

## Some Properties of Myeloma Proteins and their Papain Produced Subunits

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The 7 S myeloma proteins resolve into a number of components when subjected to electrophoresis in low ionic strength media. Their 3.5 S papain digest fractions likewise contain multiple electrophoretic components when studied under similar conditions. The fraction of lowest mobility in alkaline buffers contains the same number of components as the parent molecules. The factors responsible for this electrophoretic heterogeneity have not been ascertained.

A single myeloma protein component appears to possess most or all of the 3.5 S fragments found in papain digests of the unresolved parent system.

The 3.5 S subunit fractions of myeloma proteins produced by the actions of (1) papain, (2) pepsin and reducing agents, and (3) 8 M urea on reduced and alkylated protein, show distinct differences when subjected to electrophoresis in starch gel.

The normal 7 S  $\gamma$ -globulins and the hyperglobulinemic proteins from myeloma sera are being extensively employed in studies on the subunit composition of antibodies. Myeloma proteins display greater electrical homogeneity than normal  $\gamma$ -globulins as shown by moving boundary electrophoretic techniques<sup>1</sup>. It has been also previously noted that myeloma proteins may resolve into several distinct components upon electrophoresis in starch gel<sup>2,3</sup>. The present work indicates a relationship between the electrophoretic heterogeneity of myeloma proteins and certain of their papain produced subunits. Some of the properties of these subunits have been investigated and preliminary comparisons made between them and those produced by other methods.

### EXPERIMENTAL

Moving boundary electrophoretic studies utilized the Spinco Model H instrument. Vertical starch gel electrophoretic experiments were carried out by the method of Smithies<sup>4</sup> and employed a pH 8.6 Tris (0.045 M)-Versene (0.001 M)-Borate (0.025 M) buffer except when noted.

The myeloma (MM) proteins and their subunits were subjected to various treatments. They were reduced with 0.05 M 2-mercaptoethanol in 0.2 ionic strength, pH 8 Tris-HCl buffer for

one hour at 37°C and then alkylated by addition of iodoacetate or iodoacetamide to a concentration of 0.1 M. Reduced and alkylated proteins were separated from other reactants by dialysis or by passage through Sephadex G-25.

Conversion of MM proteins to 3.5 S fragments was effected by treatment with sulfhydryl activated papain<sup>5</sup>, by the action of pepsin followed by treatment with mercaptan<sup>6</sup>, and by treatment of the reduced and alkylated proteins with 8 M urea<sup>7</sup>. Removal of the activating sulfhydryl compound from the papain prior to the digestion of the MM proteins did not appear to influence the splitting reaction. Ultracentrifugal analyses were carried out with the Spinco Model E instrument to determine whether conversion to 3.5 S subunits was complete. The papain digestion products were separated by chromatography on CM- or DEAE-cellulose in a manner similar to that previously described<sup>5</sup>.

### RESULTS

Starch gel electrophoretograms of seven different 7 S MM proteins are shown in Fig. 1. The initials designate the patient source. It can be seen that each contains a series of components closely related with respect to mobility. The components are designated in order of decreasing cathodic mobility as MM-1, MM-2, etc. Similar patterns are obtained on electrophoresis in starch gel in pH 8.5, 0.01 M sodium diethylbarbiturate buffer. These MM proteins all migrate as relatively homogeneous peaks on moving boundary electrophoresis in 0.1 ionic strength buffers, but at low ionic strength a heterogeneity qualitatively similar to that observed in starch gel is seen. The results of moving boundary electrophoretic experiments on two of the MM proteins are shown in Fig. 2. One (Hu) is relatively acidic while the other (CO) has a much higher isoelectric point. They have electrophoretic mobilities in the pH 8.5, 0.1 ionic strength Na diethylbarbiturate buffer of  $-2.28$  and  $-0.64 \times 10^{-5}$  cm<sup>2</sup> volt<sup>-1</sup> sec<sup>-1</sup>, respectively.

The electrophoretic patterns of the papain digested (PD) proteins are included with the data of Fig. 2. When a MM protein is converted into 3.5 S units by this enzyme, two electrophoretic components, one more acidic and the other more basic than the parent protein, are seen. These fractions have been designated B and C respectively<sup>5</sup> and are analogous to the fast (F) and slow (S) subunits of other investigators<sup>8</sup>.

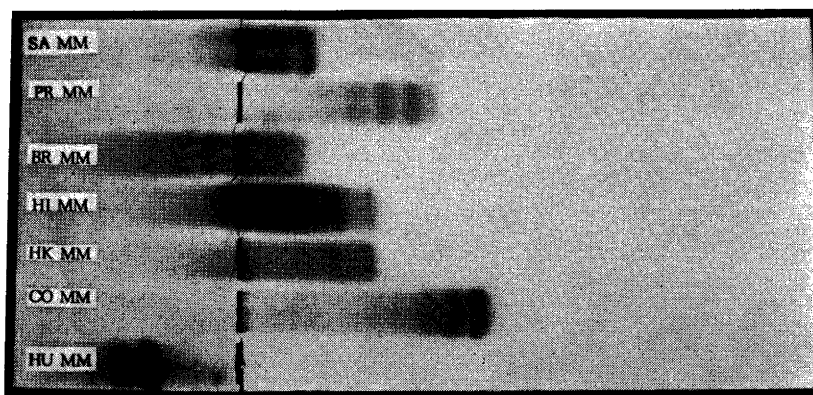


Fig. 1. Starch gel electrophoretograms of myeloma globulins. Migration to the right is cathodic.

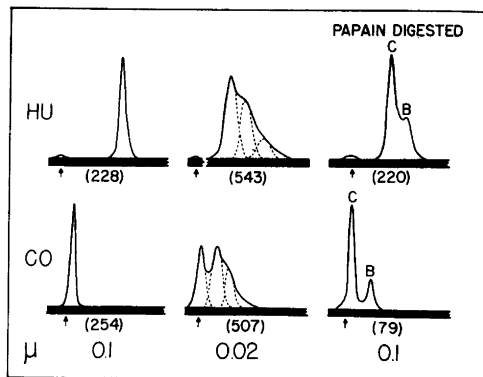


Fig. 2. Moving boundary electrophoretic diagrams of MM proteins HU and CO and their papain digestion products in pH 8.5, Na diethylbarbiturate buffer. The position of the starting boundary is indicated by an arrow and the duration of the experiment in minutes is given by the number in parenthesis. The potential gradient of the 0.1 ionic strength ( $\mu$ ) experiments was near 5.7 volts  $\text{cm}^{-1}$ ; for the 0.02  $\mu$  experiment it was 7.6 and 9.5 for HU and CO respectively.

Some separation of the electrophoretic components of certain MM systems has been achieved by chromatography on DEAE-cellulose, particularly when low ionic strength solutions have been employed in the early stages. An example of this is shown for the HI system in Fig. 3. A resolution qualitatively paralleling that observed on starch gel and on free electrophoresis at low ionic strength is noted.

A starch gel electrophoretogram of the papain digests of nine MM proteins, a pool of these papain digests (MM-PD's) and of papain digested normal  $\gamma_2$ -globulin ( $\gamma_2$ -N-PD) are shown in Fig. 4. As previously indicated<sup>9</sup>, the anodically migrating B fractions from various MM globulins are quite similar whereas the C-fractions are different. The number of cathodic C-components seen in a given

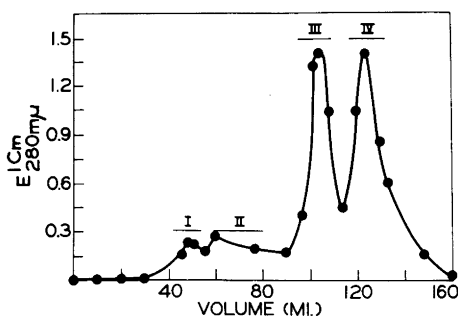


Fig. 3. Chromatography of 50 mg of myeloma protein HI on a  $1.2 \times 30$  cm. column of DEAE-cellulose. A pH 9.0, 0.02 M Tris-HCl buffer and a continuous salt gradient to 0.15 M NaCl was used to elute the protein.

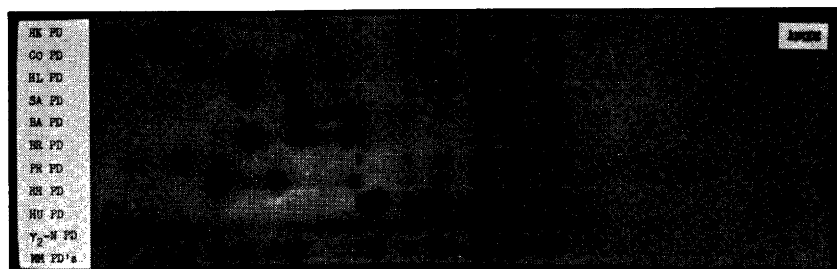


Fig. 4. Starch gel electrophoretograms of papain digests of myeloma and normal  $\gamma$ -globulins.

papain digest agrees with the number of components noted in the parent MM protein (see Fig. 1). The pooled MM papain digests (MM-PD's) possess components distributed throughout the mobility range of those of the  $\gamma_2$ -N-PD system. It is apparent that if a larger number of individual MM-PD's had been pooled a starch gel electrophoresis result similar to that for  $\gamma_2$ -N-PD would result. This reiterates a previous finding that the various  $\gamma_2$ -type MM proteins possess properties within the range of the proteins making up normal  $\gamma_2$ -globulins<sup>1</sup>.

The structural features of the various components in the acidic and basic fractions of the papain digests which are responsible for their electrophoretic mobility differences are not known. When the parent myeloma proteins and their papain digest products are reduced and then alkylated with iodoacetate, a decreased cathodic mobility for each system is noted (see Fig. 5). The position of each of the reduced and alkylated C-fraction components appears to be shifted to that of a more acidic component of the untreated fraction. These results indicate that each of the starch gel components of the C-fraction has the same number of readily cleaved, *i. e.*, labile, disulfide bonds. Papain digestion of reduced and alkylated MM protein gives a C-fraction having the same starch gel electrophoretic composition as C-fractions which have been reduced and alkylated following their isolation. Reduction and alkylation of the B-fraction likewise increases the anodic mobility of its components. However, in this case an increased number of components is also seen.

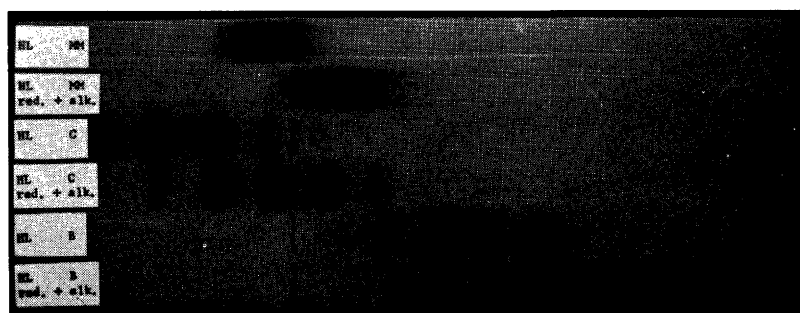


Fig. 5. Starch gel electrophoretograms of a myeloma protein and its papain digest products before and after reduction and alkylation with iodoacetic acid.

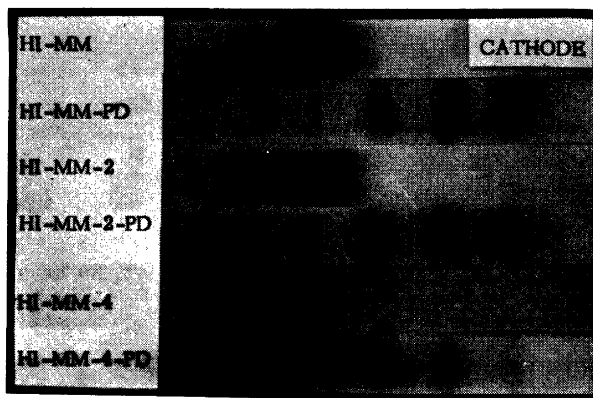


Fig. 6. Starch gel electrophoretograms of some HI-MM components and their papain digest products.

Preliminary amino acid analyses, cysteine and cystine determinations and immunochemical studies of these individual components suggest an identity with respect to these parameters. Treatment with neuraminidase produces no changes in their starch gel electrophoretic properties which indicates that differences in sialic acid content do not occur. Further studies on these systems are in progress to elucidate the basis of the electrophoretic heterogeneity noted in low ionic strength media.

A study of the effect of papain on the individual MM components resolved on electrophoresis has been hampered by the difficulty in purifying them in adequate amounts. The utilization of fractions separated by chromatographic methods of the type used to provide the data of Fig. 3, has given a partial answer to the obvious question of whether papain digestion of a single MM component will produce only one C-fraction component. The results shown in Fig. 6 indicate that the same number of basic components seen in papain digests of the whole MM protein is produced on digestion of one or two MM components. However, differences in relative amounts are indicated. It appears that the higher isoelectric point MM components yield larger amounts of similarly charged C-fraction components.

Starch gel electrophoretic studies of the 3.5 S material produced from MM proteins by the action of pepsin followed by reduction indicate differences from the papain digested systems. These results are shown in Fig. 7 A. The action of papain on the pepsin digest produces cathodically migrating components similar to those of the papain digest C-fraction. Material with the electrophoretic properties of the papain B-fraction is not produced from MM proteins by pepsin, and indeed, this fraction is cleaved by pepsin into dialyzable fragments.

Fig. 7 B shows the electrophoretic properties in a urea-formate starch gel<sup>10</sup> of the 3.5 S fragments of MM proteins produced by treatment with (1) pepsin and 2-mercaptoethanol, (2) papain, and (3) reduction and alkylation in 8 M urea. It is apparent that different subunits are produced by these methods. Reduction

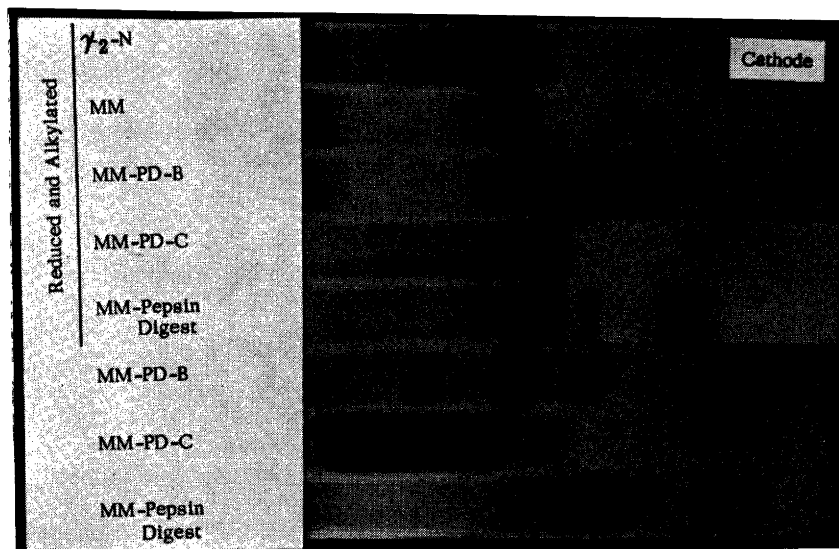


Fig. 7 A. Starch gel electrophoretograms in pH 8.5, Tris-Versene-Borate buffer of the HI-MM proteins digested with papain, with pepsin and with pepsin followed by papain.

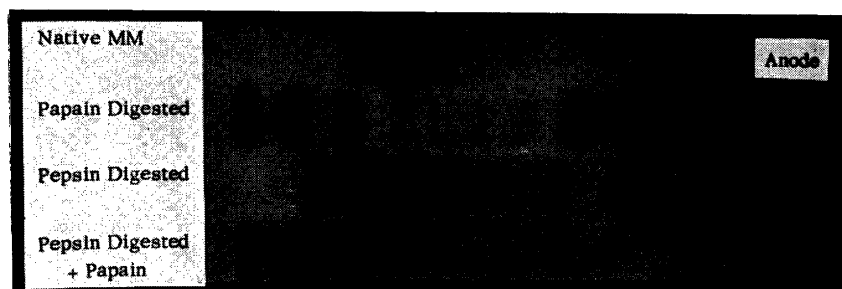


Fig. 7 B. Electrophoretograms in urea-formate starch gel of normal  $\gamma_2$ -globulins and various products of myeloma protein HI. The upper five reservoirs contain proteins that were reduced and alkylated prior to treatment with urea.

and alkylation is seen to markedly affect the 3.5 S products produced by pepsin and papain. The papain digestion C-fraction appears to have at least two subunits in common with the pepsin digest. Further relationships of the 3.5 S subunits produced by the three methods described remain to be explored.

#### DISCUSSION

Myeloma proteins appear to be composed of a family of molecules which show small differences in electrophoretic mobility in alkaline buffers of low ionic strength. Preliminary study of these components suggests that they possess many chemical and biological properties in common and differ only by slight

variations in charge. The acidic and basic fractions of the papain digests of myeloma proteins also show a number of starch gel electrophoretic components. Those of the basic fraction appear to reflect the differently charged entities in the parent protein. Unpublished data indicate that the individual components have a similar amino acid composition and immunochemical properties.

The myeloma proteins of mol. wt. near 160 000 separate into a series of components when subjected to electrophoresis in alkaline buffers of low ionic strength. Each of these components appears to consist of four 3.5 to 4 S subunits. One of them is acidic and is common to every 7 S myeloma protein. The remainder are relatively more basic and appear to be closely related but differentiable from each other on the basis of charge. The number of the latter in a given myeloma protein appears to be the same as the number of parent 7 S components which can be resolved on electrophoresis. It would appear that three such different basic subunits should be found in each parent myeloma protein component along with one of the above discussed acidic units. Concepts of the unit composition of the 7 S molecules will be dependent on a better understanding of these subunits. This knowledge must await more adequate separation of the individual 7 S MM components.

Each of the electrophoretic components of the papain digest fractions possesses the biological activity characteristic of the parent molecules. The genetic Inv factors are present in each of the C-fraction components while those of the B-fraction possess Gm-1 genetic and anticomplementary activity<sup>9</sup>.

Further more searching experimentation will be required to explain the noted interesting electrophoretic polymorphism. The results of such studies should provide insight into the properties of normal  $\gamma_2$ -globulins.

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#### REFERENCES

1. Deutsch, H. F., Morton, J. I. and Kratochvil, C. H. *J. Biol. Chem.* **222** (1956) 39.
2. Binette, J. P. and Schmid, K. *Nature* **192** (1961) 732.
3. Fahey, J. L. *J. Clin. Invest.* **42** (1963) 111.
4. Smithies, O. *Biochem. J.* **7** (1959) 585.
5. Deutsch, H. F., Stiehm, E. R. and Morton, J. I. *J. Biol. Chem.* **236** (1961) 2216.
6. Nisonoff, A., Wissler, F., Lipman, L. and Woernly, D. *Arch. Biochem. Biophys.* **89** (1960) 230.
7. Edelman, G. M. and Gally, J. A. *J. Exptl. Med.* **116** (1962) 207.
8. Edelman, G., Heremans, J., Heremans, M. and Kunkel, H. *J. Exptl. Med.* **112** (1960) 203.
9. Deutsch, H. F., Thorpe, N. O. and Fudenberg, H. H. *Immunology. In press.*
10. Smithies, O., Connel, G., and Dixon, G. *Am. J. Hum. Genet.* **14** (1962).

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