Amino Acid Analysis of Serum Proteins in Multiple Myeloma*

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It has long been recognized that the serum proteins of patients with multiple myeloma may differ markedly from those of normal serum as revealed by salt fractionation (11, 22), electrophoretic and ultracentrifugal analysis (5, 16, 18), as well as by other analytical procedures. During the past few years a number of investigators have attempted to determine whether the protein increase in the serum of multiple myeloma patients is simply an increment of normally occurring protein or whether it represents the de novo appearance of an abnormal protein (8, 10, 17, 23, 30).

Physico-chemical studies directed toward an understanding of normal and abnormal proteins of plasma and tissues have been conducted in this laboratory (1, 2, 28, 29). As part of this program a study of the proteins of the serum from patients with multiple myeloma was initiated.

Since electrophoresis and ultracentrifuge data for normal gamma globulin are known (9, 16, 20) and since analytical data for the amino acid composition of this protein have been reported (5, 26, 27), it was of interest to conduct similar studies on the myeloma proteins. This was further suggested by the fact that the Bence-Jones proteins, which often appear in the urine of these patients and which have been shown to differ antigenically from normal serum proteins (13), are very low or lacking in methionine (8, 24).

This paper summarizes physico-chemical data for the proteins obtained from the sera of eight different patients with multiple myeloma. It also presents the results of amino acid analyses for these proteins, and compares these values to those for normal gamma globulin.

MATERIALS AND METHODS

Sera used in this study were obtained from hospitalized patients and, in a few cases, at autopsy.

The myeloma proteins were separated from the serum as follows: Sera were diluted with 8 volumes of 0.1 M NaCl and dialysed overnight (2°-5° C.) with stirring against a solution of ammonium sulfate adjusted to pH 7.2 with NH₄OH and of such concentration as to give a final concentration of 1.6 M after equilibration. The precipitates were washed twice with 1.6 M ammonium sulfate and finally dissolved in either 0.15 M NaCl or 0.1 M phosphate buffer, pH 8.0. This method was effective for the preparation of proteins with mobilities in the range of β-globulins as well as for the slower ones, although protein 2 in this group was difficult to keep in solution once it had been separated from the other serum constituents. The precipitated proteins migrated essentially as single components (91–97 per cent) in the electrophoresis cell.

Protein nitrogen was determined by the standard semimicro-Kjeldahl technic.

Electrophoresis experiments were carried out under conditions described previously (7) in pH 8.6 barbital buffer of ionic strength 0.1. Protein concentrations were adjusted to 2 per cent for sera and to 1 per cent for the different precipitated fractions.

One per cent solutions of the proteins were analyzed in the Spinco analytical ultracentrifuge. Samples were prepared for analysis by dialyzing with stirring for 12 hours against a 0.001 M phosphate, 0.15 M NaCl solution at pH 7.4. Sedimentation constants have been corrected to water at 20° C., and are reported for the 1 per cent solutions.

The microbiological assay method described by Henderson and Snell (14) was used in quantitating the amino acid composition of the proteins. Acid hydrolysates were used for the assay of all the amino acids with the exception of tryptophan, in which case alkaline hydrolysates were employed. Both acid and alkaline hydrolysates were used in the estimation of tyrosine. Hydrolysis was effected by autoclaving 0.5 gm. of protein with 20 ml. of 3 N HCl or with 10 ml. of 5 N NaOH, for 10
and 15 hours, respectively, at 15 pounds pressure. The samples, in Erlenmeyer flasks, were covered with an inverted beaker during autoclaving.

Assay cultures were carried in a total volume of 2 ml. Lactic acid production was measured by titration with 0.02 N or 0.04 N NaOH. Each value reported represents several separate assays (3–6), each of which was carried out in triplicate at five concentrations. Assays usually agreed within better than 5 per cent, and in no case was the variation greater than 10 per cent. Repeat assays were usually on the same hydrolysate, due to the limited amounts of myeloma proteins available.

RESULTS

The electrophoresis and ultracentrifuge studies are summarized in Table 1. It is clear that there is no apparent correlation between the degree of hyperproteinemia, the concentration of the abnormal protein, the electrophoretic mobility, and the sedimentation constant of the abnormal protein.

It can be seen from Table 2 that there is considerable variation in amino acid composition among the several myeloma proteins. Protein 2 differs from the others in its content of several amino acids, arginine being increased, while tyrosine, leucine, and lysine are decreased. This is of particular interest, since this is also the protein which was so difficult to keep in solution and which had a main component with a sedimentation constant of 17.7 S as noted in Table 1. Similar properties have been described for part of the γ-1 protein separated from normal plasma pools by Deutsch et al. (9).

Although, for purposes of comparison, the ultracentrifuge determinations were made on protein solutions of 1 per cent concentrations, several dilutions of proteins 1 and 2 were analyzed to determine the effect of concentration on the sedimentation of these proteins. Extrapolated to zero concentration, the corrected sedimentation constant $S_0^0$ for 1 was 6.86 $\times 10^{-13}$, and for the main component of 2, 19.2 $\times 10^{-13}$.
If histograms are constructed for the amino acid composition of the myeloma proteins, γ-globulin, and other purified proteins available in the literature, it is apparent that the over-all patterns for the myeloma proteins, including those with the mobility of β-globulin, show striking similarity to that of normal γ-globulin.

On the other hand, the variations in composition noted may be indicative of small differences in the composition of the proteins, which are further revealed by differences in electrophoretic mobility, as has been reported by Smith and co-workers (26, 27) for two fractions of normal human γ-globulin.

**DISCUSSION**

Although there has been no attempt to correlate clinical observations with the analytical data in the present study, work from other laboratories indicates that, to the present time, it has not been possible to predict the type of protein abnormality, whether of the serum or urinary proteins, or both, on the basis of clinical findings.

In relating these proteins which appear in the serum to the urinary Bence-Jones proteins, in which methionine has been shown to be present in low concentrations or absent, it is significant that the serum myeloma proteins all showed the presence of methionine. Whether the presence of methionine bears any relation to the suggestion that the Bence-Jones proteins may be split-products of the myeloma serum proteins (25), or whether it may be related to a series of events in the synthesis of these proteins, remains to be determined. A recent report (12) of an experiment in which labeled glycine was given to a patient with multiple myeloma, however, indicates not only that the half-life times of the abnormal plasma protein and the urinary Bence-Jones proteins are different but that their rates of synthesis are independent.

It is difficult to assess the significance of the differences in amino acid composition, both among the myeloma proteins and between the latter and normal γ-globulin, in terms of whether or not the proteins are normal globulins. It has been demonstrated (6, 9) that normal γ-globulin is, in fact, made up of a number of proteins. In view of this, it might be expected that constituent proteins with a narrow mobility range would vary somewhat in their amino acid content when compared to γ-globulin. There is a good possibility that the myeloma proteins are indeed the same as some of the normally occurring serum proteins. This possibility is supported by the fact that all the sedimentation constants found for the myeloma proteins in the present study have also been reported for normal γ-globulin preparations (6, 9, 15). Immunologic experiments on this point have been conflicting, but, using improved technics, Kunkel et al. (17) have demonstrated that some myeloma proteins are related to at least a part of the normal γ-globulin; and Wunderly et al. (31) have recently reported that their results do not justify assuming the presence of atypical proteins. If it is true, as indicated by a number of reports (4, 18, 21, 23), that the myeloma proteins are being produced by the malignant plasma cells, it seems reasonable that small amounts of these same proteins could be formed by the smaller number of plasma cells normally found. To this end it would be desirable to have more fractions of normal γ-globulin in order to compare their amino acid compositions to those of the different myeloma proteins.

**SUMMARY**

Eight proteins have been separated by ammonium sulfate precipitation from the sera of different patients with multiple myeloma. The electrophoretic and ultracentrifugal properties of these proteins are summarized.

Analytical data for the amino acid composition of these proteins indicate differences among them, especially for one relatively insoluble protein which differed markedly from the rest in that it had an increased arginine and decreased tyrosine, leucine, and lysine concentrations. It was further distinguished by having as its main component a protein with a sedimentation constant (S0) of 19.2. In a comparison with the amino acid composition of normal gamma globulins, the myeloma proteins were in general high in lysine and proline and low in valine. The over-all similarity of the amino acid composition of these proteins to normal γ-globulin is discussed.

**REFERENCES**

29. ———. Electrophoretic and Ultracentrifugal Studies of Soluble Proteins of Rat Liver. Ibid., pp. 511-16.
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