ON THE IDENTITY OF HUMAN LYSOZYMES ISOLATED FROM NORMAL AND ABNORMAL TISSUES OR SECRETIONS*

A. MOUTON and J. JOLLES
Laboratory of Biochemistry, Faculty of Science, Paris 6°, France

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1. Introduction

In the course of our comparative studies devoted to lysozymes (EC 3.2.1.17) of different origins [1,2] our group purified and prepared in a chromatographically pure state six normal human lysozymes (from milk, tears, placenta, saliva, plasma and leucocytes) and three lysozymes from patients suffering from leukemia (from leucocytes and plasma of patients suffering from chronic myelogenous leukemia, CML lysozymes, and from the urine of patients suffering from acute myeloblastic leukemia, AML lysozyme). All these human enzymes seemed to have a very near related chromatographic behaviour and, of course, a quite different one from all other already isolated lysozymes as, for instance, bird egg-white lysozymes. Amino acid analyses of several human lysozymes yielded comparable results, suggesting the probable identity of the enzyme in all human tissues and secretions [3]. Some immunochemical results were also in favour of such an interpretation [4].

Many years ago, we described differences in the chromatographic behaviour of two hen lysozymes (from egg-white and lung) and of two dog lysozymes [5] (from spleen and kidney); quite recently we were able to indicate structural differences between two lysozymes from the same duck egg-white [6] despite the fact that they had a very similar chromatographic behaviour on Amberlite CG-50 and almost identical amino acid analyses; from these latter alone it was not possible to establish with certainty structural differences. These observations conducted us to investigate more in detail the different human lysozymes, especially their chromatographic behaviour, their specific activity, their amino acid composition and their tryptic peptides. As only small quantities of the enzymes were available, the peptides from the different lysozymes were compared by electrophoresis chromatography.

2. Materials and methods

Human milk lysozyme, normal human leucocytes lysozyme and AML lysozyme were prepared according to the procedure of Jollès and Jollès [3,7], Charlemagne and Jollès [8] and Jollès et al. [9], respectively. The ion-exchange chromatography was performed on Amberlite CG-50 with a 0.2 M phosphate buffer of pH 6.98. The biological activities were measured as previously described [10] and were all expressed as hen egg-white lysozyme. Lysozymes were reduced by mercaptoethanol and carboxymethylated [11]. The reduced enzymes were separated from the reagents by a filtration on Sephadex G-25 with 1.0 N formic acid as eluent. They were then digested with 20% by weight of trypsin (EC 3.4.4.4) at 37°, pH 7.8 (trimethylamine), 6 hr. Trypsin was pretreated during 16 hr by 0.066 N HCl at 37°. The peptides obtained after tryptic hydrolysis were separated by electrophoresis-chromatography on Schleicher and Schüll 2043 b paper. The best conditions for the tryptic peptides from lysozymes were for the electrophoresis (Wieland-Pfleiderer Original Pherograph): −4°, 3 hr, 40 V/cm, 45 mA, pH 5.35, pyridine-acetic acid - water -

* 65th communication on lysozymes; 64th communication, see J.Jollès and P.Jollès, Bull. Soc. Chim. Biol. 50 (1968) 2543. Inquiries should be addressed to Dr. J.Jollès.

dimethylformamide (100:7.5:900:37, v/v); ascending chromatography: 20°, 40 hr, pyridine-tertiary amylic alcohol - water (35:35:30, v/v); the peptides were characterized with ninhydrin (0.025% solution in alcohol when the peptides were eluted for analytical or structural studies; 0.25% solution for photographs of the peptide maps).

3. Results

3.1. *Chromatographic behaviour*

All the *normal* human lysozymes which we have isolated possess the same chromatographic behaviour during their ion-exchange chromatography on Amberlite CG-50 [2,3,8]. Fig. 1 indicates the chromatographic patterns of human milk and human leucocytes lysozymes (usually symmetrical peaks). However from this same figure it can be concluded that the AML lysozyme can be separated into two peaks (α and β) by this same chromatographic procedure. With CML lysozyme only one peak was observed, but it was not symmetrical and presented a large tail (J.Saint–Blancard, unpublished data). Several reasons can explain the presence of the two AML lysozymes: different contents of amide groups, structural differences, etc.

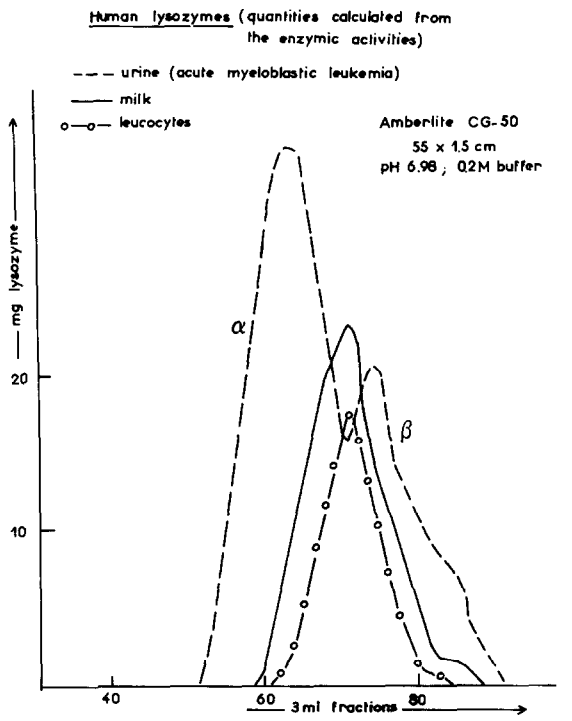

Fig. 1. Ion-exchange chromatography on Amberlite CG-50 of three human lysozymes.

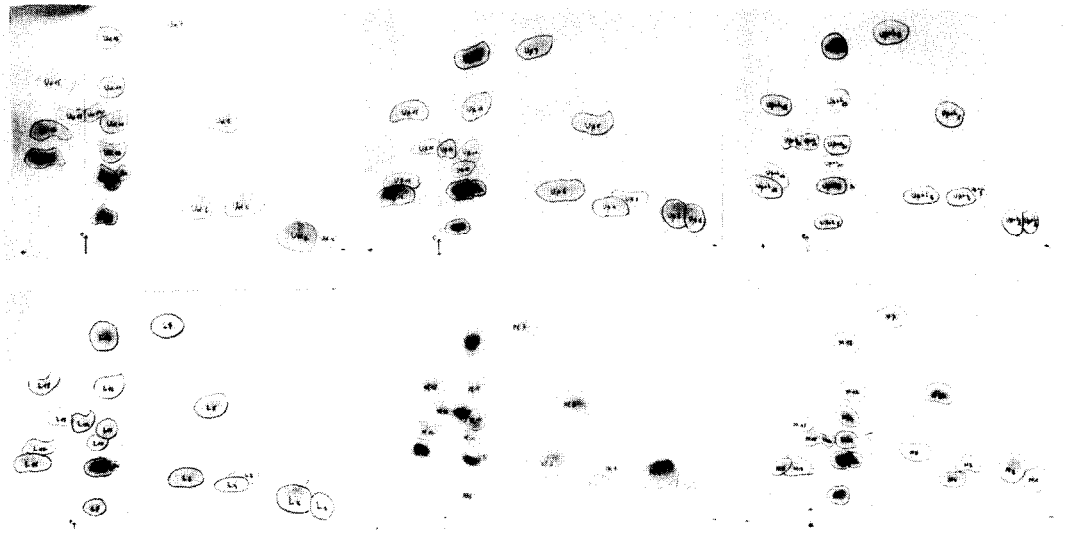

Fig. 2. Electrophoresis-chromatography of the tryptic peptides of different reduced and alkylated human lysozymes. L: from normal leucocytes; M: from milk; Pl: from normal plasma; Uα: from urine of patients with acute myeloblastic leukemia (AML), peak α; Uβ: from urine of patients with AML, peak β; Uβ + L: mixture of the two enzymes.

Table 1
Amino acid composition of human lysozymes. Residues per mole calculated on the basis of one histidine; nearest integer after hydrolyses of different time intervals.

Amino acid	Human tear lysozyme [3]	Human milk lysozyme [3]	Data of Jollès et al. [10] estimated from analysis of human salivary, placental and leucocytic lysozymes	ALM lysozyme α	ALM lysozyme β
Asp	17	17	18 ± 1	17	17
Thr	5–6	5–6	6 ± 1	4–5	5
Ser	6–7	6–7	6 ± 1	6	6
Glu	9–10	9	9 ± 1	9	9
Pro	3	3	2 – 3	3	2–3
Gly	12	12	12 ± 1	10–11	11
Ala	12–13	12–13	12 ± 1	13	13
Val	7–8	7–8	7	8	8
(Cys-)	6	6	6	6	6
Met	2	2	2	2	2
Ile	5	5	5	5	5
Leu	8	8	8	8	8
Tyr	5	5–6	5–6	6	6
Phe	2	2	2	2	2
Trp	5	5–6	5–6	5	5
Lys	5	5	5	5	5
His	1	1	1	1	1
Arg	11–12	11–12	11–12	12–13	12–13
Total	124 ± 3	124 ± 3	122 ± 5	124 ± 2	124 ± 2

3.2. *Specific activity*

Normal human lysozymes had all approximately 3–4 times the activity of hen egg-white lysozyme at equivalent concentrations when titrated with suspensions of *Micrococcus lysodeikticus* cells. The two ALM lysozymes, α and β, have however quite different specific activities which are 6–7 and 3–4, respectively.

3.3. *Amino acid composition*

The amino acid compositions of all human lysozymes (normal and from patients with leukemia) mentioned above seem to be quite comparable (table 1) as the analyses show no differences which cannot be accounted for by methodologic error. However many determinations achieved with the ALM lysozymes α and β after total hydrolyses of different time intervals (18, 48, 72 and 96 hr) suggest eventually slight differences in the Thr, Pro and Gly contents. A slight discrepancy was recently noted between the analysis of human milk lysozyme established in 1967 [3] and the amino acids found in the tryptic peptides accounting for the whole primary sequence of the enzyme (J.Jollès, unpublished data).

3.4. *Comparison of the tryptic peptides*

Fig. 2 shows the tryptic peptides of different reduced and alkylated lysozymes (three normal enzymes and two enzymes from patients with leukemia). A detailed examination of the peptide maps allows to conclude to their identity. Even with a mixture of two lysozymes (from leucocytes and ALM urine, peak β) no difference could be observed.

4. Conclusion

It is worth adding that all the *normal* human lysozymes have very near related apparent affinity constants for the bacterial substrate [12] and similar inhibition constants when N-acetylglucosamine is employed as inhibitor [13]. From all these data it seems reasonable to assume that the different human lysozymes may be identical. However in view of explaining completely the two peaks obtained during the ion-exchange chromatography of ALM lysozyme, a more detailed chemical structure study seems necessary.

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