as epinasty, root initiation, growth retardation, hastening of fruit ripening, and defoliation have been attributed to this chemical (*1*). More recently, treatment of pineapple with the same chemical has induced flowering (*2*).

Monoecious cucumbers of the cultivar "S.C. 23" were sprayed with one or more applications of a water solution of 2-chloroethanephosphonic acid at concentrations of 120, 180, and 240 parts per million (*3*). We applied the treatments to the point of runoff, beginning when the first true leaf was about 2 cm in diameter. Plants treated more than once were sprayed at 48-hour intervals.

The greatest number of continuous pistillate nodes was produced by plants treated with either two applications of 240 parts per million or four applications of 120 parts per million (Table 1). Lesser numbers of nodes were affected by the lower dose, suggesting an additive effect of the chemical. Untreated plants usually did not bear a pistillate flower until about node 9 and produced only 3.5 pistillate flowers on the first 20 nodes (Fig. 1).

It was also possible to enhance the yield of a monoecious variety with this chemical (*4*). The variety "Model," when sprayed with the chemical, produced essentially the same total yield as the five leading gynoecious varieties in a variety trial. Earliness of production was also improved.

The ability to control flowering in cucumbers will have far-reaching effects, for example, on the production of hybrid seed. To produce a hybrid, one need only plant the parental lines in separate rows, treat the female parent with 2-chloroethanephosphonic acid and let bees make the pollen transfer. If there is adequate isolation from other cucumber plantings, viable seed produced by the female parent should result from the cross.

Table 1. Effect of 2-chloroethanephosphonic acid on the production of pistillate flower-producing nodes by plants of the monoecious cucumber cultivar "S.C.23." Data were recorded for 20 nodes in all cases, and node No. 1 was the first true-leaf node; that is, the one immediately beyond the cotyledonary node.

Concentration (ppm)	Applications (No.)	Pistillate nodes	
		Continuous (No./plant)	Total (No./plant)
240	2	18.9	19.1
120	4	18.7	19.0
180	2	16.2	16.5
240	1	16.1	16.2
120	2	16.0	16.1
120	1	12.6	12.9
180	1	12.0	12.5
0	0	0.0	3.5

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3. Experiments were replicated three times on ten plants per plot for each treatment.
4. Plants were sprayed with the chemical once with a 120-ppm solution when the first true leaf was about 2 cm in diameter.
5. Published with the approval of the director of the North Carolina State University Agricultural Experiment Station. We thank Amchem Products, Inc., Ambler, Pennsylvania, for furnishing the chemical.

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Affinity Site Labeling of a Mouse Myeloma Protein Which Binds Dinitrophenyl Ligands

Abstract. *A mouse myeloma protein of the immunoglobulin A class, which specifically binds dinitrophenyl ligands, has been successfully affinity labeled (site-directed labeling) with several diazonium reagents, leading to inactivation of the combining sites. The labeling reagents reacted only with tyrosine residues of light chain origin. The 7S subunit of the myeloma protein appears to contain only one reactive site.*

Several human (*1*) and mouse (*2*, *3*) myeloma or Waldenström immunoglobulins which appear to be homogeneous and capable of binding ligands with high specificity have been described. They apparently have a homogeneous binding constant (*1*, *3*, *4*), and they satisfy all the operational criteria for chemically defining a single molecular form of antibody.

One mouse immunoglobin A myeloma protein (MOPC 315) which has been immunochemically characterized resembles normal mammalian antibodies to dinitrophenyl ligands in many of its characteristics (*3*). It binds ε-*N*(2,4 dinitrophenyl)-L-lysine as determined by equilibrium dialysis, and when the ligand is bound, it exhibits the characteristic shift in absorbancy and fluorescence quenching (*5*). Unlike induced antibodies which typically consist of molecules of varying affinities, the MOPC 315 protein appears to have uniform combining sites of high affinity ($K_A = 1$ to $2 \times 10^7 M^{-1}$) (*3*). Other mouse myeloma proteins of the immunoglobulin A class have been isolated which specifically combine with either dinitrophenyl or trinitrophenyl ligands, though these seem to have a lower combining affinity than MOPC 315.

In order to compare the combining sites of these proteins with each other and with those of specifically induced antibodies, we have applied the technique of affinity labeling (*6*). In this method, a modified ligand which is initially bound noncovalently to the combining site is used. The ligand contains a reactive group that can form a covalent bond with an amino acid residue side chain. In that it is preferentially localized in the combining site, the ligand reagent will preferentially react with side chains available in the combining site.

We now report results obtained when MOPC 315 was reacted with diazonium reagents under the conditions used to label rabbit antibodies to dinitrophenyl determinants (*7*, *8*).

The MOPC 315 tumor was grown in strain Balb/c mice; the myeloma protein was isolated from serum or ascites fluid by precipitation with ammonium sulfate at 5°C and dialyzed against water until a copious euglobulin precipitate formed. The euglobulin precipitate was either dissolved directly in the appropriate labeling buffer or in a 0.2*M* tris(hydroxymethyl)aminomethane-hydrochloric acid (tris) buffer (*p*H 8.6) prior to reduction and alkylation. The euglobulin (1 to 2 percent solution) was reduced with 0.01*M* dithiothreitol at room temperature for 1 hour, and the reduced protein was alkylated with iodoacetamide, in 10 percent molar excess over sulfhydril reagent, at *p*H 8.0 for 15 minutes at room temperature. The reduced alkylated preparation was then applied to a G-200 Sephadex column equilibrated with a *p*H 8.0 borate NaCl buffer and eluted at room temperature. Only the component having an effluent volume comparable to 7*S* γ-globulin was used.

A human γG_1 myeloma protein [γG(War)] was used as a control protein and was isolated by diethylaminoethylcellulose chromatography from a sodium sulfate precipitate of whole serum.

In all cases, the labeling reactions were conducted on protein solutions having an optical density of 4 at 280 nm. For analytical work, reaction volumes of 0.5 ml were routinely used. After reaction for various times, the

diazonium reagent was inactivated with resorcinol, the protein was precipitated with ethanol, and the precipitate was washed with ethanol and ether (*6*). Absorption spectra and radioactivity were determined as described (*6*, *8*, *9*).

The mouse and human myeloma proteins were reacted with *p*-nitrobenzenediazonium fluoroborate (PNBDF) and *m*-nitrobenzenediazonium fluoroborate (MNBDF) at *p*H 5.0 in a 0.5*M* sodium acetate buffer and with *p*-carboxybenzenediazonium fluoroborate (PCBDF) at *p*H 8.0 in borate-NaCl buffer at 2°C. The reaction was rapid, as evidenced by formation of colored azo products with both the nitrobenzene derivatives in the presence of the mouse protein; but no visible change occurred when the reagents were reacted with the human myeloma protein. By comparison with the model compound, the reaction with MOPC 315 yielded predominantly azotyrosine derivatives (Fig. 1). On the basis of the known extinction coefficients (*7*, *8*) for the ionized azotyrosine derivatives at 520 and 490 nm for the *p*-nitro- and *m*-nitrobenzene compounds, respectively, the amount of product could be quantitated after various times of reaction. With both reagents, the amount of azotyrosine formed at 10 minutes was at least 500 to 1000 times as much with MOPC 315 as with γG(War). Maximum modification was obtained with $4 \times 10^{-5}M$ PNBDF for 60 minutes and with $8 \times 10^{-5}M$ MNBDF for 3 hours. Only trace amounts of azotyrosine were formed with γG(War) under these conditions.

With PCBDF, the difference in reaction with MOPC 315 and γG(War) was much less. In both cases, a mixed azo-spectrum was found. After subtraction of the γG(War) spectrum from the MOPC 315 spectrum it was clear, however, that an enhanced formation of azotyrosine was occurring with MOPC 315 (Fig. 1). The amount of azohistidine and azotyrosine formed during the course of reaction could be quantitated by spectroscopic analysis (*9*). No difference in the relative rates for the formation of azohistidine was found for the two proteins, but the rate of azotyrosine formation was approximately four times as rapid with MOPC 315 as with γG(War). With $8 \times 10^{-5}M$ PCBDF, reaction for 12 hours appeared to give the highest differential yield of azotyrosine.

Under the conditions described, the yield of specific azotyrosine formation was 4.5×10^{-6}, 4.0×10^{-6}, and 3.3×10^{-6} mole/liter per optical density unit of the protein at 280 nm for PNBDF, MNBDF, and PCBDF respectively (*10*). In an experiment with tritiated MNBDF (^{3}H-MNBDF), the amount of specifically bound reagent was calculated to be 5.1×10^{-6} mole/liter per optical density unit. Quantitatively identical results were obtained with labeling of MOPC 315 that had been reduced and alkylated as those with untreated protein.

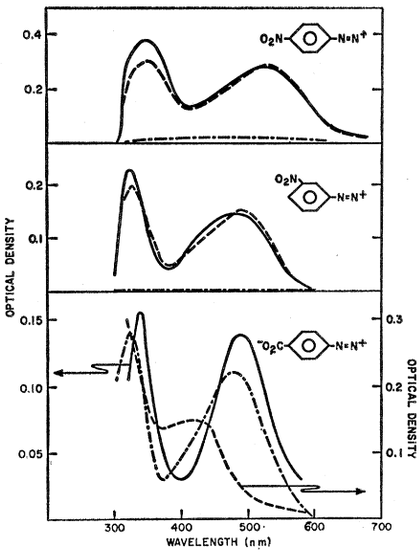

Fig. 1. Spectra of modified MOPC 315 (solid line), γG(War) (light dashed line) and the appropriate azo-*N*-chloroacetyltyrosine derivative (heavy dashed line) in alkaline solution. In each case, the spectrum is for a protein concentration of 4 optical density units at 280 nm. (Top) Products of reaction with PNBDF. (Middle) Products of reaction with MNBDF. (Bottom) Products of reaction with PCBDF. In the latter instance, the spectrum given for MOPC 315 is a difference spectrum calculated by subtracting the spectrum of γG(War) (reacted with PCBDF under identical conditions) from the spectrum of MOPC 315. For the azotyrosine spectrum of the model PCBDF derivative the spectrum of *p*-azobenzenearsonic acid-*N*-chloroacetyltyrosine was used because it closely resembles that for the *p*-carboxy compound (*9*).

Labeling experiments with PNBDF were conducted with four other mouse myeloma proteins that react with dinitrophenyl or trinitrophenyl ligands by one of several criteria (*3*). MOPC 460 precipitates with dinitrophenyl- and trinitrophenyl-substituted proteins; MOPC 292 and MOPC 378 precipitate only with trinitrophenylated proteins; MOPC 329 does not precipitate with nitrophenylated proteins but does produce a characteristic spectral shift with dinitrophenyl ligands. None of these four showed significant labeling with PNBDF under the conditions used with MOPC 315. These results emphasize the specificity of the reaction with MOPC 315 (*11*).

The presence of the nonreactive ligand ε-*N*-(2,4 dinitrophenyl)aminocaproate sharply reduced the specific labeling of MOPC 315. For example, with $2 \times 10^{-5}M$ ^{3}H-MNBDF, $5.1 \times$

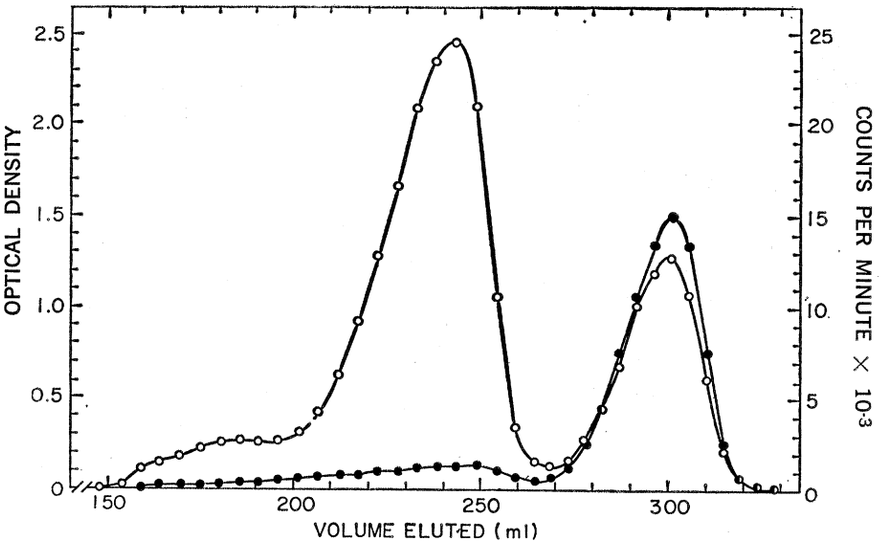

Fig. 2. Elution pattern of reduced alkylated MOPC 315, which had been labeled with tritiated MNBDF, from a G-200 Sephadex column. The solvent was 6*M* in urea and 0.1*M* in acetic acid. The light chain peak contained 26 percent of the recovered optical density and was eluted at the same volume at which light chains from γG(War) were eluted. The latter result is consistent with the light chains of the two myeloma proteins being of comparable size. Approximately 85 percent of the recovered radioactivity was found under the light chain peak of the labeled MOPC 315 protein. (Optical density at 280 nm.)

10^{-6} mole of label per liter per optical density unit was obtained, whereas under the same conditions but in the presence of $2 \times 10^{-4}M$ dinitrophenyl caproate only 1.1×10^{-6} mole/liter was labeled. A control experiment with the human myeloma protein confirmed that the dinitrophenyl caproate did not inactivate the MNBDF. The activity of the MOPC 315 labeled to a level of 4.6×10^{-6} mole/liter was compared to that of the unreacted protein.

As tested by equilibrium dialysis with tritiated dinitrophenyl caproate, the labeled preparation bound 1.8×10^{-6} mole of ligand per liter per optical density unit, whereas the unlabeled protein bound 4.6×10^{-6} mole. These results indicated that a substantial proportion of the combining sites were inactivated by the labeling reaction.

The heavy and light polypeptide chains of site labeled MOPC 315 were separated and analyzed. Whether the labeling had been performed before or after reduction and alkylation and whether PNBDF or MNBDF was used (no studies with PCBDF have yet been performed) the results were the same: essentially all of the specific labeling occurred on the light chains. In a typical elution pattern of MOPC 315 labeled with ^{3}H-MNBDF (Fig. 2) 85 percent of the recovered radioactivity was under the light chain peak. If the molecular weight and the extinction coefficient for the light chains of MOPC 315 are similar to those of other light chains (*12*) the number of moles of bound ^{3}H-MNBDF per mole of light chain is 0.46—that, is approximately 1 mole per 2 moles of light chains.

In view of these results we attempted to find out whether a population of labeled light chains could be differentiated from the unlabeled chains. Freshly isolated light chains from MOPC 315 labeled with ^{3}H-MNBDF were subjected to disc electrophoresis in alkaline 10*M* urea, and the radioactivity of the stained, sliced gel was determined (*13*). A major, single, dense band was obtained with a much fainter closely opposed slower and faster band on either side. The radioactivity was symmetrically distributed over the dense band. Thus, by this technique at least, we were unable to find evidence for two structurally distinctive types of light chain.

Overall, the labeling of MOPC 315 closely parallels the results obtained with rabbit antibodies and antibodies from other species labeled with the same reagents (*7, 8, 14*). With the rabbit antibodies the initial rate with MNBDF was very similar to that obtained with PNBDF, although the former gave a somewhat higher yield of specifically labeled binding sites (*8*). With MOPC 315, the rate with MNBDF was slightly lower than with PNBDF. With the rabbit antibodies, PCBDF reacted much more slowly (*7*), presumably because of the lower association constant for the benzoic derivative. The rate of azotyrosine formation was five times greater than that for the same preparation in the presence of a competing nonreactive ligand. With MOPC 315, the rate was four times that found with the human myeloma protein control. In both cases there was no enhanced rate of azohistidine formation. The major differences between the results obtained with MOPC 315 and previous studies are related to the uniformity of labeling, location of the label, and valence.

With the rabbit antibodies, only 25 to 50 percent of the combining sites were easily modified (*7, 8*), and the kinetics of labeling were clearly heterogeneous (*7*). With MOPC 315, 80 to 90 percent of the combining sites were rapidly labeled, and this result indicates probable uniformity of the combining sites.

In contrast to previous studies on antibodies where affinity labeling resulted in labeling of both chains (*15*), essentially all the label appeared on the light chains (Fig. 2). However, although 80 to 90 percent of the combining sites had been modified, the amount of label was equivalent to only 1 mole per two moles of light chains. This is consistent with the analysis of the 7*S* monomer which indicates 1 mole of label per mole of subunit (*10*) and with hapten binding studies on the monomer [see above and (*3*)]. The effective univalence of the monomer is in marked contrast to the divalence routinely observed with γG antibodies.

That the explanation of these unusual results may lie in the disposition of the chains, rather than in there being two different types of chains within the monomer, is suggested by our failure to detect two such hypothetical chain types in the disc electrophoresis experiment. Further studies on MOPC 315 and other similar proteins are required to determine whether these properties are peculiar to MOPC 315, to all mouse γA immunoglobulins or to all immunoglobulins of the A class. Most binding studies and all previous labeling studies have been performed only on γG proteins. The finding by Grey and Abel (*16*) that the disulfide linkages between the polypeptide chains of certain human and mouse immunoglobulins of the A class are different from those observed with γG immunoglobulins may be relevant. It suggests that the disposition of the chains may also be somewhat modified in these proteins (*16*).

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10. The extent of binding of ligands and of labeling is here presented as moles per liter per unit optical density of the proteins at 280 nm, since neither the extent of purity of the preparations used nor the molecular weight and molar extinction coefficient of the MOPC 315 protein have been absolutely determined. If we assume that the four-chain monomer is pure, has a molecular weight of 1.2×10^5 and a molar extinction of 1.56×10^4 M^{-1} cm (*3*) at 280 nm, an optical density unit is equivalent to 6.4×10^{-6} mole of protein per liter. Actually, it is estimated (H. N. Eisen, personal communication) that the euglobulin in preparations here used are about 80 percent pure; thus an optical density unit is equivalent to 5.1×10^{-6} mole of monomer per liter.
11. These results do not mean that these proteins cannot undergo affinity labeling with other reagents. By analogy with the experience obtained with rabbit antibodies, the dinitro- and trinitrophenyl analogs may well be bound with considerably higher association constants than the mononitro derivatives and may therefore be more effective labeling reagents.
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