

## Amino Acid Composition of $\alpha_1$ -, $\alpha_2$ -, and $\alpha_3$ -Caseins

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Amino acid analyses have been made of a purified  $\alpha$ -casein, of its principal component,  $\alpha_1$ -casein, and of two other purified components,  $\alpha_2$ - and  $\alpha_3$ -caseins.  $\alpha_1$ -Casein resembles  $\alpha$ -casein in content of most amino acids, but both differ from  $\alpha_2$ - and  $\alpha_3$ -caseins which have their own characteristic amino acid compositions.

### INTRODUCTION

Previous papers (1, 2) have described an improved method for the preparation of  $\alpha$ -casein and methods for fractionating  $\alpha$ -casein into components designated  $\alpha_1$ -,  $\alpha_2$ -, and  $\alpha_3$ -caseins on the basis of electrophoretic mobilities under specified conditions.  $\alpha_1$ -Casein and  $\alpha_3$ -casein have been found to differ in phosphorus, hexose, and sialic acid content and also in their specific extinction coefficients at 278  $m\mu$  which indicates that  $\alpha_3$ -casein contains more aromatic amino acids than  $\alpha_1$ -casein (2). However, determinations of tryptophan showed that  $\alpha_1$ -casein contains slightly more, rather than less, of this amino acid than  $\alpha_3$ -casein (3). We have now completed the amino acid analysis of  $\alpha_1$ -,  $\alpha_2$ -, and  $\alpha_3$ -caseins. A newly purified sample of  $\alpha$ -casein was also analyzed for purposes of comparison. Earlier amino acid analyses of  $\alpha$ -casein had been made by different methods (4, 5) on a somewhat different preparation.

### MATERIALS AND METHODS

$\alpha$ -Casein was prepared from an acid-extracted casein by a modification of the Warner method, as described by McMeekin, Hipp, and Groves (1). The  $\alpha$ -casein was then used for the preparation of  $\alpha_1$ -casein by the method of McMeekin, Hipp, and Groves (1),  $\alpha_3$ -casein by the method of Hipp, Groves, and McMeekin (2), and  $\alpha_2$ -casein

according to the procedure which Long, Van Winkle, and Gould (7) worked out for the separation of  $\lambda$ -casein, except that the final centrifugations were performed in the absence of salt.

Samples (about 2 mg.) of the air-dried, lyophilized proteins were weighed out into test tubes, 0.5 ml. of 6 *N* distilled HCl was added, and the tubes were drawn out, evacuated, and sealed. The samples were hydrolyzed for 24, 48, or 72 hr. at 110° in an oil bath. The hydrolyzates in the opened tubes were evaporated to dryness in a vacuum desiccator over soda-lime and P<sub>2</sub>O<sub>5</sub>. The residues were dissolved in 1 ml. of pH 2.2 citrate buffer (8) and transferred to the columns for analysis by the method of Moore, Spackman, and Stein (9).

Cystine was determined as cysteic acid in separate 24-hr. hydrolyzates prepared from larger samples (8–25 mg.) of protein which had been oxidized by performic acid according to Schram, Moore, and Bigwood (10). These hydrolyzates were applied to the 150-cm. column as described above. The cysteic acid peak emerging at about 45 ml. effluent volume was analyzed and calculated as cystine assuming a 90% conversion during the oxidation (10).

### RESULTS AND DISCUSSION

The analytical results are summarized in Table I. The figures for amino acids and ammonia are average values, corrected for moisture but not for small amounts of contaminant ash. The numbers in parentheses represent the number of individual analyses averaged. The extrapolated values at zero time for serine and threonine were obtained from the averaged 24- and 48-hr. hydrolyzate results, but the 72-hr. figures did not

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TABLE I  
COMPOSITION OF  $\alpha$ -CASEINS

Constituents	$\alpha$	$\alpha_1$	$\alpha_2$	$\alpha_3$	$\alpha$	$\alpha_1$	$\alpha_2$	$\alpha_3$
	g./100 g. protein				N as % of total N			
Total N	15.1	14.1	14.6	14.6				
Total P	0.99	0.85	0.87	0.35				
Asp	8.05 (7)	7.59 (6)	7.84 (9)	7.59 (5)	5.6	5.7	5.7	5.5
Thr	4.2 <sup>a</sup>	3.0 <sup>a</sup>	4.9 (9)	4.2 <sup>a</sup>	3.3	2.5	3.9	3.4
Ser	6.4 <sup>a</sup>	5.8 <sup>a</sup>	5.3 (9)	5.7 <sup>a</sup>	5.7	5.4	4.8	5.2
Glu	21.0 (7)	20.9 (6)	20.2 (8)	18.0 (6)	13.3	14.1	13.2	11.7
Pro	8.24 (7)	7.80 (7)	8.69 (7)	10.3 (6)	6.7	6.7	7.3	8.6
Gly	2.01 (7)	2.37 (6)	1.80 (7)	1.27 (6)	2.5	3.1	2.3	1.6
Ala	3.48 (7)	3.18 (7)	3.85 (9)	5.54 (6)	3.6	3.6	4.2	6.0
Cys <sup>b</sup>	0.44 (2)	0.20 (3)	0.59 (3)	1.34 (2)	0.34	0.17	0.47	1.1
Val	5.70 (4)	5.36 (3)	5.62 (9)	5.15 (6)	4.5	4.6	4.6	4.2
Met <sup>c</sup>	2.67 (4)	2.44 (6)	2.67 (9)	1.14 (5)	1.7	1.6	1.7	0.7
Ileu	5.75 (4)	5.16 (5)	6.13 (9)	6.45 (6)	4.1	3.9	4.5	4.7
Leu	7.98 (6)	8.65 (7)	6.73 (9)	6.46 (6)	5.7	6.6	4.9	4.7
Tyr	7.30 (6)	7.11 (7)	6.11 (8)	9.80 (5)	3.8	3.9	3.2	5.2
Phe	4.66 (6)	5.06 (6)	3.90 (8)	4.00 (4)	2.6	3.0	2.3	2.3
Lys	9.31 (2)	8.56 (2)	6.34 (3)	6.79 (2)	11.8	11.6	8.3	8.9
His	2.92 (2)	2.70 (2)	2.20 (3)	1.51 (2)	5.3	5.2	4.1	2.8
NH <sub>3</sub>	2.0 (2)	1.7 (2)	1.9 (3)	2.1 (2)	10.8	10.2	10.7	12.0
Arg	3.92 (2)	3.74 (2)	3.13 (3)	4.53 (2)	8.4	8.5	6.9	10.0
Try <sup>d</sup>	2.00	2.13	1.70	1.82	1.8	2.1	1.6	1.7
Total					101.5	102.5	94.7	100.3

<sup>a</sup> Extrapolated values.

<sup>b</sup> Determined as cysteic acid.

<sup>c</sup> Methionine values have been corrected for a loss of 5% during chromatography [Ref. (9)].

<sup>d</sup> Previously determined by Spies method [Ref. (3)].

TABLE II  
CHARGED GROUPS IN  $\alpha$ -CASEINS

	$\alpha$	$\alpha_1$	$\alpha_2$	$\alpha_3$
	groups/10 <sup>5</sup> g. protein			
Anionic <sup>a</sup>	268	253	252	201
Cationic	106	98	75	82

<sup>a</sup> Uncorrected for amide groups; see text.

fit the straight-line plots. Essentially the same final results for serine and threonine were obtained if the averaged analyses for 24-hr. hydrolyzates were corrected by the Rees factors for destruction of these amino acids (11). In the case of  $\alpha_2$ -casein, the analyses for serine and threonine were erratic and showed no evidence of destruction of the amino acids with time; the results of

these analyses are shown as simple averages in Table I.

The valine and isoleucine figures for  $\alpha$ - and  $\alpha_1$ -caseins are averaged results from 48- and 72-hr. hydrolyzates only, the 24-hr. values being appreciably lower; however, progressive liberation of these amino acids with time was not observed in the hydrolysis of  $\alpha_2$ - and  $\alpha_3$ -caseins, and the results listed for these proteins include the 24-hr. values.

The figures shown for ammonia were obtained from the short-column runs, no attempt being made in these experiments to differentiate between 24- or 48-hr. hydrolyzates. Also, because of the variable lability of serine and threonine, mentioned above, amide nitrogen contents deduced from the ammonia figures could only be approximations.

The amino acid composition of  $\alpha$ -casein shown in Table I is quite similar to that

previously published (4, 5) despite the differences in the methods of preparation of the samples analyzed and the methods of analysis used. Because  $\alpha_1$ -casein makes up the major portion of  $\alpha$ -casein, 74% as calculated from electrophoretic patterns (1), it was to be expected that its amino acid composition would resemble that of  $\alpha$ -casein. This is borne out by the data. Besides the many small differences in composition between  $\alpha$ - and  $\alpha_1$ -caseins, the analyses show that  $\alpha_1$ -casein contains considerably less cystine and threonine than  $\alpha$ -casein.

More striking differences are evident when comparison is made between the results for  $\alpha_1$ - and  $\alpha_3$ -caseins. Particularly noteworthy, perhaps, are the large differences for cystine, methionine, glycine, histidine, and alanine. The difference in tyrosine content serves to explain the larger extinction coefficient of  $\alpha_3$ -casein.

$\alpha_2$ -Casein contains more aspartic acid, threonine, valine, and methionine but less serine, tyrosine, phenylalanine, lysine, arginine, and tryptophan than either  $\alpha_1$ - or  $\alpha_3$ -caseins. We cannot explain why only 95% of the nitrogen of  $\alpha_2$ -casein can be accounted for.

A comparison of the approximate number of charged side-chain groups in the  $\alpha$ -caseins is presented in Table II. The total number of basic amino acid residues per unit weight of each protein is shown as "cationic groups." The anionic total is made up of glutamic and aspartic acid residues plus phosphoserine residues derived from the number of phosphorus atoms multiplied by two, the phosphoserine side chains being considered as dibasic anionic groups. It is assumed, in the absence of direct determina-

tions of amide nitrogen and also because of the uncertainty of any such figures which might be derived from considerations of destruction of hydroxy amino acids, that the approximate agreement in the analyses for ammonia reflects a similarity in content of amide groups. It is probable that about 110 amide groups can be subtracted from each of the anionic totals listed in the table. In any case, the lower net charge and lower isoelectric point of  $\alpha_3$ -casein relative to  $\alpha_1$ -casein suggested by electrophoretic data (2) appear to be substantiated by the data in Table II. Such correlation is lacking for  $\alpha_2$ -casein. Nevertheless, the data do support the general conclusion drawn from the electrophoretic evidence that  $\alpha_1$ -,  $\alpha_2$ -, and  $\alpha_3$ -caseins are distinct components of  $\alpha$ -casein.

#### REFERENCES

1. McMEEKIN, T. L., HIPPI, N. J., AND GROVES, M. L., *Arch. Biochem. Biophys.* **83**, 35 (1959).
2. HIPPI, N. J., GROVES, M. L., AND McMEEKIN, T. L., *Arch. Biochem. Biophys.* **93**, 245 (1961).
3. GORDON, W. G., AND BASCH, J. J., *Biochim. et Biophys. Acta* **48**, 397 (1961).
4. GORDON, W. G., SEMMETT, W. F., CABLE, R. S., AND MORRIS, M., *J. Am. Chem. Soc.* **71**, 3293 (1949).
5. GORDON, W. G., SEMMETT, W. F., AND BENDER, M., *J. Am. Chem. Soc.* **72**, 4282 (1950).
6. WARNER, R. C., *J. Am. Chem. Soc.* **66**, 1725 (1944).
7. LONG, J., VAN WINKLE, Q., AND GOULD, I. A., *J. Dairy Sci.* **41**, 317 (1958).
8. MOORE, S., AND STEIN, W. H., *J. Biol. Chem.* **211**, 893 (1954).
9. MOORE, S., SPACKMAN, D. H., AND STEIN, W. H., *Anal. Chem.* **30**, 1185 (1958).
10. SCHRAM, E., MOORE, S., AND BIGWOOD, E. J., *Biochem. J.* **57**, 33 (1954).
11. REES, M. W., *Biochem. J.* **40**, 632 (1946).