Stable isotope containing peptides as probes of ligand–receptor interactions

Deutero-formyl-methionyl–leucyl–phenylalanine

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The synthesis and biological evaluation of the stable isotope containing chemotactic peptide, deutero-formyl-methionyl-leucyl-phenylalanine (C^2DO-Met-Leu-Phe-OH) is reported. The results, using lysosomal enzyme release from cytochalasin B-treated rabbit neutrophils for biological evaluation indicates a 3–4 fold enhancement over CHO-Met-Leu-Phe-OH itself. This is consistent with previous speculation that the formyl proton hydrogen bonds with a critical area of the chemotactic peptide receptor of rabbit neutrophils.

Chemotactic peptide *Lysosomal enzyme release* *Receptor* *Deuterium* *Hydrogen-bonding*

1. INTRODUCTION

N^{α}-Formyl-methionyl–leucyl–phenylalanine (CHO-Met–Leu–Phe-OH) is the prototype of a series of small molecular mass oligopeptides which stimulate chemotaxis, lysosomal enzyme secretion and a variety of other cellular responses in mammalian phagocytes [1,2]. These effects are mediated by interaction of the peptide with a specific membrane receptor [3,4] the structural requirements of which have been summarized [5]. This summary, expressed as a hypothetical model of the receptor of rabbit neutrophils, included speculation about several specific interactions between the tripeptide and its receptor. In light of the almost absolute requirement for the presence of the formyl group it was postulated that the marked enhancement produced by formylation was the result of hydrogen bonding of the formyl proton with a critical area on the receptor. As a test of this hypothesis we have attempted to determine if the well-known stable isotope enhancement of hydrogen bonding seen with some enzyme–substrate interactions [6] would also be evident with this ligand–receptor interaction. Accordingly, we have synthesized deutero-formyl-methionyl–leucyl–phenylalanine (C^2DO-Met–Leu–Phe-OH). This analog has been evaluated analytically, relative to CHO-Met–Leu–Phe-OH, and for its ability to induce lysosomal enzyme secretion from rabbit neutrophils.

2. EXPERIMENTAL

The tripeptide benzyl ester, H-Met–Leu–Phe-OBzl was prepared by a rapid mixed anhydride method using isobutylchloroformate [7]. Deuteroformic acid (Merck) was coupled via a conventional anhydride procedure using

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isovaleryl-chloride as in [9]. Final deprotection was with anhydrous hydrogen fluoride/anisole (45 min, 4°C) and the compound was purified by crystallization from acetone/H_2O. The compound was compared to CHO-Met–Leu–Phe-OH by:

(i) Thin-layer chromatography (by vol.) [System (A) benzene: H_2O: acetic acid (9:1.9); system (B), *N*-butanol:H_2O: acetic acid (4:1:1); system (C) chloroform:methanol:H_2O:acetic acid (60:30:1:4)];
(ii) Melting point;
(iii) Nuclear magnetic resonance (NMR) spectroscopy in DMSO.

In addition, by amino acid analysis (Beckman 119C) the compound was also found to contain the appropriate residues in a 1:1:1 molar ratio.

Lysosomal enzyme release was determined for both CHO-Met–Leu–Phe-OH and C^2DO-Met–Leu–Phe-OH using cytochalasin-B-treated rabbit neutrophils as in [1].

3. RESULTS

The synthesis of C^2DO-Met–Leu–Phe-OH proceeded uneventfully although by a different route than reported for CHO-Met–Leu–Phe-OH [8]. Side-by-side comparison of the deuterated analog and authentic CHO-Met–Leu–Phe-OH showed that the two were indistinguishable with respect to chromatographic mobility and melting point (table 1). Furthermore, a NMR spectrum of C^2DO-Met–Leu–Phe-OH was obtained in DMSO and compared to that reported for CHO-Met–Leu–Phe-OH [10]. The results indicated that, with the exception of the total absence of the formyl proton resonance, the position and relative intensity of the other resonances were identical for both peptides.

Table 1

Analytical comparison of CHO-Met–Leu–Phe-OH and C^2DO-Met–Leu–Phe-OH

	CHO-Met–Leu–Phe-OH	C^2DO-Met–Leu–Phe-OH
Thin-layer mobility (R_f)[a]		
system A	0.66	0.66
system B	0.67	0.67
system C	0.68	0.68
Melting point (°C)	213–215	211–212

[a]System A = benzene:water:acetic acid (9:1:9, by vol.); system B = *n*-butanol:water:acetic acid (4:1:1, by vol.); system C = chloroform:methanol:water:acetic acid (60:30:1:4, by vol.).
Mixtures of the two peptides also gave a single spot in each system.

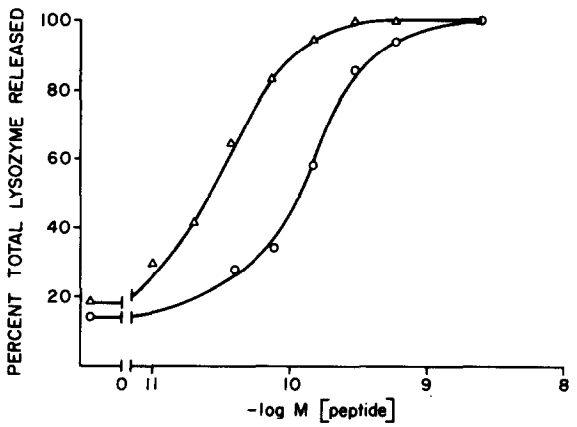

Fig. 1. Lysosomal enzyme secretion from cytochalasin B-treated rabbit neutrophils. Concentration dependency for C^2DO-Met–Leu–Phe-OH (△) and CHO-Met–Leu–Phe-OH (○). Each point is the average of 5 determinations, done in duplicate, each of which had SEM of <5%.

The results of the biological evaluation are shown in fig. 1 and clearly indicate a significantly enhanced activity for the deuterated analog. The apparent affinities (i.e., the concentration to produce 50% of the maximum effect) were 1.5×10^{-10} M (CHO-Met–Leu–Phe-OH) and 3.0×10^{-11} M (C^2DO-Met–Leu–Phe-OH), respectively. There was a similar decrease in the threshold concentration for C^2DO-Met–Leu–Phe-OH but no change in the maximum effect. Overall, this isotopic substitution results in a 4–5-fold enhancement in the biological activity of the deuterated analog *vs* CHO-Met–Leu–Phe-OH.

4. DISCUSSION

When stable isotopes are introduced into substrates at sites known to hydrogen bond, enhancement of the enzyme–substrate interaction is well documented to occur [6]. In an effort to test our hypothesis that the proton of the formyl group

was hydrogen bonding with the receptor, we attempted to demonstrate a similar enhancement following deuterium substitution into CHO-Met–Leu–Phe-OH. As indicated, this substitution did indeed enhance biological activity and to about the same extent (4–5-fold) as with enzyme–substrate interactions.

Obviously, biological activity of any complex ligand is a function both of the 3-dimensional structure of the ligand and the effectiveness of the interaction with its receptor. Any chemical alteration may therefore be expected to change either or both of these determinants. The unequivocal answer to the question posed here then requires that no change in the conformation result from the deuterium substitution. That this is the case is strongly supported by the finding that the NMR spectrum, less the formyl proton resonance, of the deuterated analog is identical to that of authentic CHO-Met–Leu–Phe-OH [10]. It should be noted, however, that the spectra were taken in DMSO and therefore do not take into account peptide–solvent interactions in physiological buffers. As further evidence of this 'identity' we note the identical mobility in a variety of thin-layer chromatography systems and the closeness of the melting points.

In summary, we have prepared C^2DO-Met–Leu–Phe-OH and found it to be indistinguishable from CHO-Met–Leu–Phe-OH in all ways except for its enhanced biological activity. This we believe is good evidence in support of the hypothesis that the formyl proton hydrogen bonds with the receptor of rabbit neutrophils. In addition to its specific use we also submit that stable isotope substitutions may be useful probes to evaluate ligand–receptor interactions in general.

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