Short Communication
Sweetness of Lysozymes

Kenji MAEHASHI and Shigezo UDADA

Department of Brewing and Fermentation, Tokyo University of Agriculture, 1-1 Sakuragaoka, Setagaya-ku, Tokyo 156, Japan

Received September 29, 1997

While examining the taste of various proteins, we found that hen egg-white lysozyme, a bacteriolytic enzyme, had sweetness. Lysozymes from other sources such as turkey and soft-shelled turtle also showed sweetness with different tastes, heavy or light. In contrast, human lysozyme was tasteless. The amino acid sequences of the various lysozymes were similar to that of hen lysozyme, but hen lysozyme did not show significant homology to sweet proteins.

Key words: lysozyme; sweet protein; sweet taste

It is known that such sweet proteins as monellin,1 thaumatin2 and mabinlin3 are exceptional for their taste, since proteins are generally tasteless because of their failure to gain access to the active site on the taste receptor due to steric hindrance. All sweet proteins were found as the principles for the sweetness of some tropical fruits and have been well studied.4 We were interested to learn of any participation of proteins in the taste of food and thus searched for tasty proteins among those purified from various sources. As a result, we found that hen egg-white lysozyme, a bacteriolytic enzyme, had sweetness. Lysozyme has been extensively studied in respect of its three-dimensional structure and the molecular mechanism for its enzymatic action, and is one of the best-characterized proteins.5 Despite the studies devoted to lysozyme, there has been only one report describing its sweet taste.6 We thought that studies on the taste property of lysozyme would contribute to understanding the mechanism of sweet perception.

Hen, turkey, quail and guinea fowl lysozymes obtained from egg-white, soft-shelled turtle lysozyme also obtained from egg-white, and human tear and milk lysozymes were compared with respect to their taste. Turkey lysozyme and α-lactalbumin were purchased from Sigma Co., while the other lysozymes were prepared in our laboratory by the method described previously7,8 that involved heat treatment at pH 4.5 and chromatography on CM-Sepharose CL-6B. The lysozyme fractions prepared from the various egg-white samples, and from human tear and milk were found to be nearly homogenous by a SDS-PAGE analysis.

The sweetness of each lysozymes shown in the Table is presented as the ratio of the threshold value of sucrose to that of the lysozyme on a weight basis. Sweetness was evaluated as described by Kohmura et al.9 The hen lysozyme exhibited intense sweetness with weak astringency and a strong, long-lasting aftertaste, suggesting that it became tightly adsorbed to the tongue surface.

The turkey lysozyme was similar in taste. The guinea fowl and soft-shelled turtle lysozymes showed clear sweetness and their tastes were somewhat different from that of hen lysozyme. While the six lysozymes examined were all of the chicken type, exhibiting similarity in their primary structure as described later, the taste of each could be divided into three groups: heavy sweetness exhibited by the hen, turkey and quail lysozymes, light sweetness by the guinea fowl and soft-shelled turtle lysozymes, and tastelessness by the human lysozyme. However, α-lactalbumin, which is a major protein in the milk of many species, a functional component of lactose synthetase, and significantly homologous to hen lysozyme in both its amino acid and nucleotide sequences, was practically tasteless. The Table also indicates that the lysozyme sweetness was independent of the bacteriolytic activity, because the sweetness intensity did not match the lytic activity; in particular, the human lysozyme having lytic activity was tasteless.

The amino acid sequences of the lysozymes and α-lactalbumin were aligned to obtain maximum homology, as shown in the Figure. Since the five lysozymes from avian and reptilian eggs were sweet, and the two proteins from mammals were tasteless, we thought it possible to predict the active site of the sweetness in the amino acid sequence by examining the common amino acids which are found only in the sweet lysozymes and are highlighted by the black background in the Figure. Considering the neighboring sequence of the common amino acids and the taste difference of the sweet lysozymes, in addition to the non-sweet protein sequence, we might eliminate nine regions; i.e., Tyr23, Gly26, Val29, Asn37-Phe38, Leu56, Trp62, Ile78, Ile98, and Lys116-Gly117 from the probable 15 active site regions. Based on the foregoing assumption, Asp52 and Glu35, which are known as the active sites for lytic activity, did not seem to be the region of sweetness.

There was no similarity (less than 16% homology) in the amino acid sequences among the sweet proteins; i.e., monellin, thaumatin and mabinlin. We then compared the amino acid sequence of hen lysozyme with the sequences of other sweet proteins or other lysozymes by using a homology-search program. Strong similarity was only found between the hen lysozyme and the other lysozymes shown in the Table. Since monellin has been reported to have some homology to certain protease inhibitors,10 we examined whether hen lysozyme had any inhibitory activity toward trypsin and bromelain. No inhibition was, however, found.

To whom correspondence should be addressed.
Table. Characteristics of Various Lysozymes

| Source          | Sweetness* | Taste                          | Lytic activity (%)** | Homology (%)*** |
|-----------------|------------| ################################|----------------------|-----------------|
| Hen             | 20         | strong, long-lasting aftertaste with weak astringency | 100                  | 100             |
| Turkey          | 20         | "                            | 74                   | 94              |
| Quail           | 10         | "                            | 94                   | 97              |
| Guinea fowl     | 14         | persistent licorice-like taste with weak astringency | 68                   | 92              |
| Soft-shelled turtle | 13   | "                            | 114                  | 68              |
| Human           |            | tasteless                      | 85                   | 60              |
| Bovine milk     |            | tasteless                      | <1                   | 38              |
| α-Lactalbumin   |            | tasteless                      |                      |                 |

* Relative sweetness based on comparison with sucrose on a threshold value basis.
** Activity toward Micrococcus luteus cells in a M/15 phosphate buffer at pH 6.2.
*** Amino acid homology of the various proteins to the hen lysozyme.

Fig. Structural Comparison of Various Lysozymes.

To dissect the sweetness of the lysozyme molecule, an analysis by protein engineering of the region of the lysozyme necessary for manifesting sweetness is now in progress.

Acknowledgments
We are grateful to Dr. Kenzo Kurihara of Hokkaido University for his critical reading of the manuscript and useful comments. We thank Dr. K. Homma of this University for providing human milk, Dr. T. Araki of Kyushu-Tokai University for the amino acid sequence of the soft-shelled turtle lysozyme before publication, and the Hattori-Nakamura Co. turtle nursery for soft-shelled turtle eggs.

References
1) J. A. Morris and R. H. Cagan, Biochim. Biophys. Acta, 26,
   114-122 (1972).