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The Amino Acid Composition of Bence-Jones Protein

GUNNAR ÅGREN

Department of Medical Chemistry, University of Uppsala, Sweden

Dent and Rose [1] recently published a chemical study of Bence-Jones protein and reported that the sample isolated by them contained no methionine. In a preliminary note from this laboratory [2] a comparison was made (paper chromatography) of the amino acid composition of two samples of Bence-Jones protein, one prepared from urine, the other from a tumor extirpated for diagnostic purposes, both from the same person. The two samples of protein contained most of the common amino acids with exception of methionine and hydroxyproline. However, it was pointed out that the paper chromatographic method did not allow the identifying of methionine in a hydrolysate from a protein with the very low methionine content reported by previous workers. A microbiological amino acid analysis was therefore carried out on both of our samples of Bence-Jones protein.

MATERIAL AND METHODS

Preparation of material *. Both samples were prepared from the same patient with multiple myelomatosis. Most of a fresh small tumor extirpated for diagnostic purposes was ground and extracted with 0.9 per cent NaCl. The solution, adjusted to a pH of about 5 with acetic acid, was rapidly heated to 100°. From the hot, filtered solution the Bence-Jones protein precipitated at about 60°. The precipitate was washed with distilled water and dried with ethanol and ethyl ether. The urine was also made slightly acid with an acetate buffer, heated to 100°, filtered and allowed to cool to about 60°. The precipitate was washed and dried as above. Hydrolysates of tryptophan were prepared with barium hydroxide in nickel containers according to Miller and Ruttinger [3]. Acid hydrolysates, used for the determinations of the other amino acids, were prepared by hydrolyzing samples of protein for 6 hours at 15 pounds pressure with 3 *N* HCl in sealed tubes.

* The preparations were carried out by professor Blix in this department and kindly supplied to me.

Microbiological procedures. Only a few milligrams of protein from the tumor were available. A microadaptation of the microbiological procedure as described from this laboratory [4] was accordingly used throughout this investigation. Microorganisms, basal medium, ranges of standard curves, and incubation times are given in Table 1. The procedure for culture and inoculum has been described in previous papers [5,6]. A casein

Table 1. Experimental condition for the microbiological analyses.

Amino acid	Medium	Microorganism	Standard curve mμg per 50 μl	Incubation time, hours
Alanine	Sauberlich and Baumann [7]	*L. citrovorum* (8091)	125—1000	24
Arginine	Henderson and Snell [8*]	*L. casei*	62—500	48
Aspartic acid	Steele *et al.*[9]	*L. mesenteroides* P—60	125—1000	48
Cystine	Sauberlich and Baumann [7]	*L. citrovorum* (8081)	31—250	24
Glutamic acid	Steele *et al.*[9]	*L. mesenteroides* P-60	62—500	48
Glycine	Dunn *et al.*[10]	*R. citrovorum* (8081)	62—500	48
Histidine	Henderson and Snell [8]	*L. mesenteroides* P—60	62—500	48
Isoleucine	Sauberlich and Baumann [7]	*L. citrovorum* (8081)	62—500	24
Leucine	Henderson and Snell [8]	*L. arabinosus* 17—5	31—250	48
Lysine	—»—	*L. mesenteroides* P—60	62—500	72
Methionine	—»—	*L. arabinosus* 17—5	31—250	29
Phenylalanine	—»—	—»—	62—500	48
Proline	—»—**	*L. mesenteroides* P—60	62—500	48
Serine	—»—*	*S. faecalis* R (8043)	62—500	48
Threonine	Steele *et al.*[9]	*L. mesenteroides* P—60	62—500	48
Tryptophan	—»—	—»—	62—500	48
Tyrosine	Henderson and Snell [8]	—»—	62—500	48
Valine	—»—	*L. arabinosus* 17—5	62—500	48

* Single-strength medium diluted with an equal volume of water [3,5].

** pH of basal medium 6.0.

hydrolysate was always included as an extra control in the determinations of the amino acids. For each amino acid several separate assays were carried out with an average deviation of approximately ± 10 per cent for all amino acids. In each series five assay levels were used. The amino acids used as standards were dried *in vacuo*, at room temperature, and kept *in vacuo* in a desiccator containing silica gel. DL-forms of threonine and valine were employed. The natural isomers of the other were used.

Other analyses. The water content was measured by drying to constant weight at 100°. The ash was estimated by ignition to constant weight in a muffle furnace. The nitrogen content was determined by a micro-Kjeldahl procedure.

RESULTS AND DISCUSSION

In Table 2 the microbiological results are presented according to the usual procedure [11]. In the case of the urine protein, it is possible to account for 100 per cent of the weight of the protein and 96 per cent of the nitrogen. The

Table 2. Amino acid composition of Bence-Jones protein from a tumor and the urine of the same patient.

The total nitrogen corrected for ash and moisture was 16.1 per cent for the urine protein (UP). The ash content was 0.47 per cent and moisture 9.2 per cent. All values for the urine protein in Table 2 are expressed on an ash- and moisture-free basis. The figures for the tumor protein (TP) are calculated on the basis of the nitrogen content in the hydrolysate and on the assumption of 16.1 per cent of total nitrogen in the TP.

	G per 100 g protein		G residue per 100 g protein		G nitrogen per 100 g protein	
	UP	TP	UP	TP	UP	TP
Alanine	6.0	3.5	4.8	2.8	0.935	0.550
Arginine	5.7	5.4	5.1	4.9	1.830	1.740
Aspartic acid	6.1	7.8	5.3	6.7	0.640	0.820
Half-Cystine	3.8	0.67	3.2	0.57	0.445	0.079
Glutamic acid	16.6	13.8	14.6	12.1	1.580	1.310
Glycine	2.3	1.8	1.7	1.4	0.430	0.336
Histidine	1.0	1.2	0.88	1.1	0.271	0.325
Isoleucine	4.2	2.6	3.6	2.2	0.450	0.260
Leucine	10.6	8.8	9.2	7.6	1.135	0.943
Lysine	4.4	4.2	3.9	3.7	0.845	0.807
Methionine	0.40	0.1	0.35	0.01	0.038	0.009
Phenylalanine	2.7	2.0	2.4	1.8	0.230	0.170
Proline	6.4	5.6	5.4	4.7	0.780	0.685
Serine	16.7	8.1	13.8	7.2	2.220	1.078
Threonine	9.5	6.9	8.1	5.9	1.120	0.815
Tryptophan	3.9	2.5	3.5	2.3	0.535	0.343
Tyrosine	7.3	4.0	6.6	3.6	0.562	0.306
Valine	11.0	6.9	9.3	5.9	1.315	0.825
Total	118.6	85.9	101.7	74.5	15.4	11.4

amide nitrogen is not included in these figures. The corresponding figures for the protein from the tumor are 75 per cent and 71 per cent. These figures are calculated from the nitrogen content of the tumor protein hydrolysate and on the assumption that the total nitrogen content of the tumor protein also is 16.1 per cent. The very limited amount of tumor protein available did not permit any nitrogen and ash analyses. To a certain degree this complicates a comparison of the amino acid composition of the two sources of protein. Even so it is quite obvious that dissimilarities exist. This observation indicates the need for a certain degree of precaution in considering the connection between function and amino acid composition of Bence-Jones proteins isolated

from the urine. The amount of methionine found in our two samples of Bence-Jones protein was below the limit of detection of the chromatographic method for the quantities of hydrolysate previously examined [2].

The total nitrogen and ash contents of our urine protein is in good agreement with those found by Dent and Rose [12]. Contrary to the finding of these authors our two preparations contained methionine. The generally accepted molecular weight for Bence-Jones protein is 35 000 [13]. The calculated value for one molecule of methionine per molecule of protein is 0.43 per cent, in good agreement with the 0.40 found in our analysis. The most recent complete figures for the amino acid composition of Bence-Jones protein isolated from the urine are given by Roberts *et al.*[14] These authors also found methionine in their preparation. In general, there is comparatively good agreement with the values found in this investigation. Of special interest is the high content of serine and threonine found by Roberts *et al.* and in this investigation. The American authors stress the similarity in amino acid composition between their sample of Bence-Jones protein and the proteins from different strains of tobacco mosaic virus, with regard to the very high amount of aliphatic hydroxy amino acids. The high content of these acids in Bence-Jones protein is another structural analogy, in addition to the absence or very low content of methionine in both types of material [12]. Dent and Rose who first suggested that multiple myelomatosis might be due to the invasion of the body by a virus, also thought that the Bence-Jones protein, when combined with nucleic acid in the cells, was the virus itself. This brings us back to the fact that while we could account for 100 per cent of the urine material in form of amino acids, the corresponding figure for the tumor protein was only 75 per cent. Accordingly, it would seem to be of considerable value to further test the virus hypothesis by determining whether the unaccountable part of the tumor material is nucleic acid.

SUMMARY

Quantitative estimations of 18 amino acids have been carried out on two samples of Bence-Jones protein, one isolated from urine, the other from an extirpated tumor belonging to the same patient. Both proteins were characterized by a low content of methionine and by very high amounts of serine and threonine. The analysis accounted for 100 per cent of the weight of the urine protein and 75 per cent of the protein from the tumor. Possible reasons for this discrepancy are discussed.

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