COMPARISON BETWEEN HUMAN AND BIRD LYSOZYMES: NOTE CONCERNING THE PREVIOUSLY OBSERVED DELETION

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1. Introduction

We recently published the primary structure of human milk lysozyme (EC 3.2.1.17) [1,2]. In order to optimize homologous relationships, an insertion and a deletion were suggested to occur in the sequence of human lysozyme when compared to bird lysozymes [2,3]. The most probable position of the insertion seemed to be residue no. 48 and the corresponding tryptic peptide was studied in detail (tryptic peptide no. 5b; for the numbers of the peptides quoted in this paper, see [1] or [2]). From considerations of homology [1,3,4] the deletion was localized after residue no. 100. The primary structure of human leukaemia lysozyme isolated from the urine of a patient with chronic monocytic leukaemia [3] was similar to that of human milk lysozyme [1]. Quite recently, Thomsen et al. [5] were able to demonstrate by an elegant method (personal communication; to be published) that human leukaemia lysozyme does not show a deletion after residue no. 100 when compared to bird lysozymes. The peptide beginning from residue no. 98 contained the sequence: Arg–Val–Val–Arg–Asp–Pro– indicating that there are 2 residues of Val (and not only 1) between the 2 residues of Arg.

It seemed important to reinvestigate the corresponding sequence of the normal human milk lysozyme, more especially because lysozyme from tissues or secretions of leukaemia patients (but not of healthy persons) contains two main active lysozyme fractions which were characterized by ion-exchange chromatography on Amberlite CG-50 [6]. Furthermore preliminary results on the structure of baboon milk lysozyme (J. Jollès and J. Hermann, unpublished data) were in favour of the presence of 2 valine residues in this area, occurring, however, in a slightly different sequence.

2. Materials and methods

The preparation of human milk lysozyme and of its tryptic and chymotryptic peptides as well as the methods used for quantitative amino acid analyses and chemical structure determinations were previously discussed [1,2].

3. Results

3.1. *The valine content of human milk lysozyme*

From previous detailed investigations with 3 different lots of human milk lysozyme [7], a valine content of 6.8–7.0 residues per mole for 18 hr hydrolysis and 7.25 residues per mole for 48 hr hydrolysis were obtained on the basis of 1 residue of histidine per mole of lysozyme. As the nearest integer value 8 residues per mole was choosen, as even after 72 hr hydrolysis the valine content did not exceed this value.

3.2. *The valine content of tryptic peptide 2 (beginning from residue no. 99)*

The valine content was determined after 18 and 48 hr hydrolyses on the basis of 1 residue of arginine per mole of tryptic peptide 2; it was 0.8 and 1.05 residue, respectively. Thus we suggested for this

Table 1
Primary structure of human milk lysozyme. Comparison with hen egg-white lysozyme.

Human milk	1	Lys-Val-Phe-Glu-Arg-Cys-Glu-Leu-Ala-Arg-Thr-Leu-Lys-Arg-Leu-Gly-
Hen egg-white	1	Lys-Val-Phe-Gly-Arg-Cys-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-His-Gly-
Human milk	17	Met-Asp-Gly-Tyr-Arg-Gly-Ile-Ser-Leu-Ala-Asn-Trp-Met-Cys-Leu-Ala-
Hen egg-white	17	Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-Leu-Gly-Asn-Trp-Val-Cys-Ala-Ala-
Human milk	33	Lys-Trp-Glu-Ser-Gly-Tyr-Asn-Thr-Arg-Ala-Thr-Asn-Tyr-Asn-Ala-Gly-
Hen egg-white	33	Lys-Phe-Glu-Ser-Asn-Phe-Asn-Thr-Gln-Ala-Thr-Asn-Arg-Asp-Thr- ⊖
Human milk	49	Asp-Arg-Ser-Thr-Asp-Tyr-Gly-Ile-Phe-Gln-Ile-Asn-Ser-Arg-Tyr-Trp-
Hen egg-white	48	Asn-Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-Trp-Trp-
Human milk	65	Cys-Asn-Asp-Gly-Lys-Thr-Pro-Gly-Ala-Val-Asn-Ala-Cys-His-Leu-Ser-
Hen egg-white	64	Cys-Asp-Asn-Gly-Arg-Thr-Pro-Gly-Ser-Arg-Asn-Leu-Cys-Asn-Ile-Pro-
Human milk	81	Cys-Ser-Ala-Leu-Leu-Gln-Asp-Asn-Ile-Ala-Asp-Ala-Val-Ala-Cys-Ala-
Hen egg-white	80	Cys-Ser-Ala-Leu-Leu-Ser-Ser-Asp-Ile-Thr-Ala-Ser-Val-Asn-Cys-Ala-
Human milk	97	Lys-Arg-Val-Val-Arg-Asp-Pro-Gln-Gly-Ile-Arg-Ala-Trp-Val-Ala-Trp-
Hen egg-white	96	Lys-Lys-Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp-Val-Ala-Trp-
		130
Human milk	113	Arg-Asn-Arg-Cys-Gln-Asn-Arg-Asp-Val-Arg-Gln-Tyr-Val-Gln-Gly-Cys-Gly-Val
Hen egg-white	112	Arg-Asn-Arg-Cys-Lys-Gly-Thr-Asp-Val-Gln-Ala-Trp-Ile-Arg-Gly-Cys-Arg-Leu
		129

peptide the structure Val–Arg. However we recently submitted this peptide to longer hydrolysis times: after 72 hr, 1.8 residues of valine per arginine residue were found. Peptide 2 was also submitted to the Edman degration. After the first step leading to the formation of PTH-Val, no free arginine could be detected and the presence of residual Val–Arg was characterized; after a second step, PTH-Val was again found as well as free arginine. Thus tryptic peptide 2 contained a particularly strong Val–Val linkage resistant to a 48 hr hydrolysis; its structure was thus Val–Val–Arg.

4. Conclusion

Table 1 indicates the primary sequence of human milk lysozyme where no deletion can be observed after residue no. 100 when the formula is compared to that of hen lysozyme. Furthermore no difference occurs in this area between the normal (milk) and leukaemia [5] human lysozymes.

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