THE AMINO ACID COMPOSITION OF DESOXYRIBONUCLEASE I*

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The amino acid composition of crystalline desoxyribonuclease (DNase I) is reported in this communication. To our knowledge, the amino acid composition of this enzyme has not been published since its crystallization by KUNITZ[1]. On the basis of ultraviolet spectroscopy, KUNITZ[1] deduced that DNase I contained approximately 8% tyrosine and 2% tryptophane, but did not pursue such analytical studies further.

EXPERIMENTAL PROCEDURES

Materials

Crystalline DNase I was obtained from the Worthington Biochemical Co. This product was recrystallized twice from $(NH_4)_2SO_4$ in accordance with the procedure of KUNITZ[1] and then dialyzed against distilled water prior to analysis. No significant change in the enzymic activity per mg of protein resulted from these manipulations. A sample of this enzyme preparation was submitted for elementary analysis**.

Ion-exchange resins. Dowex-50***, 4% cross-linked, was used in the separation of the amino acids. Dowex-2§, 8% cross-linked, was used in the estimation of cystine as cysteic acid.

Methods

The determination of the amino acid composition was carried out according to the method developed by STEIN AND MOORE[2]. In each experiment 4–5 mg of an enzyme preparation, which had been dried previously over P_2O_5 at a pressure of 1 mm Hg at 30°C, was refluxed with 6 *N* HCl for 24 or 36 hours. Hydrochloric acid was then removed by distillation *in vacuo* and the residue was dissolved in 2–4 ml of 0.2 *M* sodium citrate buffer, pH 2.2, containing BRIJ 35§§ and thiodiglycol§§§. This solution was chromatographed on Dowex-50, 4% cross-linked, using a column 0.9 cm wide and 150 cm long. The amino acids were eluted in the manner described by STEIN AND MOORE[2]. Effluent fractions of 1 ml were collected mechanically at a rate of approximately 6 ml per hour. The concentration of amino acid in each fraction was determined photoelectrically by the ninhydrin method[3]. In a preliminary experiment the identity and purity of the amino acid contained in a particular effluent fraction was verified by paper chromatography, using only the tube containing the peak fraction of each elution curve. Cysteic acid was estimated independently by the method of SCHRAM *et al.*[4], and tryptophan was estimated according to SPIES *et al.*[5].

RESULTS

The profile of a typical elution curve obtained with a 36-hour hydrolysate is shown in Fig. 1. This curve varies slightly from that reported by STEIN AND MOORE[6] in the

* Supported in part by a grant from the United States Atomic Energy Commission.

** Microchemical analysis by Dr. C. W. BEAZLEY, Micro-Technical Laboratories, Skokie, Ill. The following results were found: C, 49.00%; N, 15.27%; S, 3.28%; Ash, 0.2%. The values for C and N have been corrected for $(NH_4)_2SO_4$ content on the basis of the difference between total S and amino acid-S content.

*** Obtained from the Dow Chemical Co., Midland, Mich.

§ Obtained from the Microchemical Specialties Co., 1834 University Ave., Berkeley 3, Calif.

§§ Obtained from the Atlas Powder Co., Wilmington, Del.

§§§ Obtained from the Carbide and Carbone Chemicals Corp., 30 East 42nd Street, New York 17, N.Y.

References p. 623.

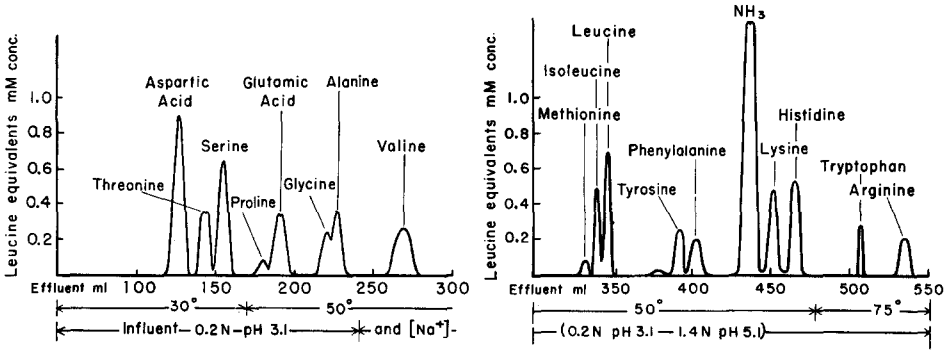

Fig. 1. Amino acid elution curve obtained with a hydrolysate of DNase I.

case of ribonuclease because of a difference in the rate of flow. Furthermore, only a partial separation of glycine and alanine was achieved and no sharp peak of cystine–cysteine was obtained constantly. Only in 2 experiments was there an indication of a fraction of cystine–cysteine. There was no evidence of the presence of cysteic acid or taurine.

Results of the quantitative amino acid determinations are shown in Table I. The values presented in columns 2–4 are the averages of four separate experiments in which the time of hydrolysis was either 24 hours (2 runs) or 36 hours (2 runs).

The elementary composition of the enzyme preparation employed here resembles rather closely that of KUNITZ's[1] preparation, although the latter preparation had a lower S-content. This difference in S-content is attributed to the presence of detectable inorganic sulfate in form of $(NH_4)_2SO_4$ in the enzyme preparation employed in these experiments. The combined S-content contributed by methionine and the cysteinyl residue accounts for only 0.78% of the total sulfur content. The cysteinyl-S was determined independently[4]. The protein-S of the preparation used in the present experiments, namely 0.78%, is somewhat lower than that reported for DNase I by KUNITZ[1]. This comparison is made on the assumption that KUNITZ's preparation was not contaminated by non-protein S and that this value, 1.09% S, constitutes only protein-S. A similar difference between total S-content and the S-content calculated as a composite of the constituent S-containing amino acids was noted by STEIN AND MOORE[6] for ribonuclease.

The total N-content of the DNase I preparation (15.27%) is somewhat lower than the total amino acid-N calculated from the constituent amino acids. This difference may be, in part, due to an absorption of NH_3 during the collection of the effluent fraction.

The noteworthy feature of the pattern of amino acid composition of DNase I is the relatively low content of glutamic acid, phenylalanine, and cystine–cysteine. In all other respects, the concentration of individual amino acids appears to be within the limits generally encountered.

Based on a molecular weight of 63,000, the number of amino acid residues per molecule were calculated. It was found that one molecule of DNase I contains approximately 540 amino acid residues. The molecular weight of DNase I is somewhat higher than the maximal molecular weight which permits a highly accurate calculation of the quantity in question and is dependent upon the accuracy of the analytical method,

References p. 623.

TABLE I

AMINO ACID COMPOSITION OF DNase I

Amino acid	*g Amino acid per 100 g protein**		*g Amino acid residues per 100 g protein*		*g N per 100 g protein*		*Number of amino acid residues per molecule*	*Number of residues to nearest integer*
Aspartic acid	13.40	(13.88)**	11.52	(11.94)	1.41	(1.46)	(65.4)	65
Threonine	6.13	(6.94)	5.21	(5.81)	0.72	(0.80)	(36.2)	36
Serine	8.43	(10.35)	7.00	(8.59)	1.12	(1.38)	(62.2)	62
Proline	3.25	(3.50)	2.73	(2.94)	0.40	(0.43)	(19.1)	19
Glutamic acid	9.32	(10.04)	8.20	(8.84)	0.89	(0.96)	(43.2)	43
Glycine***	3.06	3.06	2.33	2.33	0.57	0.57	25.8	26
Alanine***	4.87	4.87	3.90	3.90	0.77	0.77	34.6	35
Valine	7.71	7.71	6.55	6.55	0.92	0.92	41.9	42
Cystine-cysteine	1.28	1.28	1.19	1.19	0.15	0.15	3.4	3
Methionine	2.49	(2.77)	2.19	(2.44)	0.23	(0.26)	(11.7)	12
Isoleucine	4.28	4.28	3.68	3.68	0.46	0.46	20.5	21
Leucine	8.62	8.62	7.41	7.41	0.92	0.92	41.3	41
Tyrosine	7.43	(8.33)	6.69	(7.50)	0.57	(0.64)	(29.0)	29
Phenylalanine	5.25	5.25	4.67	4.67	0.45	0.45	20.0	20
Ammonia (total)	3.70	3.70						
Lysine	7.72	7.72	6.79	6.79	1.48	1.48	33.4	33
Histidine	5.31	5.31	4.67	4.67	1.44	1.44	21.5	22
Tryptophan§	1.57	1.57	1.43	1.43	0.22	0.22	4.8	5
Arginine	6.77	(7.04)	6.09	(6.34)	2.18	(2.26)	(25.6)	26
Amide NH_3	2.25	2.25			1.85	1.85	83.4	(83)§§
Total			92.25	97.02	16.75	17.42		540

* Dry and ash-free protein. The figures presented are corrected for 0.2 % ash and for the calculated amount of $(NH_4)_2SO_4$, presuming that the difference between the total content of S and the concentration of methionine- and cysteinyl-S is contributed by contaminating $(NH_4)_2SO_4$.

** Values in parentheses are corrected values taking into account decomposition during hydrolysis and using the data provided by STEIN AND MOORE[6] for the magnitude of such a decomposition. The value for methionine has been corrected for a loss of 10% occurring during chromatography.

*** The values for glycine and alanine have been extrapolated from the elution curve (Fig. 1).

§ The recovery of tryptophan is only 40% to 60% of the actual amount present. This amino acid was estimated by an independent method[5], using unhydrolyzed DNase I.

§§ The amide groups are not included in the summation of amino acid residues.

but Column 6 (Table I) provides an order of magnitude of the distribution of amino acid residues. The following formula is thus presented: $Asp._{65}$, $Thr._{36}$, $Ser._{62}$, $Prol._{19}$, $Glu._{43}$, $Gly._{26}$, $Ala._{35}$, $Val._{42}$, $Cyst.\text{-}Cyste._{3}$, $Meth._{12}$, $Ileu._{21}$, $Leu._{41}$, $Tyr._{29}$, $Phe._{20}$, $Lys._{33}$, $His._{22}$, $Try._{5}$, $Arg._{26}$, $(-CONH_2)_{83}$. The molecular weight calculated from the integral number of residues is 61,566.

SUMMARY

1. The amino acid composition of DNase I has been determined.
2. On the basis of the data obtained it was calculated that one DNase I molecule contained 540 amino acid residues.
3. The minimum molecular weight calculated from the amino acid composition was 61,566.

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Received September 11th, 1956