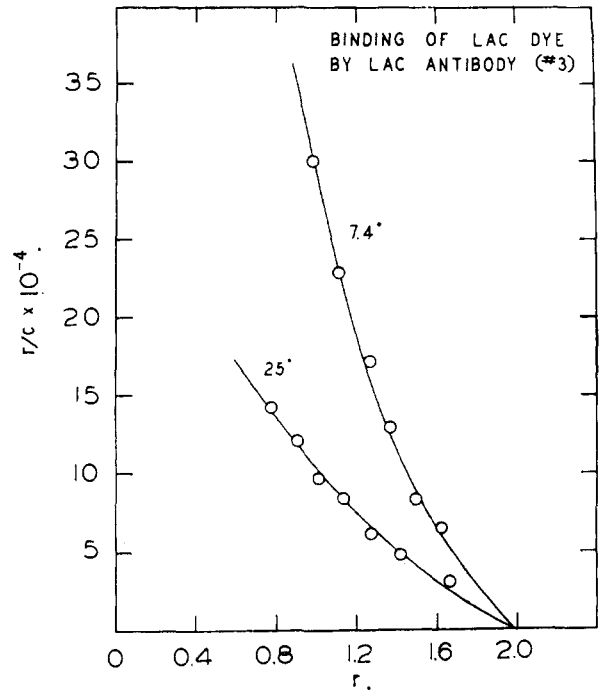

Fig. 2.—Binding results at 25 and 7.4° for the reaction between Lac dye and purified anti-Lac antibody (#3). The points are experimental and the curve for 25° is theoretical.

be adequately described by a Gauss error function in terms of the free energy of binding. Except for the measurements at 7.4° the present data can be satisfactorily fitted by such a function with a value of 1.5 for the heterogeneity index σ. The curves shown for the three sets of points involved are theoretical curves calculated assuming values of σ and n of 1.5 and 2.0, respectively. The average value of the association constant, K_0, which must also be known for the calculation, was taken as equal to the experimental value of r/c for r equal to 1. The reason for the failure to describe adequately the data at 7.4° with σ equal to 1.5 is not clear. It is of interest to note that the heterogeneity of the purified antibody specific for the Lac group is considerably less than that observed previously with the anionic hapten. In the latter case a value of 2.3 for σ was required to fit the experimental results.

The values of the thermodynamic quantities shown in Table I were calculated in the usual way using the indicated association constants. Comparison with our earlier results shows that the present values of ΔF^0 fall within the range observed before in spite of the considerable difference in the chemical nature of the hapten groups under comparison. This difference does, however, manifest itself in the comparative values of ΔS^0 and ΔH^0. Whereas the anionic hapten gave a value of ΔS^0 of about zero for the association reaction, the combination of the Lac dye with its homologous antibody yields a substantially lower value, −9 e.u. per mole of dye. Such a negative value is not unexpected in view of the fact that it refers to an association reaction, although the loss of translational and rotational degrees of freedom is probably not the only contribution to the net entropy change for the process of combina-

tion. Since complex formation between the anionic dye and its antibody most probably involves charge neutralization, the difference in the entropy results may be reasonably attributed to the positive entropy contribution which arises from the release of ion-bound water on ion-pair formation.

TABLE I

THERMODYNAMIC RESULTS FOR THE BINDING OF LAC DYE BY PURIFIED ANTI-LAC ANTIBODY[a]

Plac no.	n	σ	25°: $K_a \times 10^{-4}$, l./mole	25°: $-\Delta F^0$, kcal./mole	7°: $K_a \times 10^{-4}$, l./mole	7°: $-\Delta F^0$, kcal./mole	$-\Delta H^0$, kcal./mole	ΔS^0, e.u./mole
2	2.0	1.5	15.7	7.09	44.8	7.25	9.7	−8.8
3	2.0	1.5	10.4	6.85	29.0	7.01	9.7	−9.5

[a] The lower temperatures for Plac #2 and Plac #3 were 7.1 and 7.4°, respectively. The value of 1.5 for σ applies, in the case of Plac #3, only to the results obtained at 25°. The values of the thermodynamic functions are expressed as per mole of Lac Dye.

The large negative value of ΔH^0, about −10 kcal./mole of hapten, is of considerable interest, particularly since by far the greatest portion of it, as will be seen from the inhibition results, is due to the interaction between the disaccharide portion of the hapten and the antibody. This result emphasizes the capacity of the antibody to provide attractive interactions, for this type of molecule, which in their total energetic effect greatly exceed the competitive interactions between solvent molecules and the disaccharide. The latter interactions, which are primarily hydrogen bonding, are of considerable magnitude as is evident from the high solubility of sugars, including lactose, in aqueous solution. We surmise from these considerations that the R groups of antibody, and of proteins in general, can form hydrogen bonds with hapten, and with each other, of such stability as to withstand the disruptive competitive influence of water molecules.

The similarity in the ΔF^0 values for the two kinds of haptens in spite of the large difference in ΔH^0 is noteworthy. The reason for this may be, as was originally suggested by Pauling,[10] that the process of antibody formation and release into the circulation provides an effective selection of only those antibody molecules whose affinity does not exceed some maximum value. A lower limit for the affinity could, of course, result from the procedure employed to isolate the antibody, namely, specific precipitation.

Absorption Spectrum of Bound Dye.—It is evident from the spectra of bound Lac dye and free dye shown in Fig. 3 that complex formation in this system leads to a spectral shift similar to that observed in our earlier study. This consists of an increase in the molar extinction coefficient, ϵ, at the wave length of maximum absorption, accompanied by a broadening of the absorption band. Although the effect is less pronounced in the case of the Lac dye than in the case of the anionic dye, we interpret the spectral shift of the bound Lac dye to be due, as before, to the formation of a hydrogen bond between the azo group of the dye and a proton-donating group of the antibody.

(10) L. Pauling, THIS JOURNAL, 62, 2643 (1940).

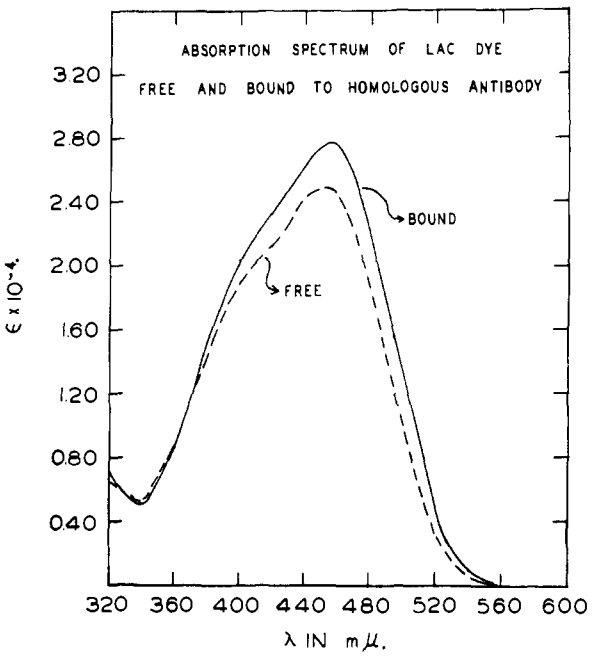

Fig. 3.—The absorption spectra of Lac dye free and bound to anti-Lac antibody.

Inhibition by Structurally Related Haptens.—Further information about the scope and selectivity of the combining region of the antibody has emerged from inhibition experiments. These experiments were designed to give the association constants for combination with antibody of the compounds listed in Table II. The values were obtained in an indirect manner, namely, by measuring the capacity of these colorless compounds to inhibit the binding of Lac dye by the antibody. The calculation of the constants utilized equations 4 and 5 of our previous paper. Several preliminary experiments were done to establish the concentrations of the inhibitors which would yield the most accurate results. The concentrations which were finally selected are shown in Table II. Except for methyl β-D-glucoside and maltose, they gave a degree of inhibition which minimized the uncertainty in the calculation of the average association constants due to experimental error and the heterogeneity of the antibody.

A close examination of Table II allows a number of interesting conclusions to be drawn regarding the combining region of the antibody:

(1) The fact that the ΔF^0 value for *p*-nitrophenyl β-lactoside is only slightly greater (0.4 kcal.) than that for the Lac dye indicates that the size, or volume, of the energetically effective region is probably not much larger than that required to accommodate this hapten. This inference agrees well with our earlier estimate of the size of the combining region specific for the anionic hapten group.

(2) The large negative values of ΔF^0 for methyl β-lactoside and lactose demonstrate that the interaction between the disaccharide portion of the haptenic group and the antibody provides the dominant contribution to the stability of the complex. From this conclusion we further deduce, as has already been mentioned, that hydrogen bonding

TABLE II

THE INHIBITION OF LAC DYE BINDING BY STRUCTURALLY RELATED MOLECULES AT 25°

p-(*p*-Dimethylaminobenzeneazo)-phenyl β-Lactoside

Inhibitor	Initial concn. in dye soln., moles/l.	Plac #12 ($K_0 = 13.4 \times 10^4$, $-\Delta F^0 =$ 7.00 kcal./mole) $K_I \times 10^{-4}$, l./mole	Plac #12 $-\Delta F^0$, kcal./mole	Plac #13 ($K_0 = 11.9 \times 10^4$, $-\Delta F^0 =$ 6.92 kcal./mole) $K_I \times 10^{-4}$, l./mole	Plac #13 $-\Delta F^0$, kcal./mole
p-Nitrophenyl β-lactoside	1.00×10^{-4}	6.75	6.60	7.26	6.63
Methyl β-lactoside	2.00×10^{-4}	2.02	5.88	2.38	5.98
Lactose (64% β)	4.00×10^{-4}	1.10	5.52	....	..
Methyl β-D-galactoside	0.0600	0.00747	2.56	0.0114	2.81
Galactose (69% β)	.0600	.00457	2.27	....	..
Cellobiose (66% β)	.100	.00275	1.96	....	..
Methyl α-D-galactoside	.200	.00134	1.54	0.00130	1.52
Methyl β-D-glucoside	.200	.00051	0.97	.00059	1.05
Maltose (64% β)	.200	.00033	0.70	....	..

is the main intermolecular force involved in the Lac system. It seems most unlikely that any interaction other than multiple hydrogen bonding could be as energetically effective as is required by the competitive role of the solvent.

(3) By a comparison of methyl β-lactoside, methyl β-D-galactoside and methyl β-D-glucoside we estimate that the β-galactoside unit contributed about 5 kcal./mole whereas the β-glucoside unit contributes only 3 kcal./mole. Thus the terminal hexoside is the dominant one of the two hexosides of the haptenic group although both play a major energetic role. These conclusions are entirely consistent with those drawn by Goebel, Avery and Babers[3] from their observations on cross-reactivity and the inhibition of specific precipitation in their disaccharide systems.

(4) The sharp specificity of the antibody with respect to the configuration of the terminal hexoside is clearly demonstrated by the difference of 3.6 kcal. between lactose and cellobiose. These sugars differ only in the reversal of the positions of the hydrogen atom and the hydroxyl group on carbon atom 4 in the terminal hexoside, and yet their association constants differ by a factor of 400. The difference in free energy is probably compounded of two factors. One would be the loss of a hydrogen bond and the other steric repulsion arising from the presence of an hydroxyl group in a position for which, in the original antigenic group, the antibody needed to provide accommodation only for the much smaller hydrogen atom.

(5) Finally, the specificity of the antibody with respect to the configuration of the linkage between the hexosides, α or β, may be noted. This is shown by the lower affinity of methyl α-D-galactoside compared to methyl β-D-galactoside and by the comparison between maltose and cellobiose. In both instances the homologous β-configuration is more stable than the α-configuration by about one kcal.

Acknowledgment.—I am indebted to Mr. Robert Marks for carrying out the organic syntheses and antibody purification and to Mrs. F. Karush for the binding and spectrophotometric measurements.

PHILADELPHIA, PA.