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Studies on Histones

8. A DEGRADATION PRODUCT OF LYSINE-RICH HISTONE*

By E. W. JOHNS

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

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Previous work from this Laboratory (Johns, 1964) has shown that the lysine-rich histones, f1, can be prepared by extracting calf-thymus nucleoprotein with 5% (0.75*N*) perchloric acid. An acetone precipitation method was also described for the further fractionation of this group which gave a lysine-rich histone (A), with an analysis comparable with that of the fraction A produced by column chromatography by Crampton, Stein & Moore (1957). *N*-Terminal-amino acid analysis showed that this fraction had virtually no free *N*-terminal amino acids. Phillips (1963*a*) has shown that the *N*-terminal amino acids of the lysine-rich histones are probably acetylated.

Another lysine-rich fraction of unusual composition (about 80% of lysine, alanine and proline) reported to be present in calf-thymus histones is the $\alpha 1$ fraction first described by Cruft, Hindley, Mauritzen & Stedman (1957) and later as f1(b) by Johns (1962). The yield of fraction f1(b), however, was small (0–5% of the total yield of f1) and variable, unlike the other histone fractions, and as it had threonine as the main *N*-terminal amino acid it was thought that it might be a product of acid degradation. The lysine-rich histone (A) mentioned above has therefore been degraded with hydrochloric acid and the products were examined. A preliminary account of this work has been given (Johns, 1963).

* Part 7: Johns (1964).

EXPERIMENTAL AND RESULTS

Preparation of histone. Lysine-rich histone (A) (70 mg.), prepared from group f1 by acetone precipitation as described by Johns (1964), was dissolved in 4 ml. of water. Constant-boiling HCl (3 ml.) was then added with stirring. The solution was heated rapidly to 100° and maintained at this temperature for 30 sec. Water (10 ml.) was then added and the solution cooled rapidly to room temperature. Trichloroacetic acid solution (3.0*M*) was then added and the protein precipitated in stages at final concentrations of 0.3*M*, 0.6*M*, 0.9*M*, 1.2*M* and 1.8*M*. The precipitates were washed in 40 ml. of acetone containing 0.1 ml. of conc. HCl, then three times in acetone and dried under vacuum. The amino acid analyses and yields are given in Table 1, together with the analyses of the starting material, fraction $\alpha 1$ prepared by Cruft *et al.* (1957) and f1(b) prepared by Johns (1962).

Amino acid analyses. Total amino acid analyses were carried out as described by Phillips & Johns (1959). *N*-Terminal-amino acid analyses were carried out as described by Phillips (1958) by a modification of the fluorodinitrobenzene method of Sanger (1945).

DISCUSSION

Although the fractions described above are the products of acid hydrolysis of f1 they are of interest because of their bearing on the structure of deoxyribonucleoprotein.

Phillips & Simson (1962) have shown, by examination of peptides obtained from the arginine-rich

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Table 1. *Amino acid analyses of lysine-rich histones and fractions derived from them by acid hydrolysis*

The amino acids are expressed as moles/100 moles of all amino acids found, and no corrections have been made for hydrolytic losses. Amide was not determined. The yields of *N*-terminal amino acids are given in μ moles/g. of protein.

protein.	Starting material*	Final concn. of trichloroacetic acid					$\alpha 1$ †	f1(b)‡
		0.3M	0.6M	0.9M	1.2M	1.8M		
		Fractions precipitated with trichloroacetic acid after acid hydrolysis						
Amino acids								
Aspartic acid	6.0	7.5	7.1	1.7	1.8	1.5	0.3	0.6
Glutamic acid				3.1			0.4	1.0
Glycine	7.0	6.9	7.8	6.6	4.3	3.2	3.7	3.4
Alanine	25.9	23.7	24.5	26.5	30.0	30.9	32.3	29.8
Valine	5.4	5.1	5.8	4.9	4.3	2.9	3.1	1.8
Leucine + isoleucine	5.3	5.3	7.4	3.4		0.5	0.6	1.4
Serine	5.7	6.3	6.6	5.4	3.6	3.6	2.8	3.5
Threonine	6.1	5.3	5.6	6.5	4.8	4.4	4.3	4.5
Phenylalanine	0.6	0.4	0.8	0.0	0.4	0.0	0.0	0.0
Tyrosine	0.8	2.0	0.8	0.3	0.8	0.3	0.0	0.0
Proline	9.3	8.2	7.3	10.0	12.8	14.6	14.1	12.0
Histidine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Lysine	26.4	27.3	24.5	30.9	36.9	37.7	38.5	41.4
Arginine	1.5	2.2	1.9	0.7	0.3	0.3	0.0	0.4
<i>N</i> -Terminal amino acids								
Alanine	0.25	—	11.8	—	—	—	—	22.5
Proline	0.0	—	0.0	—	—	—	—	13.0
Glycine	0.05	—	8.3	—	—	—	—	11.5
Serine	0.25	—	11.8	—	—	—	—	17.0
Threonine	0.15	—	6.5	—	—	—	—	67.0
Others§	1.3	—	20.2	—	—	—	—	11.5
Main <i>N</i> -terminal group	Acetyl	—	Alanine and serine	—	—	—	—	Threonine
Weight (g./mole of <i>N</i> -terminal amino acids)	500000	—	17000	—	—	—	—	7000
Yield (percentage of starting material)	—	3	57	7	11	2	—	—

* Fraction (A), prepared from f1 (Johns, 1964).

† Fraction $\alpha 1$, recalculated in moles/100 moles, from the analysis by Cruft *et al.* (1957).

‡ Johns (1962).

§ Others include lysine, aspartic acid and glutamic acid.

|| Phillips (1963a).

histones, that there is an irregular arrangement of the basic amino acids in the sequence, varying from juxtaposition to regions in which there are seven non-basic amino acids between basic amino acids. It has also been shown (Phillips, 1963b) that the slightly lysine-rich histones f2(a) have similar irregular primary structure. It is therefore no longer possible to accept a regular structure as suggested by Davison & Butler (1956) in which every phosphate group of DNA is attached to a basic amino acid side chain, separated by three non-basic residues. The isolation from the product of a short period of acid hydrolysis of f1(A), of fractions which are precipitated with 1.2M- and 1.8M-trichloroacetic acid, amounting to over 12 % of the starting material, and containing over 36 % of lysine, supports the evidence that the primary structure of the histones does not conform to this type of regular structure, and that in portions of

the peptide chain many of the basic amino acids may be next to each other or separated by only one or two non-basic residues.

It would also appear possible that the fractions $\alpha 1$ and f1(b) previously described as lysine-rich histones are in fact products of acid degradation. Threonine is the main *N*-terminal amino acid of f1(b), and Desnuelle & Casal (1948) have shown that threonine and serine *N*-terminal amino acids are also produced by the partial acid hydrolysis of casein.

SUMMARY

1. The products of partial acid hydrolysis of a lysine-rich histone, f1(A), have been examined by total and *N*-terminal-amino acid analyses.

2. One of the products contained over 80 % of alanine, lysine and proline, and is similar to lysine-rich histones previously reported.

3. The results also show that the primary structure of the lysine-rich histone is probably irregular with respect to the lysine residues.

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Kinetics and Mechanism of Catalysis by Proteolytic Enzymes

2. KINETIC STUDIES OF THROMBIN-CATALYSED REACTIONS AND THEIR MODIFICATION BY BILE SALTS AND OTHER DETERGENTS*

BY E. F. CURRAGH AND D. T. ELMORE

Department of Biochemistry, Queen's University, Belfast, N. Ireland

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Thrombin, which converts fibrinogen into fibrin, is capable of hydrolysing suitable derivatives of amino acids. The present work was undertaken to compare the specificities and kinetics of action of bovine thrombin and bovine pancreatic trypsin (Baines, Baird & Elmore, 1964). Most of the potential substrates examined belong to one of the series of compounds (I) or (II). It has been shown that bile salts have an inhibitory effect on the blood-clotting process (Garagnani & Facchini, 1956). This might be of clinical significance in obstructive jaundice when the plasma concentration of bile salts is increased. We have studied the

effects of bile salts and other detergents on the kinetics of several thrombin-catalysed reactions. A preliminary account of some of this work has appeared (Curragh & Elmore, 1963).

EXPERIMENTAL

Materials

The thrombin used in the present investigation was 'topical thrombin' obtained from Parke, Davis and Co. Ltd.; its activity was assayed with α -N-toluene-*p*-sulphonyl-L-arginine methyl ester (II*b*; R = O·CH₃, *n* = 3) and expressed in the units defined by Ehrenpreis & Scheraga (1957). Syntheses of several of the potential substrates and inhibitors have been reported by Baines *et al.* (1964).

* Part 1: Baines, Baird & Elmore (1964).