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PREDICTED SECONDARY STRUCTURE OF CALCITONIN IN RELATION TO THE BIOLOGICAL ACTIVITY

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SUMMARY

The secondary structure of the natural analogues of calcitonin have been predicted using the methods of Burgess et al., Chou and Fasman, and Lim. The predicted structures were similar in the N-terminal part of the chain, but were variable in other regions of the molecules. The higher hypocalcaemic potency of ultimobranchial calcitonins as compared to thyroidal calcitonins may be related to the higher potential for helical structure in the former.

INTRODUCTION

The knowledge of the secondary structure of calcitonin (CT) would be useful for resolving structure-activity relationship, unfortunately only little is known about it. We have used the predictive methods to define the structural potentials of the 5 natural analogues of CT isolated from thyroid glands and the 4 from ultimobranchial bodies. The common and variable structural domains found have been analyzed in relation with some biological properties.

METHODS

We used 3 predictive methods, the statistical methods of Burgess et al., (1) and Chou and Fasman (2), and the stereochemical method of Lim (3).

The ambiguities in the Chou and Fasman method have been corrected by using the modification reported by Lenstra et al., (4). They relate to the nucleation sites of helices and sheets, which may vary in position as well as in length depending on the nucleation used. The conformational parameter P_t, the average potentials <Pα>, <Pβ>, <Pc> were used according to the definition of Chou and Fasman (2).

As to the method of Burgess et al. the conformational potentials of a residue depend on the 4 residues on either side, the structure of the N-terminal and C-terminal 4 residues cannot be defined.

According to Lim method, only helices and sheets can be predicted, and sheets are searched for on the fragments of the chain which cannot be helical ; then, predicted helices may overlap potential sheets.

RESULTS

The predicted secondary structure of human, rat, pig, cow, sheep, salmon I, II, III and eel CT are shown in Table I.

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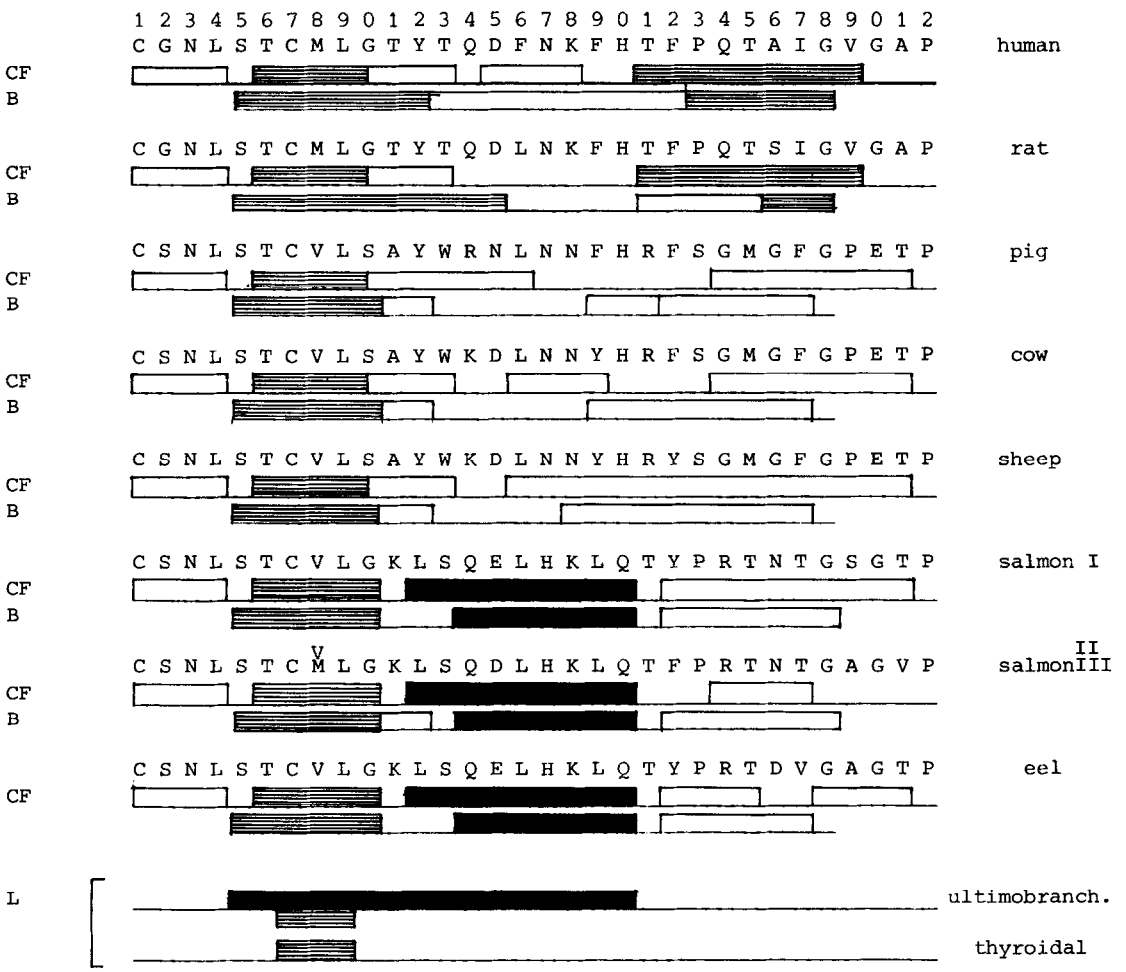
TABLE I : Predictions patterns of calcitonin, according to Chou and Fasman (CF) Burgess et al. (B), Lim (L) rules. α -helix residues are denoted by ( ), β -sheet residues by ( ), R-coil residues by (——), β -turn residues by ( ).



In the N-terminal part of the chain, the predictions were similar for each CT : ie, a β -turn in position 1-4 with :

CSNL Pt = 0.93, $\langle P_c \rangle$ = 1.23, $\langle P_\alpha \rangle$ = 0.91, $\langle P_\beta \rangle$ = 0.97

or CGNL Pt = 0.88, $\langle P_c \rangle$ = 1.27, $\langle P_\alpha \rangle$ = 0.84, $\langle P_\beta \rangle$ = 1.00

and a β-sheet in position 5-10 $\langle P_\beta \rangle$ = 1.24 (CF), 6-10 (B) or 7-9 (L). the potential β-sheet 7-9 of ultimobranchial CT was overlapped by the α-helix 4-20 (L).

- In the central part of the ultimobranchial CT, an α-helix was predicted. It is located in position 12-20 $\langle P_\alpha \rangle$ = 1.17 or $\langle P_\alpha \rangle$ = 1.22 (CF), 14-20 (B) or 4-20 (L).

- In the C-terminal part of human and rat CT a β-sheet in position 21-29 $<P_\beta> = 1.17$ or $<P_\beta> = 1.15$ (CF), 23-28 or 26-28 (B) was predicted, but only by these two methods.

- Multiple β-turns regions appeared in the central part of the thyroidal CT, and in the C-terminal region of the ultimobranchial and the arteriodactyl (pig, cow, sheep) CT.

DISCUSSION

Previous works showed that human (HCT) and porcine (PCT) calcitonin exist predominately in an extended random coil form in aqueous solutions (5) (6), although a weak degree of ellipticity was found for PCT (6). In 2-chloroethanol PCT contains about 50 % of helical structure (6). Thus, calcitonin seems to be a flexible molecule with a conformation highly dependent on its environment.

Such a behavior was previously reported for various peptides (7). For short peptides, a good agreement of the secondary structure predictions with experimental measurements performed in a proteinaceous like environment (using surfactants) has been found (8). Such a result is supported by the principles of the methods, because they are related to the structural properties of polypeptide fragments within a protein. Thus, the predicted structures for calcitonin have to be considered as relative to a proteinaceous environment.

ANALYSIS OF THE PREDICTION

- In the N-terminal region : . As the β-turn, predicted in position 1-4, allows the chain to reverse itself, it would be related to the disulfide bridge 1-7.

. The β-structure potential (in position 5-10, 6-10 or 7-9) was present for each analogue, although in ultimobranchial CT, according to Lim rules, the β-sheet 7-9 was overlapped by the helix 4-20.

. In arteriodactyl CT (cow, sheep, pig) the tetrapeptide 8-11 exhibited a significant helical potential :

$$<P_\alpha> = 1.18 > <P_\beta> = 1.14 \text{ (CF)}.$$

If a region exhibits both helical and β forming potential, one conformation is usually preferred depending on the environmental conditions (2). This may be examplified by the helical or β-sheet structure of region 19-27 of glucagon depending on the environment (9). Then, the helical potential of the region 8-11 of PCT might be related to the 10 % ellipticity of this molecule as elicited in a diluted aqueous solution (6), whereas a β-structure cannot be stabilized by intermolecular β-sheet interactions.

- In the central region of the ultimobranchial CT, a helical structure was predicted, according to the 3 methods, whereas clusters of β-turns appeared in thyroidal CT. This result suggested a clear structural distinction between thyroidal CT on the one hand and ultimobranchial CT on the other hand, while the evolutionary tree of calcitonins (10), based upon the analysis of the sequences, showed the human hormone closer to the piscine than the arteriodactyl one.

It should be noticed that the regions 8-16 and 19-23 of arteriodactyl CT had a weak helical forming potential $\langle P_\alpha \rangle = 1.03$ and $\langle P_\alpha \rangle = 1.01$ respectively. Chou and Fasman suggested that when regions have $\langle P_\alpha \rangle = 1$ they may be helical in 2-chloroethanol (2) ; then these regions may be responsible of the 50 % ellipticity found in 2-chloroethanol solution of PCT (6).

- In the C-Terminal region of human and rat CT, a β-sheet was predicted according to Burgess et al. and Chou and Fasman, but not according to Lim rules. Clusters of β-turns were predicted in other CT, but they were not always located at homologous positions.

In this region, the disparity of the results does not allow us to give any accurate structural potentials.

Relation to biological properties

The understanding of the nature of an hormone-receptor interaction should depend on the description of the conformation of the hormone when bound to the receptor. This latter could not be anticipated by the results of predictive methods, since it is not known if the secondary structure of the hormone is conserved while interacting with the receptor, though, such maintenance of secondary structure has been suggested for structural domains of glucagon and insulin (11) (12). The preferential conformation of the hormone in the biological environment may be of importance concerning the enzymatic degradation processes and the structural changes which may occur before or by receptor interactions. It thus would seem useful to consider the predicted secondary structure for calcitonin in relation to some biological properties.

- In the N-terminal region, the importance of the residue in position 8 has been investigated. In both HCT and salmon III CT, oxidation of the Met^8 to its sulfoxide drastically reduced their biological activity (13). This result suggests the importance of an hydrophobic residue in position 8, hypothesis further documented by the increased activity of HCT-Val^8 analogue relative to HCT (14). The hydrophobic nature of the amino-acid in position 8 appeared necessary with regard to the interaction with the receptor, as shown by the very weak receptor binding of HCT-Met^8 sulfoxide (15).

It is interesting to note that the presence of an hydrophobic residue in position 8 was also important for both the helical and β-sheet potentials in the N-terminal region.

- The hypocalcaemic potency of ultimobranchial CT is 20-40 higher than that of thyroidal. We have analyzed the structural changes displayed by synthetic analogues of CT, in order to test whether a helical structure potential of the central part of CT molecule could be correlated with a higher hypocalceamic potency. These synthetic analogues are related to the HCT sequence, one or more amino-acids being replaced by the homologues of the salmon I sequence. The aromatic residues Tyr^{12}, Phe^{16}, Phe^{19} have been replaced by Leu residues (16).

HCT-Leu^{16} and HCT-Leu^{19} analogues had the same activity as HCT, whereas HCT-Leu^{12} and HCT-$Leu^{12, 16, 19}$ were respectively 5 and 10 times more active.

According to Lim rules, the change Tyr^{12}, Leu^{12} alone is sufficient to induce an α-helix for the segment 4-20 in HCT-Leu^{12} and HCT-$Leu^{12, 16, 19}$.

According to Chou and Fasman, this substitution is the most significant as an α-breaker like Tyr is replaced by a strong α-former Leu. The helical potential of fragment 12-20 which is $\langle P_\alpha \rangle = 0.98$ in HCT and $\langle P_\alpha \rangle = 1.01$ for HCT-Leu^{16} and HCT-Leu^{19}, becomes $\langle P_\alpha \rangle = 1.06$ for HCT-Leu^{12} and $\langle P_\alpha \rangle = 1.11$ for HCT-$Leu^{12, 16, 19}$.

According to Burgess rules, the structure of the fragment 15-18 of HCT-$Leu^{12, 16, 19}$ should be helical.

In natural and synthetic analogues, it seems, that a high hypocalceamic potency is linked to a helical structure potential. The higher biological effectiveness of ultimobranchial CT relative to thyroidal CT has been related to a longer half-life in vivo and resistance to degradation by plasma or tissues extracts (17) (18) (19), as well as a higher affinity for specific receptors (15) (20). It should be interesting to study which of these mechanisms are the most concerned with differences of structural potentials in the central region of the molecule.

CONCLUSIONS

From the predictive methods, the N-terminal region of each CT has an ordered structure potential and the central region of ultimobranchial CT, a helical structure potential. A parallelism between this predicted helical structure and the hypocalcaemic potency has been found. The nature of the amino-acids in position 8 and 12 has been described as an important factor for both the biological potency and the prediction of definite secondary structures.

Thus, although the conformation of CT bound to its specific receptor cannot be described using only predictive methods, such an approach seems of interest for

the structure-activity relationship. Indeed, it should be useful to study the in vitro relative receptor-binding capacity and degradation rate of different synthetic analogues of CT, for which the structural forming potential should be predicted either enhanced or decreased as function of amino-acids substitutions.

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