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Role of proline in the imprinting developed by dipeptides – in Tetrahymena. Possible role in hormone evolution

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Abstract. The effects of proline and serine dipeptides containing phenylalanine, alanine and leucine on the behaviour of receptors of Tetrahymena were investigated. Only proline-containing dipeptides were able to develop positive imprinting, and the activity depended on which other amino acid was present in the dipeptide. In contrast to the positive imprinting effect of the dipeptides Pro-Phe and Pro-Ala, the dipeptide Pro-Pro and Pro-Leu caused negative imprinting. Serine dipeptides produced negative imprinting in all cases. The possible importance of proline in the evolution of hormone specificity is discussed.
Key words. Proline; imprinting; receptor evolution; hormone evolution; Tetrahymena.

The first encounter of a sensitive cell with a hormone leads to hormonal imprinting[1–4], and results in a durable and altered binding capacity of binding sites/receptors. This is followed by a change in the response of the cell. In higher organisms the imprinting takes place around birth, so it affects the receptors during their maturation period and thus the functions of the developing cells[5]. In unicellular organisms, the binding sites become stronger and turn into specific receptors[3,4,6,7]. The information acquired by imprinting is transferred – by a mechanism that is not completely known at present – to daughter cells. Thus the functioning of receptors is determined by the initial imprinting. Amino acid, polypeptide or steroid hormones are all able to induce the development of imprinting[3].

During evolution, hormone receptors were probably derived from nutrient receptors, with the receptors of amino acid and polypeptide type hormones originating as receptors of amino acids[8,9]. In the unicellular ciliate Tetrahymena, some amino acids have the capacity to induce imprinting, and imprinting treatment with amino acids is able to influence the hormone receptors[10,11].

Dipeptides and oligopeptides vary in their ability to induce imprinting, according to amino acid content and sequence, and the chain length. Moreover some amino acids lead to more marked changes in binding or in the functional alterations developed by imprinting. Previous experiments[12] showed that proline plays an important role in imprinting with oligopeptide chains. Ishii[13] has already called attention to the importance of proline in the development of hormone specificity in higher organisms in his experiments on hormone evolution. Therefore we studied the role of proline in the development of imprinting, using various dipeptides.

Materials and methods

Populations of Tetrahymena pyriformis GL cells, cultured in 0.1% yeast extract and 1% Tryptone containing medium (Difco, Michigan, USA) for 24 h at 28 °C were used, in the logarithmic phase of growth. Doubling time was approximately 3 hours. The following dipeptides (Serva Feinbiochemica, Heidelberg, Germany) were used:

1) Prolyl-dipeptides: Pro-Pro; Pro-Phe; Pro-Ala; Pro-Leu.
2) Seryl peptides: Ser-Phe; Ser-Ala; Ser-Leu.
3) Leu-Leu and Ala-Ala.

Imprinting developed by the prolyl and seryl-dipeptides was assayed by treating populations of Tetrahymena cells with the dipeptide at 10^{-6} M for 1 hour. This was followed by rinsing 3 times with PBS. The cells were then transferred into fresh culture medium and cultured for one day. After this, the cells were fixed with 4% formaldehyde in PBS, pH 7.2, for 5 min, washed with PBS, and incubated with FITC labelled prolyl or seryl dipeptides (FITC: fluoresceine isothiocyanate Isomer I. BDH, Poole, England). The peptide concentrations of the FITC-dipeptide conjugates were 10^{-6} M. Duration of incubation was 1 hour at room temperature. The cells were then washed thrice with PBS; drops were applied to slides and allowed to dry, and the intensity of fluorescence was determined with a Zeiss Fluoval cytofluorimeter. In each group 20 cells were measured. The analogue signals emitted by the cytofluorimeter were transformed into digital signals which were registered by a Hewlett Packard HP 41CX calculator. The built-in mathematical programme presented the average values and standard deviations of the groups and the value of the intergroup significance (Student's t-test). The experiments were each repeated three times, which means that each column in the table represents the mean value for 60 cells.

The binding capacity of cells imprinted by seryl- and prolyl-dipeptides containing Ala or Leu was also assayed using FITC-Ala-Ala or FITC-Leu-Leu conjugates.

In addition to the imprinted groups, the binding parameters were also measured in untreated control groups. These values were taken as 100%, and the values for imprinted groups were related to them.

Results and discussion

The results of our experiments indicate that pretreatment (imprinting) with different dipeptides is able to influence the subsequent binding of the identical or non-identical dipeptides to Tetrahymena (table). The differences from the control were always significant, either positively or negatively. The encounter with dipeptides is able to influence the binding of dipeptides to Tetrahymena in the progeny generations, thus dipeptides have the capacity to induce imprinting.

In the experiments, dipeptides containing proline were compared with those without, in order to clarify the specificity of the effect of proline. The experiments show clearly that only prolyl dipeptides induced positive imprinting, and only when the proline was combined with phenylalanine or alanine (table, groups 1 and 3.) When proline was combined with leucine or a second proline residue, negative imprinting resulted (table, groups 4 and 2.) It is crucial in the development of imprinting by prolyl peptides which other amino acid is present in the dipeptide. It was striking that the dipeptide of proline (Pro-Pro) presented negative imprinting. In previous experiments, we found that the dipeptide Phe-Pro did not show positive imprinting[10], in contrast to the dipeptide Pro-Phe investigated here. It is probable that the site of proline in the peptide is important: positive imprinting depends on proline being in the amino-terminal position. The serine containing dipeptides caused negative imprinting (table, groups 5–7.)

The amino acids linked to proline are important not only for the imprinting activity but also for the binding of dipeptides. Tetrahymena cells imprinted with Pro-Phe have a significantly higher binding capacity for Pro-Phe than for Pro-Pro. On the other hand, Tetrahymena cells imprinted with Pro-Ala bind Pro-Pro more readily than Pro-Ala. This indicates not only that there are differences in the binding of combinations, but that the dipeptides which were used for imprinting are not necessarily the most readily bound. The fact that Ala-Ala is very poorly bound, and Pro-Ala is bound less than Pro-Pro, suggests that alanine has the potency to decrease binding to receptors, alone or when coupled to proline.

In previous experiments[12] it was observed that synthetic opioid oligopeptides containing proline developed imprinting in Chang liver cells, whereas a tetrapeptide which did not contain proline was ineffective. Ishii[13] established in his study of the evolution of gonadotropic hormones that in the luteinizing hormone (LH) the content of proline increased during phylogenesis. Ishii[13] stated that chicken LH contained 15 prolines and 10 of them had identical positions to those in bovine LH, which contains 20 prolines. Ishii suggests that the progressive increase of proline residues in LH molecules is responsible for the narrow species specificity of the hormone. This tendency towards an increase in the number of prolines was not observed in follicle stimulating hormone (FSH) and this hormone also shows less species-specificity. When Fahrenholz et al.[14] studied the binding relations of vasopressin agonists and antagonists to hepatic vasopressin receptors they found an extraordinary intolerance to conformational changes elicited by substitution of the proline residue. The above-mentioned experiments indicate that proline, an unusual amino acid which has the amino group inside the rigid ring structure[15], could play a prominent role in the development of specific reactions, including imprinting. If we accept the theory[2,3] that if the ability to induce positive imprinting played a decisive role during the selection of molecules that subsequently became hormones, their proline content could have been important.

Our results show that imprinting may not only be positive, but negative. At the evolutionary level we do not understand the significance of negative imprinting. It may have played a role in the selection of molecules for their suitability, showing that some structures are unsuitable as chemical signals. Both negative and positive imprinting may have been involved in the evolutionary process matching molecules suitable for pairs of receptor–hormone systems.

Binding of FITC-dipeptides to imprinted Tetrahymena

Group number	Imprinted by	Binding of FITC-dipeptide	% of control†
1	Pro-Ala	Pro-Ala	145.82*
		Pro-Pro	235.20*
		Ala-Ala	54.54*
2	Pro-Leu	Pro-Leu	72.32*
		Pro-Pro	85.35**
		Leu-Leu	64.00*
3	Pro-Phe	Pro-Phe	188.51*
		Pro-Pro	140.46*
4	Pro-Pro	Pro-Pro	85.17**
5	Ser-Ala	Ser-Ala	63.54*
		Ala-Ala	70.41*
6	Ser-Leu	Ser-Leu	75.39*
		Leu-Leu	70.99*
7	Ser-Phe	Ser-Phe	56.02*

Significance: *$p < 0.01$; **$p < 0.5$; †s.d. = under ±5% in each group.

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