STRUCTURAL COMPARISONS OF LEUKOCYTE INTERFERON AND PRO-OPIOMELANOCORTIN CORRELATED WITH IMMUNOLOGICAL SIMILARITIES

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1. Introduction

Pro-opiomelanocortin is a polyprotein [1] that generates several hormones, among them adrenocorticotropic hormone (ACTH) and endorphin. Immunological cross-reactivities between these peptides and human leukocyte interferon have been reported [2]. Thus, antibodies raised against $ACTH_{1-13}$ or against γ-endorphin seem to recognize structural similarities in leukocyte interferon. A similarity is further suggested by a reported release of ACTH activity from this interferon upon peptic proteolysis [2].

Sequence comparisons of interferon and pro-opiomelanocortin structures are necessary to evaluate the immunological and functional results. We therefore compared leukocyte interferons with pro-opiomelanocortins, since we have studied fragments of the prohormone [3] and have a program for peptide comparisons [4]. Included in the comparison were the amino acid sequences reported for 2 human leukocyte interferons, α_1 [5] and α_2 [6], and for bovine [7] and human [8,9] pro-opiomelanocortins. The interferons belong to a group of several related interferons [5,6 10] and differ by 17% in amino acid sequence [6].

The results show that a region within the prohormone, from the end of ACTH to β-endorphin, displays sequence similarities with α-interferons. In this segment, 6 alignments with interferon show sequence identities of ~25% in stretches of up to around 50 residues in length. These results support the immunological conclusion of similarities. However, the alignments are different and partly overlapping in both molecules. Hence, they do not support a genetic relationship but may indicate convergence in structure. Such a possibly non-divergent relationship among immunological cross-reactants is a novel idea.

2. Materials and methods

The structures of human leukocyte interferon α_1 [5] and α_2 [6] were compared with those of human [8,9] and bovine [7] pro-opiomelanocortins, utilizing all possible segments [11], or spans [12], of 30–50 residues. Background coincidences were judged by comparisons of randomly generated sequences from the same structures. Identities between the real sequences were related to those in the random comparisons, giving estimates of probabilities for chance occurrence [4]. However, probability values depend on method of calculation and on number of comparisons. Values given here refer to probabilities of a single hit belonging to the random distribution, without correction for the number of comparisons. With this system, similarities above the 0.01 probability level are usually not of interest for peptides of this size [13].

3. Results

3.1. *Sequence similarities*

Utilizing spans of 30 and 50 residues, respectively, in comparisons of human pro-opiomelanocortin with human leukocyte interferon α_1, 5 sets of alignments between the molecules show regions with 7–8 identities/ 30 residues, or 9–11 identities/50 residues. All these regions are shown in fig.1. No other similarity between the whole molecules was detected at this level (<0.01 in the probability expressions used).

Exchanging interferon α_2 for α_1 in the comparison also yields 3 of the alignments detected with interferon α_1 at this probability level. In addition, a fourth alignment fits the same region of pro-opiomelanocortin,

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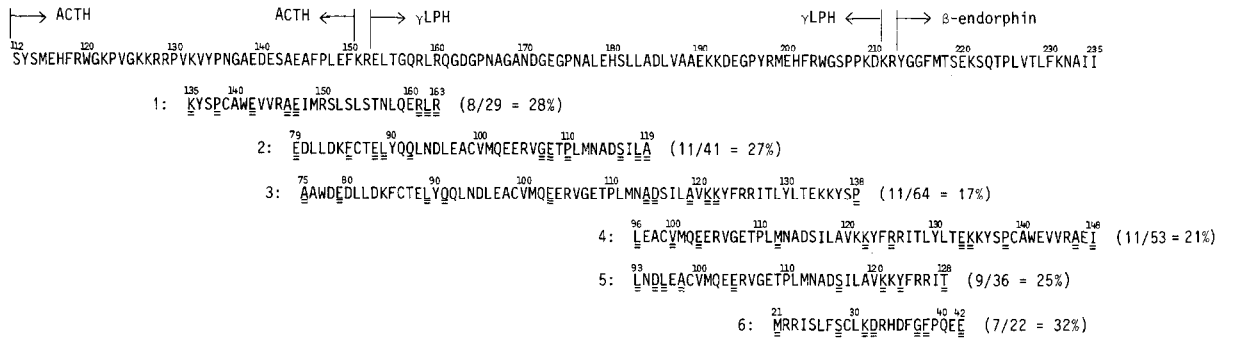

Fig.1. The most similar regions between human pro-opiomelanocortin (top row) and human leukocyte interferon (6 bottom rows). The regions shown are those containing 6 of 8 total similarities at the lowest probability level (<0.01 in the probability expression used). They include an internal part of pro-opiomelanocortin (starting inside ACTH and ending inside β-endorphin) and different internal parts of interferon that are aligned below the prohormone to show the identities (with double underlining). Borders of ACTH, γLPH and β-endorphin are indicated. All positional numbers refer to the respective complete sequences. Values within parentheses show the degree of sequence identity for each alignment. Alignments 1–5 are the only ones at this probability level in 30–50-residue span comparisons of the human prohormone and interferon α_1. They are shown with the sequences from interferon α_1. Similar comparisons with interferon α_2 instead, or with the bovine prohormone also yield alignments 1,2,3 (with interferon α_2), and 1,4 (with the bovine prohormone). Interferon α_2 also yields alignment 6 (this alignment therefore shown with the sequence of interferon α_2). In addition to the alignments shown, all comparisons only give 2 similarities at this probability level outside the depicted region of pro-opiomelanocortin. They include the human prohormone residues 7–29 towards interferon α_2 46–68 (7 identities in 23 residues) and the bovine prohormone residues 82–105 towards interferon α_1 103–126 (7 identities in 24 residues).

and a final one another region of the prohormone. All these alignments are listed in fig.1 and its legend. Exchanging the bovine pro-opiomelanocortin for the human prohormone gives only 3 alignments at this probability level, but 2 of these are again in the ACTH and endorphin regions as also shown in fig.1.

Combined, all comparisons show 8 alignments that give the highest sequence identities between regions of pro-opiomelanocortins and human α-interferons. Six of these correspond to the segment from ACTH to β-endorphin in the prohormone. Several of these 6 alignments were detected in different comparisons, involving spans of 30 or 50 residues, the bovine or human prohormone, and the α_1 or α_2 interferons. The alignments show sequence identities between interferon and hormone parts in the order of 20–25% for lengths of ~30–50 residues. Only 2 alignments show corresponding values for other regions of the prohormone (one alignment between the bovine prohormone and interferon α_1, one between the human prohormone and interferon α_2).

Consequently, there are sequence similarities between internal parts of pro-opiomelanocortin and α-interferons clustered into the region corresponding to end of ACTH/γLPH/start of β-endorphin in the prohormone. Interestingly, alignments 1 and 4 (fig.1) contain sequence identities in those regions of interferon predicted to correspond to antigenic determinants [14].

3.2. *Interpretation of the alignments detected*

The probability values for the different alignments in fig.1 do not allow interpretations as to the nature of the similarities. Identities are higher than those obtained in other regions but are not significant for distinction from random events.

Therefore, other aspects than the probabilities alone are of interest. It is then noticed that each of the 6 alignments in fig.1 are partly overlapping with at least 2 others. Furthermore, the shifts in either molecule between the alignments are all different and mutually exclusive for a common pattern as shown by the positional numbers in fig.1. Finally, different alignments are detected depending on whether the α_1 or α_2 interferons, and the bovine or human prohormone are compared. All these properties differ from those in another peptide comparison also involving low degrees of similarity [4], where positions and peptide origins instead supported each other in being compatible with a distant genetic relationship. In

the present case, however, correlations of the alignments in fig.1 do not support any such relationship. Even partial and multiple duplications can hardly explain the complicated pattern of shifts in fig.1.

Consequently, the combined data suggest that these pro-opiomelanocortins and α-interferons have some internal sequence similarities but that these cannot be taken to support divergence from a common origin. If anything, the patterns instead suggest an aggregation of non-related similarities. This picture would be compatible with a convergent evolution to some common structural properties, just as mutually exclusive limited similarities in another protein (the adenovirus hexon capsid protein) have been interpreted to indicate functional constraints on the sequence variability [15].

4. Discussion

4.1. *Immunology–structure correlation*

Earlier detected immunological cross-reactivities between interferon and ACTH or γ-endorphin have suggested a structural relationship [2]. This is also supported by the demonstration of sequence similarities between pro-opiomelanocortin and α-interferon (fig.1).

However, 2 aspects of the structural properties may be important:

(i) No region of maximal structural similarity involves the N-terminal part of ACTH (cf. fig.1), although immunological cross-reactivities were noticed between $ACTH_{1-13}$ and leukocyte interferon [2]. Consequently, immunological cross-reactivity may reflect structural similarities of just a few residues rather than the main similarities detected between the 2 molecules. This fact further demonstrates that few residues appear especially critical for ACTH functional activity, in agreement with studies of synthetic analogues [16].

(ii) More importantly, although a correlation exists between immunological and structural properties [14], the immunological relationship is without support for reflecting a divergent genetic relationship. This appears to be a novel aspect of immunological cross-reactivities, which are often taken to indicate sequence divergence in classifications [17,18]. These results, however, suggest that classifications cannot always be based on antigenic relationships. At least not when, as here, different molecular types are compared. Instead, it appears possible that immunological activities, exactly as sequence comparisons, can pick up structures corresponding to convergent (or random) evolutionary changes.

4.2. *Functional mechanism for interferon*

The immunological properties have suggested that interferon may function via mechanisms related to opiomelanocortin hormones [2]. This conclusion is compatible with the similarities inside the molecules in fig.1. From a functional point of view, the possibility of convergence rather than divergence, if significant, may be especially important, since it would indicate a common functional solution in spite of different structural origins.

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