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'ACETALDEHYDE-ENKEPHALINS': ELUCIDATION OF THE STRUCTURE OF THE ACETALDEHYDE ADDUCTS OF METHIONINE-ENKEPHALIN AND LEUCINE-ENKEPHALIN

Michael C. SUMMERS*, Michael J. GIDLEY and Jeremy K. SANDERS
*Biochemistry Department, St Mary's Hospital Medical School, Paddington, London W2 and University Chemical Laboratories, Lensfield Road, Cambridge, England

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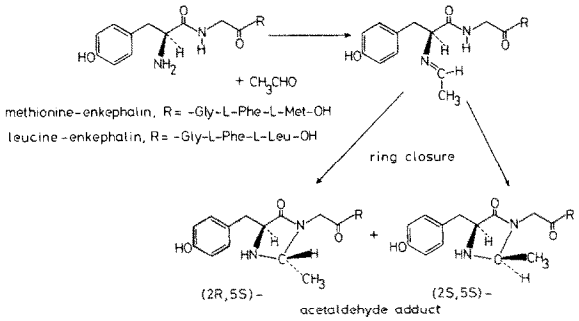
1. Introduction

The two major endogenous opioid pentapeptides, methionine-enkephalin and leucine-enkephalin [1,2] rapidly react with acetaldehyde, under very mild conditions, to give an 'acetaldehyde-enkephalin' adduct, referred to as 'acetaldehyde-enkephalin [Met^5] and 'acetaldehyde-enkephalin [Leu^5], respectively [3]. These adducts are sufficiently stable that their chemical structure and biological activities can be determined. We have shown that 'acetaldehyde-enkephalin' adduct formation causes a pronounced loss of the opiate activity of both parent opioid pentapeptides [3,4]. Because of this loss of activity we considered it important to determine the chemical structure of the acetaldehyde adducts, and the influence that acetaldehyde adduct formation has on the solution conformation of the enkephalins. In this first report, an analysis of the 100 MHz proton nuclear magnetic resonance (^{1}H NMR) spectra of methionine-enkephalin and its corresponding stable acetaldehyde adduct has established that the latter arises by formation of a 2-methylimidazolidin-4-one ring structure in which the acetaldehyde moiety forms a molecular bridge between the α-amino group of the N-terminal tyrosine residue and the amide nitrogen of the $Tyr^{(1)}$–$Gly^{(2)}$ peptide bond. This is outlined in scheme 1, and detailed in the following discussion.



Scheme 1

2. Materials and methods

2.1. *Synthesis of methionine-enkephalin and 'acetaldehyde-enkephalin [Met^5]*

Methionine-enkephalin was prepared by standard liquid-phase synthesis as in [5]. 'Acetaldehyde-enkephalin [Met]' was prepared by incubating methionine-enkephalin with acetaldehyde in 0.05 M phosphate buffer (KH_2PO_4/Na_2HPO_4) (pH 7.0) at room temperature, and purified by the procedure in [3]. Other acetaldehyde-peptide adducts and 'acetone-enkephalin [Met^5]' were synthesised by standard methods [3].

2.2. *Spectroscopic measurements*

^{1}H NMR spectra of 100 MHz were recorded on a Varian XL-100 spectrometer equiped with a decoupler unit, and operated at 30°C. NMR parameters: 100 scans; acquisition time, 4.0 s; 8 k data points. Integrated and 80 MHz ^{1}H NMR spectra were obtained using a Varian CFT-20 spectrometer.

Chemical shifts (in δ values) are reported in parts per million (ppm) downfield from external tetramethylsilane.

3. Results and discussion

Structure elucidation of the 'acetaldehyde-enke-

phalin' adducts has been primarily achieved by the use of ^{1}H NMR spectroscopy. However, reliable interpretation of the data has necessitated not only an analysis of the adducts indicated in the rubric to this paper, but also an investigation of the ^{1}N NMR spectrum of several other 'acetaldehyde-peptide' adducts and, in particular, the acetaldehyde derivative of the tripeptide

L–Tyr–Gly–Gly–OH (detailed in [3]);

this adduct has been used extensively in our 100 MHz ^{1}H NMR investigations because it retains many of the essential structural features of the 2 opioid pentapeptides, but produces a considerably simpler spectrum.

The ^{1}H NMR spectrum of methionine-enkephalin acetate is presented in fig.1. The 100 MHz spectrum, as shown, compares very well with previously recorded spectra at 270 [6], 300 [7,8] and 360 MHz [9,10] using solutions of methionine-enkephalin in deuteriated dimethylsulphoxide (d_6-DMSO). Chemical shift assignments are in broad agreement and any reported major discrepancies, such as the assignment of individual amide NH resonances, have been attributed to a pH and concentration dependence on the conformation of methionine-enkephalin in solution [11–13]. Proton assignments and coupling constants have been detailed in [6–10] and will, therefore, not be repeated here; proton resonances pertaining to the present discussion are included in fig.1,2. A noteworthy feature of the spectrum of methionine-enkephalin is the low field signal at 8.6 ppm of the amide NH of the Tyr$^{(1)}$–Gly$^{(2)}$ peptide bond in which both its position and broadening may be attributed to the proximity of the α-NH_2 of the N-terminal tyrosine residue [10,12–15]. Other important features include the α proton resonances of the constituent amino acids occurring at 3.8, 4.2 and 4.5 ppm corresponding to Gly$^{(2,3)}$, Met$^{(5)}$ and Phe$^{(4)}$, respectively. The α proton of Tyr$^{(1)}$ is not completely resolved from the αCH_2 resonances of Gly$^{(2)}$ and Gly$^{(3)}$ at 100 MHz.

By contrast, the ^{1}H NMR spectrum of 'acetaldehyde-enkephalin [Met^5]', shown in fig.2, indicates several important differences to that observed for methionine-enkephalin in fig.1. Most striking is the disappearance of the amide NH of Gly$^{(2)}$ and the appearance of new proton resonances at ~4.3 and ~1.1 ppm. Integration confirmed the loss of one amide NH proton, the appearance of one additional proton at 4.3, and 3 protons at 1.1 ppm. Furthermore, ^{1}H NMR studies, at both 80 and 100 MHz, on the 'acetaldehydetripeptide (Tyr–Gly–Gly–OH)'

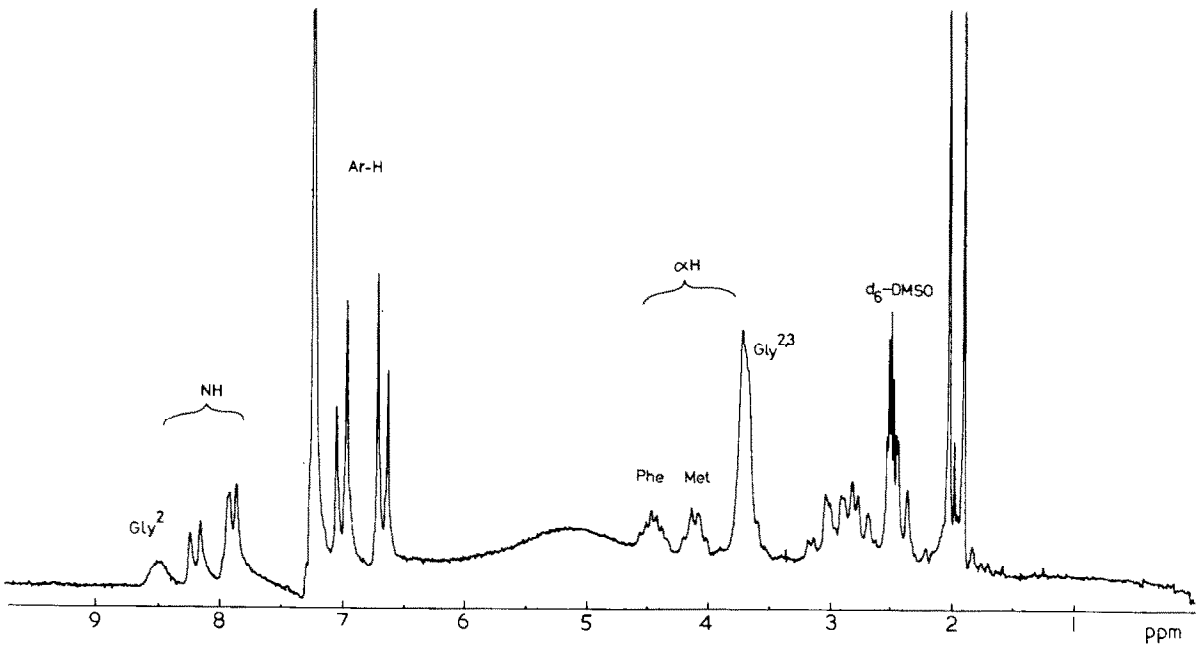


Fig.1. 100 MHz ^{1}H NMR spectrum of methionine-enkephalin (10 mg/ml) in deuteriated dimethylsulphoxide (d_6-DMSO) at 30°C.

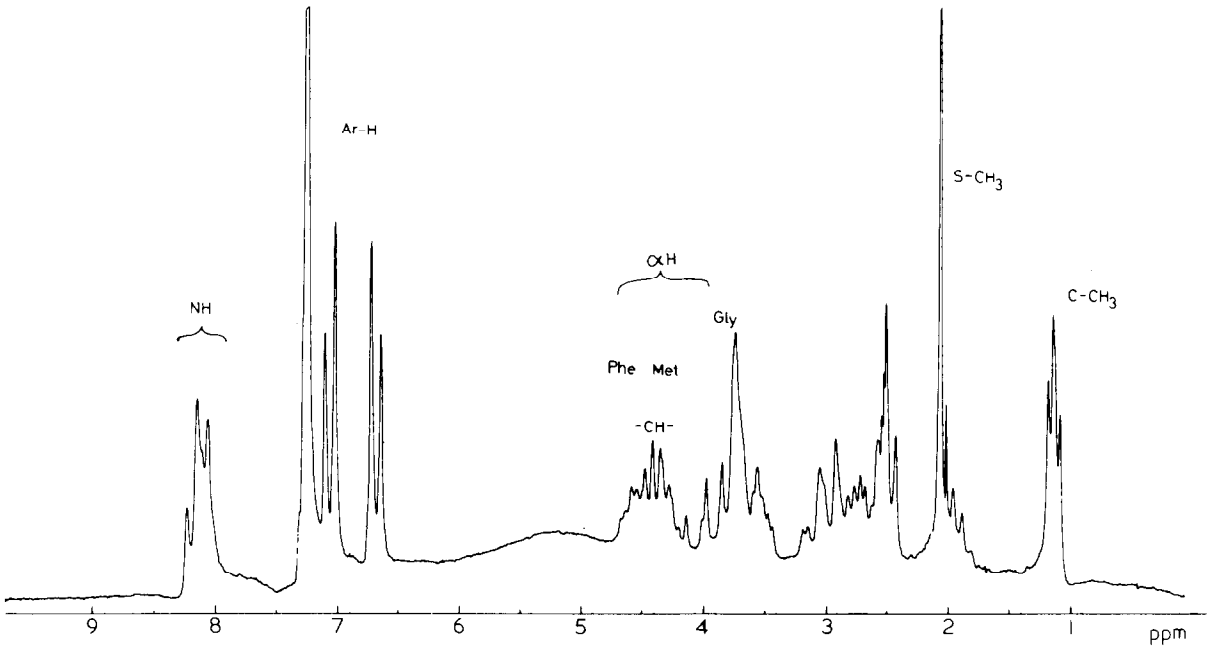


Fig.2. 100 MHz ^{1}H NMR spectrum of 'acetaldehyde-enkephalin-[Met]' (8mg/ml) in d_6-DMSO at 30°C.

alluded to earlier, and on 'acetaldehyde-enkephalin [Met^5]' at 80 MHz have shown that the methyl signal of the latter is a double doublet (1.15 and 1.10 ppm, J = 6.0 Hz). A similar methyl splitting pattern was observed with the 'acetaldehyde-tripeptide' (1.20 and 1.15 ppm, J = 5.8 Hz) and, in addition, the methine proton resonance at 4.4 ppm was shown to be an overlapping double quartet (J = 5.8 Hz). Selective proton decoupling of 'acetaldehyde-enkephalin [Met^5]' gave results consistent with the same double quartet pattern (J = 6.0 Hz). The major spectroscopic differences observed in the ^{1}H NMR spectra of 'acetaldehyde-enkephalin [Met^5]' can, therefore be attributed to a –CH–CH_3 unit with a vicinal coupling constant of 6.0 Hz, and the full ^{1}H NMR data are consistent with a 2-methylimidazolidin-4-one ring structure. Furthermore, the chemical shift of the methine proton is typical for a C-2 proton of a C-5 substituted imidazolidin-4-one [16], and the observed splitting pattern is also consistent with the formation of a diastereoisomeric mixture of the (*2R,5S*) and (*2S,5S*)-2-methylimidazolidin-4-one derivative of methionine-enkephalin (see scheme 1); a diastereoisomeric mixture is expected, since in the absence of asymmetric induction ring closure is presumed to occur with equal facility to either the *re* or *si* face [17] of the initially formed Schiff base.

One interesting feature in the spectrum of 'acetaldehyde-enkephalin [Met^5]' is the appearance of two 'rogue' peaks at 3.80 and 3.95 ppm. The same pattern is seen with the 'acetaldehyde-tripeptide' and must therefore arise from the N-terminal sequence, Tyr–Gly–Gly–OH. 'Acetone-enkephalin-[Met^5]' does not display a comparable pattern, which suggests that the additional resonances observed with the 'acetaldehyde-peptides' are a consequence of either the formation of a new chiral centre or an extra proton at C-2 of the 2-methylimidazolidin-4-one ring structure. The protons responsible for these additional signals may provide important information on the solution conformation of the two diastereoisomers of 'acetaldehyde-enkephalin [Met^5]' and we are currently investigating this aspect using 300 MHz ^{1}H NMR spectroscopy and derivatives of methionine-enkephalin in which Gly$^{(2)}$ and Gly$^{(3)}$ have been replaced, in turn, by α-deuteriated analogues.

Thus, the ^{1}H NMR data firmly establish the structure of 'acetaldehyde-enkephalins' as the 2-methylimidazolidin-4-one derivative of the parent pentapeptides, and that related peptides with similar N-terminal amino acid sequences undergo the same facile reaction with acetaldehyde [3]. In particular,

we have shown that β-endorphin, one of the major opioid peptides of pituitary origin [18,19] similarly loses opiate activity when incubated with aqueous solutions of acetaldehyde [3], and presumably forms the corresponding 'acetaldehyde-β-endorphin' adduct.

Acknowledgements

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