A VAL–VAL SEQUENCE FOUND IN A HUMAN MONOCYTIC LEUKEMIA LYSOZYME

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Received 9 February 1972

1. Introduction

Chromatographic differences between normal and leukemic lysozymes have been observed [1, 2]. The peptide maps of the tryptic digests did not differ [2], however. The primary structures of lysozyme isolated from human milk [3] and from the urine of a patient with chronic monocytic leukemia [4] were found to be identical.

In our laboratory amino acid analysis of a urinary lysozyme from a case of monocytic leukemia demonstrated the presence of 9 valine residues instead of 8 as previously reported. No other discrepancies were found (table 1).

2. Methods

The lysozyme was isolated as described by Johansson and Malmquist [6]. The lyophilized preparation contained no contaminating proteins detectable by agarose gel electrophoresis. Likewise, a rabbit antiserum against the purified protein contained no detectable antibodies against other proteins present in the urine sample used, or against serum proteins.

Amino acid analysis was performed on a Beckman amino acid analyzer, Model 120C. Hydrolysis was carried out according to Moore and Stein [7], Matsubara and Sasaki [8], and Spencer and Wold [9], except that the air oxidation was omitted after the hydrolysis in the Moore and Stein procedure. The hydrochloric acid used was prepared from analytical grade fuming hydrochloric acid (Merck, Darmstadt, W. Germany) and distilled and deionized water 1:1 (v/v). The hydrolyzates were evaporated to dryness as described by Brunfeldt and Thomsen [10].

Automated Edman degradation [11] was carried out in a Beckman sequencer, Model 890B. The sequence of the first 79 residues was established by repeated degradations of the native lysozyme, the reduced and carboxymethylated [12] lysozyme, and a fragment obtained after cyanogen bromide cleavage [13] of the RCM* lysozyme. So far, no deviation from the earlier reported sequence [3, 4] was found.

RCM human leukemia lysozyme (135 mg) in 0.2 M sodium hydroxide (25 ml) was reacted with 1,2-cyclohexanedione at a 10-fold molar excess over guanidino groups [14]. The cyclohexanedione was vacuum sublimated immediately before use [15]. After 3 hr at room temp an equal volume of acetic acid was added and the modified lysozyme desalted on a column (2.5 × 75 cm) of Sephadex G-25 fine. Elution was performed with 50% acetic acid. The CHD-RCM lysozyme (135 mg) was digested at 37° with trypsin in 0.2 M ammonium hydrogencarbonate, pH 7.8, (13 ml) 1×10^{-3} M in calcium chloride. The digestion was initiated by the addition of 2.3 mg TPCK treated

* Abbreviations: RCM = reduced and carboxymethylated. CHD = cyclohexanedione modified. TPCK = tosylamido-2-phenylethyl-chloromethyl ketone.

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Table 1
The amino acid composition of a human monocytic leukemia lysozyme. Residues per mole, calculated relative to aspartic acid, which is set to 18.0.

Amino acid	Native			RCM	Performic acid oxid [5]	Nearest integer	Human milk lysozyme [3] and human leukemia lysozyme [4]
	a)	b)	c)	a,d)	a)		
Trp	1.4	4.9 e)				5	5
Lys	5.1	5.1		5.0	4.9	5	5
His	1.1	1.1		1.0	1.0	1	1
Arg	14.1	14.4		14.0	13.7	14	14
$CySO_3H$			8.2		6.9		
CMCys				8.0			
Asp	18.0	18.0	18.0	18.0	18.0	18	18
$Met(O_2)$					2.0		
Thr	5.2 e)	5.3 e)	4.3	5.2 e)	5.2 e)	5	5
Ser	5.9 e)	5.6 e)	3.2	6.3 e)	6.1 e)	6	6
Glu	9.1 e)	9.4 e)	9.1 e)	9.1 e)	9.0 e)	9	9
Pro	4.2 f)	6.1 f)	2.1	2.1	1.3	2	2
Gly	11.0	11.3	11.2	10.9	10.8	11	11
Ala	14.1	14.3	13.9	13.9	13.8	14	14
$\frac{1}{2}$Cys	3.8					8 g)	8
Val	9.0	9.3	9.3	8.8	8.6	9	8
Met	2.0	2.0	1.0	1.8		2	2
Ile	4.9	4.9	5.4	4.9	4.8	5	5
Leu	8.0	8.1	7.9	8.0	8.0	8	8
Tyr	5.9	6.1		5.9	5.6	6	6
Phe	2.1	2.2	1.4	2.1	2.0	2	2
Total						130	129

a) Hydrolyzed for 24 hr at 110° in 6 N HCl [7] air oxidation was omitted after the hydrolysis.
b) Hydrolyzed for 24 hr at 110° in 6 N HCl containing 3% mercaptoacetic acid [8].
c) Hydrolyzed for 24 hr at 110° in 6 N HCl containing 2% dimethylsulfoxide [9].
d) Average of 3 determinations.
e) Corrected according to experiments with hen egg-white lysozyme, Trp +17%, Thr +10%, Ser +20%, Glu −4%.
f) Proline + cysteine.
g) Determined as cysteic acid or *S*-carboxymethylcysteine.

bovine trypsin (Merck, Darmstadt, W. Germany) dissolved in 100 µl buffer. The same amount was added after 2 and 4 hr. The final enzyme–substrate ratio was approx. 1:20 (w/w). The mixture was lyophilized after 6 hr of digestion.

The tryptic digest was submitted to a preliminary fractionation on Sephadex G-50 fine (2.5 × 81 cm) in 50% acetic acid followed by ion-exchange chromatography on SP-Sephadex C-25 (1.0 × 36 cm) and QAE-Sephadex A-25 (1.0 × 21 cm). The SP-Sephadex column was developed at 10.8 ml × hr^{-1} × cm^{-2} with a linear gradient of pyridine–acetic acid [16]. The developing system for chromatography on QAE-Sephadex consisted of 3 linear gradients used in succession. The first was made by mixing equal volumes [17] of 0.1 M triethylamine–0.1 M *N*-methylmorpholine–0.05 M pyridine adjusted to pH 9.4 with acetic acid and 0.1 M triethylamine–0.1 M *N*-methylmorpholine–0.15 M pyridine adjusted to pH 6.6 with acetic acid. The second was made by mixing equal volumes of the latter buffer and 0.5 M acetic acid, and the third was generated from 0.5 M

acetic acid and 2.0 M acetic acid as above. The QAE-Sephadex column was developed at 20 ml × hr^{-1} × cm^{-2}. All buffers contained 0.1% mercaptoethanol or thiodiglycol [18]. Peptides were detected with ninhydrin after alkaline hydrolysis [19].

3. Results

The isolated fractions were subjected to automated Edman degradation using a technique similar to that described by Sauer et al. [20]. One particular fraction contained 3 different peptides. Eight degradation cycles allowed deduction of the following sequences:

1. $\overset{2}{\text{Val}}$–Phe–Glu–Arg–Cys–Glu–Leu–$\overset{9}{\text{Ala}}$–
2. $\overset{14}{\text{Arg}}$–Leu–Gly–Met–Asp–Gly–Tyr–$\overset{21}{\text{Arg}}$–
3. $\overset{(98)}{\text{Arg}}$–Val–Val–Arg–Asp–Pro–Gln–$\overset{(105)}{\text{Gly}}$–

Peptides 1 and 2 could be placed within the confirmed sequence, 1–79. One prolyl was identified as residue no. 71, but the sequence of peptide 3 did not agree with the sequence around this position. The second proline residue in human milk and human leukemia lysozyme was reported to occupy position no. 102, but in this region only one valine residue was found [3, 4].

$\overset{98}{\text{Arg}}$–Val–Arg–Asp–Pro–Gln–$\overset{104}{\text{Gly}}$–

As this sequence is identical with that of peptide 3, except for one valine residue, it is concluded that the additional valine demonstrated by amino acid analysis is present in a Val–Val sequence at positions 99–100.

Acknowledgements

We are indebted to the Danish Medical Research Council and to Valdemar Selmer Trane and Elisa Trane's Foundation for grants in support of this work.

References

[1] D.Charlemagne and P.Jollès, Nouvelle Rev. Franç. Hématol. 6 (1966) 355.
[2] A.Mouton and J.Jollès, FEBS Letters 4 (1969) 337.
[3] J.Jollès and P.Jollès, Helv. Chim. Acta 54 (1971) 2668.
[4] R.E.Canfield, S.Kammerman, J.H.Sobel and F.J.Morgan, Nature New Biology 232 (1971) 16.
[5] C.H.W.Hirs, Methods Enzymol. 11 (1967) 197.
[6] B.G.Johansson and J.Malmquist, Scand. J. Clin. Lab. Invest. 27 (1971) 255.
[7] S.Moore and W.H.Stein, Methods Enzymol. 6 (1963) 819.
[8] H.Matsubara and R.M.Sasaki, Biochem. Biophys. Res. Commun. 35 (1969) 175.
[9] R.L.Spencer and F.Wold, Anal. Biochem. 32 (1969) 185.
[10] K.Brunfeldt and J.Thomsen, in: Methods for Determination of Amino Acid Composition of Milk Proteins, ed. K.Brunfeldt (The Danish Institute of Protein Chemistry, 1966) p. 16.
[11] P.Edman and G.Begg, European J. Biochem. 1 (1967) 80.
[12] A.M.Crestfield, S.Moore and W.H.Stein, J. Biol. Chem. 238 (1963) 622.
[13] E.Steers, Jr., G.R.Craven, C.B.Anfinsen and J.L.Bethune, J. Biol. Chem. 240 (1965) 2478.
[14] K.Toi, E.Bynum, E.Norris and H.A.Itano, J. Biol. Chem. 242 (1967) 1036.
[15] J.Lenard and P.M.Gallop, Anal. Biochem. 29 (1969) 203.
[16] W.A.Schroeder, Methods Enzymol. 11 (1967) 351.
[17] R.M.Bock and N.-S.Ling, Anal. Chem. 26 (1954) 1543.
[18] J.I.Harris, Methods Enzymol. 11 (1967) 390.
[19] C.H.W.Hirs, Methods Enzymol. 11 (1967) 325.
[20] R.Sauer, H.D.Niall and J.T.Potts, Jr., Federation Proc. 29 (1970) 728.