Note
Importance of the Carboxy-terminus of Human Interleukin-11 in Conserving Its Biological Activity

Kenji Miyadai, Jun Ohsumi, Chigusa Yoshimura, Ichiro Kawashima, and Yasuhiro Ito*
Biomedical Research Laboratories and *Research Institute, Sankyo Co. Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan
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Biological activities of the carboxy-terminal (C-terminal) deletion mutants of human interleukin-11 (IL-11) were analyzed. Removal of only 1 amino acid residue (leucine) from the C-terminus caused nearly an 80% loss of its biological activity. This shows the importance of C-terminus of human IL-11 in terms of conserving the biological activity.

Key words: interleukin-11; deletion mutant; biological activity

We have reported on the isolation of a cDNA encoding a novel adipogenesis inhibitory factor (AGIF) from a human bone marrow-derived stromal cell line and showed the identity of this factor with interleukin-11 (IL-11). IL-11 was originally identified in medium conditioned by a macaque bone marrow-derived stromal cell line, PU-34, and it was cloned as a growth factor for the IL-6 dependent plasmacytoma cell line T1165. IL-11 is a multi-functional cytokine with a wide spectrum of in vitro biological activities in the hematopoietic, lymphopoietic, hepatic, adipose, neuronal, and osteoclast cells, either alone or in synergy with other hematopoietic growth factors (for reviews see refs. 3 and 4). It was reported that IL-11 is synthesized as a precursor consisting of 199 amino acids and processed into the mature form of 178 amino acids by cleavage between alanine (−1) and proline (+1). A structure-function analysis of IL-11 was reported whereby it was shown that at least 4 amino acid residues at the carboxy-terminal (C-terminal) portion of human IL-11 molecule are indispensable for in vitro function.

This paper describes generation and evaluation of a series of C-terminal deletion mutants of IL-11 lacking 1, 2, 3, 4, 5, and 10 amino acid residues (JC1, JC2, JC3, JC4, JC5, and JC10). The C-terminal deletions of IL-11 were generated by oligonucleotide-directed mutagenesis on a single-strand DNA template using an oligonucleotide-directed mutagenesis system (Amersham). A BamHI-BglII fragment carrying the complete coding region of IL-11 from the plasmid pSBr-2.2 was subcloned into the M13mp10 vector. Six oligonucleotides were synthesized to generate the following mutations. (JXaa means the deleted amino acid residue(s) from C-terminus of IL-11):

JC1 (Leu):
5'CTGTCGAAAGACCTCTGGTACCCGGGGGACCAAGGCCAC 3'
5'CAGGTTGCTAAGGCTACCTTGACCTGGGGGCCAAAGCCAC 3'
JC2 (JAAG-Leu):
5'CTGTCGAAAGACCTCTGGTACCCGGGGGACCAAGGCCAC 3'
5'CAGGTTGCTAAGGCTACCTTGACCTGGGGGCCAAAGCCAC 3'
JC3 (1Thr-Arg-Leu):
5'GAAGCTGCTGGTCAGTGAACCCGGGGGACCAAGCCAC 3'
5'CAGGTTGCTAAGGCTACCTTGACCTGGGGGCCAAAGCCAC 3'
JC4 (JLys-Thr-Arg-Leu):
5'GAGGCTGCTGGTCAGTGAACCCGGGGGACCAAGCCAC 3'
5'CAGGTTGCTAAGGCTACCTTGACCTGGGGGCCAAAGCCAC 3'
JC5 (Leu-Lys-Thr-Arg-Leu):
5'GGGCTGCTGGTCAGTGAACCCGGGGGACCAAGCCAC 3'
5'CAGGTTGCTAAGGCTACCTTGACCTGGGGGCCAAAGCCAC 3'
5'CTGACTGGGCGCTGCTGACCCGGGGGACCAAGCCAC 3'

The individual mutations were finally checked by sequencing using the dye-exchange chain termination method. The mutants were subcloned into the IL-11 expression in vitro transcription vector pGEM-3Z-20-2 by replacing the Xhol-Smal fragment coded the C-terminus of IL-11. IL-11 mutant proteins were produced using the TNT coupled reticulocyte-lysate system (Promega). These mutant proteins were analyzed by SDS-PAGE and functionally analyzed.

Fig. 1. SDS PAGE of Cell-free Synthesized IL-11 Mutants Truncated at the C-Terminus.
Zero point five micrograms of each DNA was transcribed and translated using TNT coupled reticulocyte-lysate system (Promega), and full-length IL-11 and the truncated forms were separated by SDS PAGE (12.5%) and visualized by fluorography. JC0 represents the full-length IL-11 control, JC1, JC2, JC3, JC4, JC5, and JC10 denote the IL-11 polypeptides lacking 1, 2, 3, 4, 5, and 10 amino acid residues from the C-terminus, respectively.

Fig. 2. Biological Activity of Full-length and C-terminally Truncated IL-11 Polypeptide.
Serial dilutions of translation cocktails containing cell-free synthesized IL-11 were used in a T1165 cell proliferation-assay measured by 3H-thymidine incorporation. Each dilution value is corrected on the basis of the 3H-methionine radioactivity measured by the imaging-analyzer (Fuji Film, BAS-1000). The data are expressed as means ± S.D. (n = 3).

Abbreviations: C-terminal, carboxy-terminal, IL-11, interleukin-11; AGIF, adipogenesis inhibitory factor, SDS PAGE, SDS polyacrylamide gel electrophoresis.

* To whom correspondence should be addressed.
plasmids expressing C-terminal deletion mutants of IL-11 were transcribed with T7 RNA polymerase and translated in the reticulocyte lysate in the presence of 35S-methionine (Amersham). Translated proteins were analyzed on SDS-polyacrylamide gel (12.5%) electrophoresis (SDS-PAGE) and fluorographed.

Figure 1 shows an increased electrophoretic mobility of the 35S-methionine-labeled IL-11 mutants in comparison with the full-length IL-11 (ΔC0), which was also synthesized by the in vitro coupled transcription and translation system. Figure 2 shows the IL-11 dependent proliferation of murine plasmacytoma cell line T1165 as measured by 3H-thymidine incorporation. One × 10⁴ T1165 cells were cultured in 200 μl of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and varying concentrations of IL-11 for 44 h in 96-well plates. Two hours before cell harvest, 20 μl samples of saline containing 0.5 μCi 3H-thymidine (Amersham) were added. The cells were harvested and the 3H-thymidine incorporation was measured. Assays were run in triplicate and the results were expressed as a mean value ± standard deviation. As a control, we used purified recombinant human IL-11 standard, and the half-maximal thymidine incorporation was seen at 0.20 ng/ml (data not shown). When translated full-length IL-11 (ΔC0) was added to the T1165 cells, a dilution-dependent proliferation was detected and the half-maximal thymidine incorporation (2500 dpm/well) was seen at a dilution value of 0.0005. From these results it can be inferred that the concentration of the translated ΔC0 was 0.40 μg/ml. Incidentally, the concentration of translated ΔC1, ΔC2, ΔC3, ΔC4, ΔC5, and ΔC10 estimated from the 35S-methionine radioactivity by the imaging-analyzer (Fuji Film, BAS-1000) was 0.28, 0.43, 0.47, 0.47, 0.42, and 0.50 μg/ml, respectively. To compare each activity, each dilution value needed to be compensated for by the estimated concentration value. The C-terminal truncated mutant (ΔC1) had its half-maximal point (2500 dpm/well) at a dilution value of 0.002. These results show that the removal of only 1 amino-acid residue from the C-terminus (ΔC1) leads to nearly an 80% reduction in IL-11 activity. In addition, elimination of 10 amino-acid residues (ΔC10) completely abolished the IL-11 activity. It has already been reported that removal of 4 amino-acid residues from the C-terminus lead to a 96% reduction in IL-11 activity. These results show that not only the 4 amino-acid residues fragment of IL-11 but also a single amino-acid residue belonging to this cytokine is important for its biological activity.

The above results have yielded the following two interpretations. One is that the C-terminal amino-acid residues(s) of IL-11 are essential for its biological activity through its receptor binding or folding. The other is that C-terminal deletion mutants of IL-11 are destroyed by certain proteases present in the assay medium more easily than that of the full-length IL-11 (ΔC0).

When these C-terminal truncated IL-11s were expressed in COS cells using the pSRα vector, the amounts of secreted IL-11 lacking 1, 2, 3, 4, 5, and 10 amino-acid residue(s) from their C-termini were 67%, 60%, 7%, 0%, 0%, and 0%, respectively of that of the full-length IL-11 (ΔC0), while the amount of each IL-11 detected in the cells was almost the same. On the other hand, the secreted IL-11s (ΔC1, ΔC2, and ΔC3) were not degraded in the culture medium after incubation at 37°C for 24 h (data not shown).

The latter results are indicative of C-terminal deletion mutants (at least ΔC1, ΔC2, and ΔC3) being degraded less easily than the full-length IL-11 (ΔC0), and thus ascertains that the C-terminus of IL-11 is important not only for its biological activity but also for its transportation and secretion in cells. The C-terminal truncated IL-11s may fold incorrectly and the transportation-secretion system in cells would not recognize them, or a degradation system such as BIP might recognize these misfolded proteins preferentially. It is noteworthy however that these hypotheses are not inconsistent with the report insisting that removal of 4 amino-acid residues from the C-terminus of IL-11 did not adversely affect the global folding but may affect some local structure change.

In conclusion, the removal of 1 amino-acid residue from the C-terminus of IL-11 resulted in nearly an 80% loss in its activity. This result shows that the importance of the C-terminus of human IL-11 in terms of conserving the biological activity.

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References