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N-terminal-methionylated interleukin-1β has reduced receptor-binding affinity

Paul Wingfield, Pierre Graber, N. Rao Movva, Angela M. Gronenborn* and H. Robson MacDonald+

*Biogen SA, PO Box 1211, Geneva 14, Switzerland, *Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG* and *+Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland*

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The receptor-binding affinity of recombinant-derived interleukin-1β containing unprocessed N-terminal methionine (MAPV-) was 10-fold lower than protein containing the authentic N-terminal sequence (APV-). Structural analysis of the methionylated and non-methionylated proteins by NMR spectroscopy detected no (or minor) conformational differences. The differences in binding affinity, therefore, suggest that the additional N-terminal methionine causes a small, direct or indirect, perturbation of the receptor-binding region.

Interleukin-1; Interleukin-1 receptor; NMR; Methionine aminopeptidase; N-terminal processing

1. INTRODUCTION

The purification and characterization of recombinant-derived interleukin-1β (IL-1β) have been recently described [1,2]. The purified protein was found to be a mixture of 80% correctly processed protein (N-terminus = Ala) and 20% of an N-terminal-methionylated form. The presence of N-terminal Met in a mature polypeptide is not uncommon and may be due to the difficulty which the processing enzyme methionine aminopeptidase experiences in removing Met when it is followed by a residue with large radius of gyration [3]. For recombinant-derived proteins produced in *E. coli* the removal of Met may also be compromised by the very high levels of accumulated protein which may simply saturate the processing enzyme(s). For IL-1β this is almost certainly true, since the purified protein can be readily de-methionylated in vitro using purified methionine aminopeptidase [4]. Partial removal of Met has also been reported for some other recombinant-derived proteins produced in *E. coli* [5,6].

Here, we have used a competition binding assay [7,8] to compare the receptor-binding affinities of N-terminal-methionylated and non-methionylated wild-type IL-1β and also that of a mutant in which His 30 has been replaced by an Arg residue [7]. Surprisingly, in both instances, the methionylated proteins showed 10-fold lower receptor-binding affinities compared to the corresponding non-methionylated forms.

Correspondence address: P. Wingfield, Biogen SA, PO Box 1211, Geneva 14, Switzerland

2. MATERIALS AND METHODS

2.1. *Preparation of methionylated and non-methionylated IL-1β proteins*

N-terminal-methionylated and non-methionylated IL-1β wild-type proteins were separated by chromatofocusing as described [2]. The mutant IL-1β His 30 ⟶ Arg was fractionated in a similar manner except that the sample, dialysed against 20 mM Tris-HCl (pH 8.0) was applied to a fast liquid chromatography (FPLC) MonoP column equilibrated with 25 mM Bis-Tris-acetate (pH 7.3).

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Polybuffers were removed by hydrophobic chromatography using a phenyl-Superose HR 5/5 (Pharmacia) column. Protein concentrations were determined by ultraviolet absorbance: an $A^{1\%}_{1cm}$ = 0.63 at 280 nm was used [1].

2.2. *Preparation of methionine aminopeptidase and in vitro digestion of IL-1β*

Methionine aminopeptidase was purified from *Salmonella typhimurium* and used for the in vitro processing of N-terminal-methionylated IL-1β as in [4].

2.3. *NMR spectroscopy*

Sample preparation and measurements were made as detailed elsewhere [7,8].

2.4. *Analytical measurements*

Isoelectric focusing on thin-layer polyacrylamide gels and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were performed according to [1,2].

2.5. *IL-1 receptor-binding assay*

The assay procedure has been described in detail elsewhere [7,8].

3. RESULTS AND DISCUSSION

As reported in [2], purified N-terminal-methionylated and non-methionylated wild-type IL-1β exhibit single bands on SDS-PAGE (M_r = 17500) and the methionylated protein has an isoelectric point (pI = 6.55) approx. 0.25 pH units lower than the non-methionylated protein. Similar results were obtained with the corresponding IL-1β mutant proteins (not shown).

The methionylated wild-type IL-1β had an approx. 10-fold lower receptor-binding activity compared to the non-methionylated protein (fig.1A). To show that the reduced receptor binding was specifically due to the presence of N-terminal Met, N-terminal-methionylated IL-1β was digested with an N-terminal-specific peptidase, methionine aminopeptidase, purified and utilized as described by Miller et al. [4]. As expected, removal of N-terminal Met resulted in a pI increase of 0.25 pH units with a concomitant 10-fold increase in receptor-binding affinity (fig.1A). Enzyme treatment of the non-methionylated IL-1β had no effect on binding affinity (fig.1A).

Fig.2 shows a comparison of the 500 MHz ^{1}H-NMR spectra from the methionylated form of IL-1β and from the protein without the N-terminal Met. As can easily be appreciated both spectra are very similar, thus demonstrating that no gross conformational differences exist between the two forms of IL-1β. The resonances arising from the additional Met are indicated at the top of the figure. The new methyl resonance is clearly visible in the one-dimensional spectrum, whereas the other resonances belonging to the additional Met were identified in a two-dimensional HOHAHA spectrum (not shown). A comparison of the two-dimensional spectra confirmed that only very small differences exist between the two protein forms. We could identify two shifts in α-proton resonances and two in the methyl region; both are, however, extremely small (<0.05 ppm). Incidentally, one of the residues experiencing a shift is a

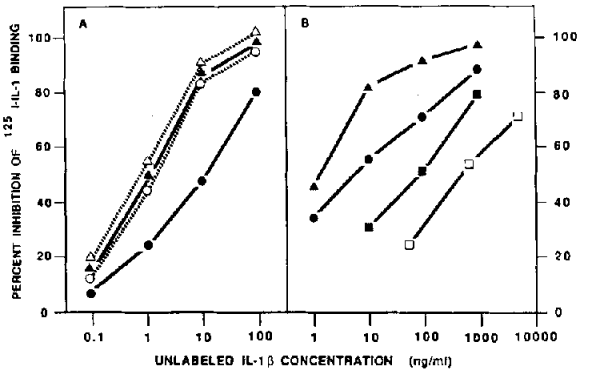

Fig.1. Competition binding assay of N-terminal-methionylated and non-methionylated IL-1β. The indicated concentrations of unlabeled IL-1β proteins were mixed at 4°C with ^{125}I-IL-1α (1 ng/ml) prior to addition of EL4-6.1 cells (5 × 10^5/tube). After 4 h at 4°C, bound radioactivity was evaluated by centrifugation of cells through an oil gradient. Data are expressed as percent inhibition of ^{125}I-IL-1 binding compared to untreated controls. (A) N-terminal non-methionylated IL-1β treated (△) and non-treated (▲) with methionine aminopeptidase are compared with N-terminal methionylated IL-1β treated (○) and non-treated (●) with protease. (B) N-terminal non-methionylated IL-1β with a His 30 ⟶ Arg substitution (■) and N-terminal-methionylated mutant IL-1β (□) are compared with the corresponding wild-type proteins.

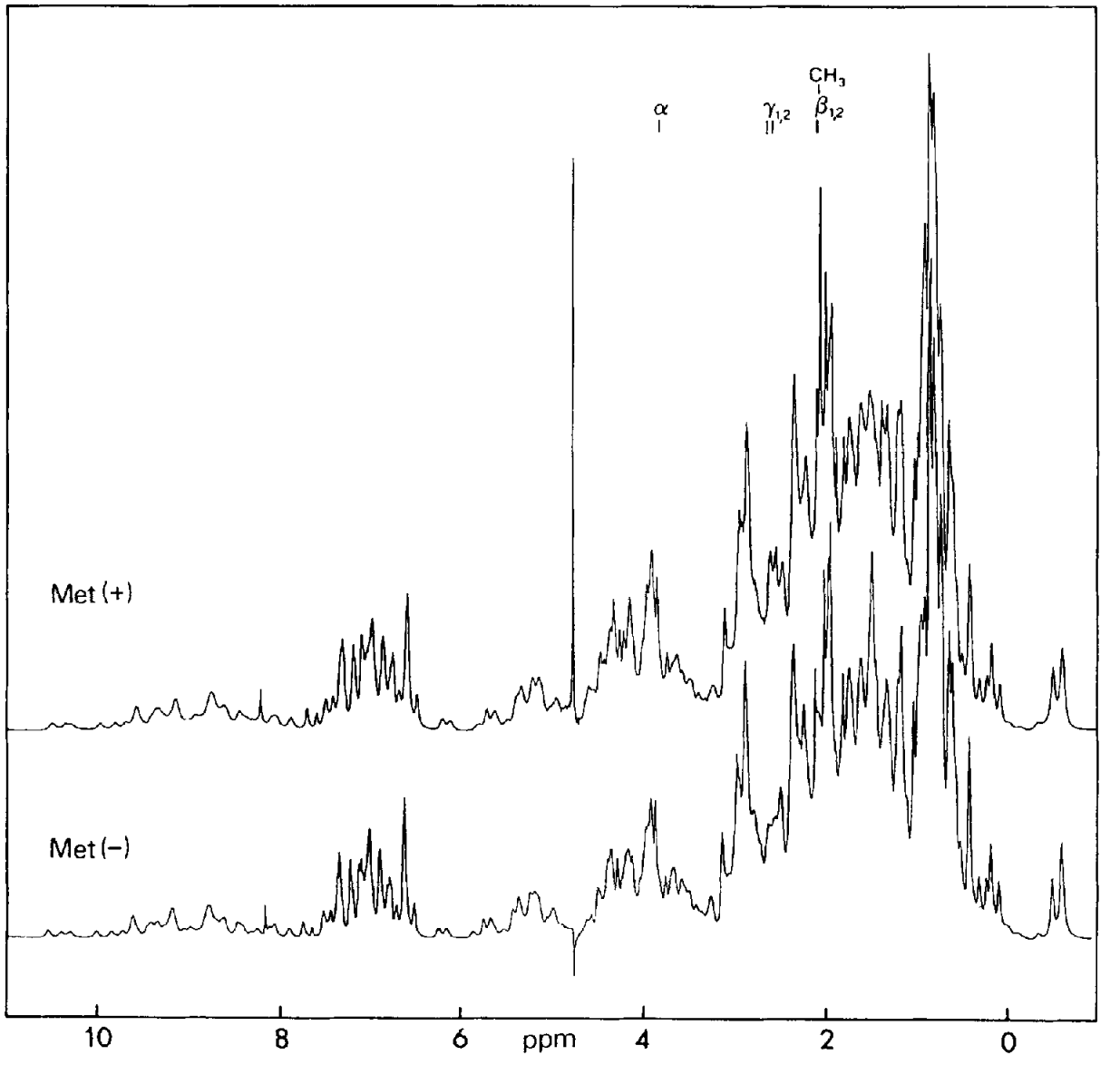

Fig.2. 500 MHz ^{1}H-NMR spectra of N-terminal-methionylated [Met(+)] and N-terminal non-methionylated [Met(−)] IL-1β at 25°C.

threonine (Thr b), whose spin system was identified in an earlier study [8].

In a previous report it was shown that the IL-1β mutant His 30 ⟶ Arg has a 100-fold lower receptor-binding affinty compared to wild-type protein [7]. Furthermore, analysis by NMR spectroscopy indicated that this activity difference was not due to gross conformational changes [7]. The mutant protein used in the aforementioned study, similar to wild-type IL-1β, consisted of approx. 20% N-terminal-methionylated and 80% non-methionylated protein. It was, therefore, of interest to see whether the presence of N-terminal Met also affected receptor binding of this protein. The result obtained, which indicated a 10-fold reduction in receptor-binding affinity (fig.1B), was similar to that obtained with the corresponding wild-type proteins. The methionylated IL-1 mutant

has, therefore, almost a 1000-fold lower receptor-binding affinity compared to the wild-type non-methionylated protein. This simple additive decrease in binding affinity may indicate that the two protein modifications are producing independent, and probably indirect, perturbations of the receptor-binding region. If both N-terminal Ala and His 30 were directly involved in receptor binding, we might have expected larger, i.e. synergistic, rather than additive decreases in binding affinity as a result of modifying both residues.

Obviously, in the absence of real structural information on IL-1β and its receptor, we can only speculate on the mechanism by which modification of the N-terminus of IL-1β perturbs receptor binding. However, several recent reports are relevant to this issue. Schrader et al. [10] have drawn attention to the N-terminal sequence homologies among several cytokines, including IL-1β and interleukin-2 (IL-2). The N-terminal sequence of IL-1β, namely Ala-Pro, is the most common sequence, and the conservation of N-terminal Ala is suggested to have functional and/or structural significance. The results described herein would seem to support this prediction, at least for IL-1β. In the case of IL-2, however, Yamada et al. [5] have reported that there is no difference in biological activity between N-terminal-methionylated and non-methionylated protein. In this context, Wingfield et al. [1] also saw little difference in biological activity between methionylated and non-methionylated IL-1β. (Although the methionylated IL-1β was consistently at least 2-fold less active than the non-methionylated protein, this was not considered significant in view of the inherent variability in the assay used.) In the case of IL-2, it would be of interest to compare the receptor-binding affinities of the methionylated and non-methionylated proteins.

Various biologically active N-terminal deletion analogues of IL-1 have been reported [11,12], suggesting that the N-terminal region has no direct functional role. Since these reports were of a somewhat preliminary nature and since the chemical and physical properties of neither the control nor deletion analogues used were described, one must say that the structural and/or functional role of the N-terminal region remain to be established.
established.

4. CONCLUSIONS

Recombinant-derived proteins produced in *E. coli* may contain N-terminal Met as a result of incomplete processing of initiating *N*-formylmethionine (see [13] for discussion). The presence of this additional residue on the N-terminus would be a concern if the protein in question were to be used in clinical studies [14,15]. Although there is no immediate clinical application envisaged for IL-1β, the reduced receptor-binding affinity of the N-terminal-methionylated protein would make it seem worthwhile to use only the purified non-methionylated protein for detailed structural/function studies. The methods and procedures outlined here and in the cited references provide routes to pure recombinant-derived IL-1β with the chemical, and presumably the biological, properties of the authentic protein.

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REFERENCES

[1] Wingfield, P., Payton, M., Tavernier, J., Barnes, M., Shaw, A., Rose, K., Simona, M.G., Demaczuk, S., Williamson, K. and Dayer, J.-M. (1986) Eur. J. Biochem. 160, 491–497.
[2] Wingfield, P., Graber, P., Rose, K., Simona, M.G. and Hughes, G.J. (1987) J. Chromatogr. 387, 291–300.
[3] Sherman, F., Stewart, J.W. and Tsumasawa, S. (1985) BioEssays 3, 27–31.
[4] Miller, C.G., Strauch, K.L., Kukral, A.M., Miller, J.L., Wingfield, P.T., Mazzei, G., Werlan, R., Graber, P. and Movva, N.R. (1987) Proc. Natl. Acad. Sci. USA, in press.
[5] Yamada, T., Kato, K., Kawahara, K. and Nishimura, O. (1986) Biochem. Biophys. Res. Commun. 135, 837–843.
[6] Staehelin, T., Hobbs, D.S., Kung, H.-F. and Pestka, S. (1981) Methods Enzymol. 78, 505–511.
[7] MacDonald, H.R., Wingfield, P., Schmeissner, U., Shaw, A., Clore, G.M. and Gronenborn, A.M. (1986) FEBS Lett. 209, 295–298.
[8] Lowenthal, J.W. and MacDonald, H.R. (1986) J. Exp. Med. 164, 1060–1074.

[9] Gronenborn, A.M., Clore, G.M., Schmeissner, U. and Wingfield, P. (1986) Eur. J. Biochem. 161, 37–43.
[10] Schrader, J.W., Ziltener, H.J. and Leslie, K.B. (1986) Proc. Natl. Acad. Sci. USA 83, 2458–2462.
[11] De Chiara, T.M., Young, D., Semionow, R., Stern, A.S., Batula-Bernardo, C., Fiedler-Nagy, C., Kaffka, K.L., Kilian, P.L., Yamazaki, S., Mizel, S.B. and Lomedico, P.T. (1986) Proc. Natl. Acad. Sci. USA 83, 8303–8307.
[12] Rosenwasser, L.J., Webb, A.C., Clark, B.D., Irie, S., Chang, L., Dinarello, C.A., Gehrke, L., Wolff, S.M., Rich, A. and Auron, P.E. (1986) Proc. Natl. Acad. Sci. USA 83, 5243–5246.
[13] Marston, F.A.O. (1986) Biochem. J. 240, 1–12.
[14] Hsiung, H., Mayne, N.G. and Becker, G.W. (1986) Bio/Technology 4, 991–995.
[15] Glasbrenner, K. (1986) J. Am. Med. Assoc. 255, 581–587.