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NMR studies of new arginine vasopressin analogs modified with α-2-indanylglycine enantiomers at position 2 bound to sodium dodecyl sulfate micelles

Emilia Lubecka, Anna Kwiatkowska, Jerzy Ciarkowski, Emilia Sikorska *

Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

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ABSTRACT

In this paper, we use NMR spectroscopy and molecular modeling to examine four new vasopressin analogs modified with α-2-indanylglycine (Igl) at position 2, [L-Igl^2]AVP (**I**), [D-Igl^2]AVP (**II**), [Mpa^1,L-Igl^2]AVP (**III**) and [Mpa^1,D-Igl^2]AVP (**IV**), embedded in a sodium dodecyl sulfate (SDS) micelle. All the analogs display antiuterotonic activity. In addition, the analogs with D-Igl reveal antipressor properties.
Each analog exhibits the tendency to adopt β-turns at positions 2, 3 and/or 3, 4, which is characteristic of oxytocin-like peptides. Mutual arrangement of aromatic residues at positions 2 and 3 has been found to be crucial for binding antagonists with the OT and V_{1a} receptors. The orientation of the Gln^4 side chain seems to be important for the V_{1a} receptor affinity. In each of the peptides studied, the Gln^4 side chain is folded back over the ring moiety. However, it lies on the opposite face of the tocin moiety in analogs with L and D enantiomers of Igl.

1. Introduction

Arginine vasopressin (AVP), a neurohypophyseal hormone and a neuromodulator, is a nonapeptide with a disulfide bridge between Cys residues at positions 1 and 6. It plays a very important role in controlling resorption of water by the distal tubules of the kidney and regulating the osmotic pressure of blood [1,2]. Besides, AVP is responsible for stimulation of the adrenocorticotropine secretion [3] and stability of the body temperature [4]. It exhibits also oxytocic activity [5]. Moreover, recent studies have shown that vasopressin, together with oxytocin, might also take part in autism and provide an effective treatment of autism's repetitive and affiliative behaviors [6,7].

The biological effects of vasopressin are mediated by four different receptor subtypes: V_2 (renal), V_{1a} (vasopressor), V_{1b} (pituitary) and OT (uterine), being typical members of class A GPCR, which are membrane-spanning proteins [8]. The current model for peptide hormone interactions with their receptors suggests that the bioactive conformation of the peptide is induced upon association with the cell membrane followed by a two-dimensional diffusion process, whereby the peptide is recognized and then interacts with the receptor [9,10]. Therefore, exploring the conformational and dynamic properties of a ligand in a membrane-mimicking environment can contribute to better understanding of the molecular features involved in their interactions with the target receptor.

It is believed that the tyrosine residue at position 2 of AVP plays a part initiating the pressor response of AVP [11]. The substitution of Tyr with its D enantiomer produces an analog with only partial agonist activity, whereas the deletion or O-alkylation of Tyr^2 hydroxyl group results in antagonistic properties [12]. Similarly, replacement of Tyr^2 of AVP with bulky or sterically restricted substituents is generally favorable for generation of effective antagonists [13,14].

In this paper, we use NMR spectroscopy and molecular modeling to examine four new vasopressin analogs modified with α-2-indanylglycine (Igl) (Fig. 1) at position 2, [L-Igl^2]AVP (**I**), [D-Igl^2]AVP (**II**), [Mpa^1,L-Igl^2]AVP (**III**) and [Mpa^1,D-Igl^2]AVP (**IV**) embedded in a sodium dodecyl sulfate (SDS) micelle. Although, dodecyphosphocholine (DPC) provides a zwitterionic surface on the micelle that better mimics the biological membranes of the vertebrates, we decided to use the SDS micelle with negatively charged head groups, because the AVP analogs modified with Igl are poorly soluble positively charged peptides, which advises towards incorporation of net negative charges on the SDS micelle surface in order to improve peptide/micelle solubility [15].

The α-indanylglycine was earlier successfully applied for the synthesis of potent and totally enzyme-resistant bradykinin antagonists [16]. In turn, the deamination of cysteine at position 1, usually enhances antidiuretic activity and V_2/V_{1a} selectivity [17]. However, most of oxytocin antagonists describe to date contain Mpa (Fig. 1) instead of Cys^1 [18].

All the studied analogs exhibit only negligible antidiuretic activity. The [L-Igl^2]AVP (**I**) and [Mpa^1,L-Igl^2]AVP (**III**) analogs are moderately potent but selective OTR antagonists. In turn, those modified with enantiomer D of Igl have dual activity—they show high antioxytocic potency at low and full oxytocic agonism at high concentrations [19]. This phenomenon is difficult to explain. However, similar effect has been reported for

Abbreviations: AVP, arginine vasopressin; GPCR, G-protein coupled receptor; Igl, α-2-indanylglycine; Mpa, 3-mercaptopropionic acid; OT, oxytocin; SDS, sodium dodecyl sulfate.

* Corresponding author.
E-mail address: milka@chem.univ.gda.pl (E. Sikorska).

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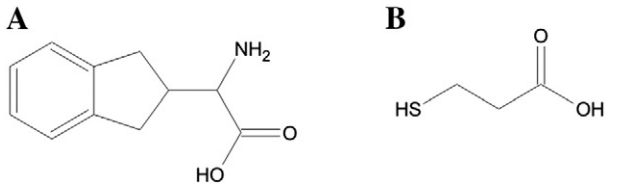

Fig. 1. (A) 2-α-Indanylglycine (Igl) and (B) 3-mercaptopropionic acid (Mpa).

naloxone, an opioid antagonist at low concentration and partial agonist at a high one. The changeover of the activity of naloxone from antagonistic to agonistic is probably the result of a slight desensitization and down regulation of the opioid receptors [20].

2. Materials and methods

2.1. Peptide synthesis and purification

The peptides were synthesized using the standard 9-fluorenylmetoxycarbonyl (Fmoc) methodology (full details including protecting groups, deprotection and cyclication have been reported recently [19]). After HPLC purification, their purity was higher than 98% as determined by analytical HPLC. The MALDI TOF mass spectrometry confirmed that the purified peptides were the desired products.

2.2. Sample preparation

The SDS-d_{25} was purchased from Sigma Aldrich. The samples in the SDS micelle were prepared at a concentration of about 3 mM of a peptide in 0.7 ml of a partially deuterated phosphate buffer (90% H_2O and 10% D_2O) of pH = 7.4 containing about 35 mg of SDS-d_{25}. The SDS-d_{25}:peptide ratio was adjusted to approximately 1:40. The SDS-d_{25} concentration exceeded considerably the critical micelle concentration of SDS (8.3 mM), to be sure that the peptides were indeed micelle-bound.

2.3. NMR measurements

All the NMR experiments were recorded on a 500 MHz Varian spectrometer equipped with a Performa II gradient generator unit, WFG, Ultrashims, a high stability temperature unit and a 5 mm $^1H\{^{13}C/^{15}N\}$

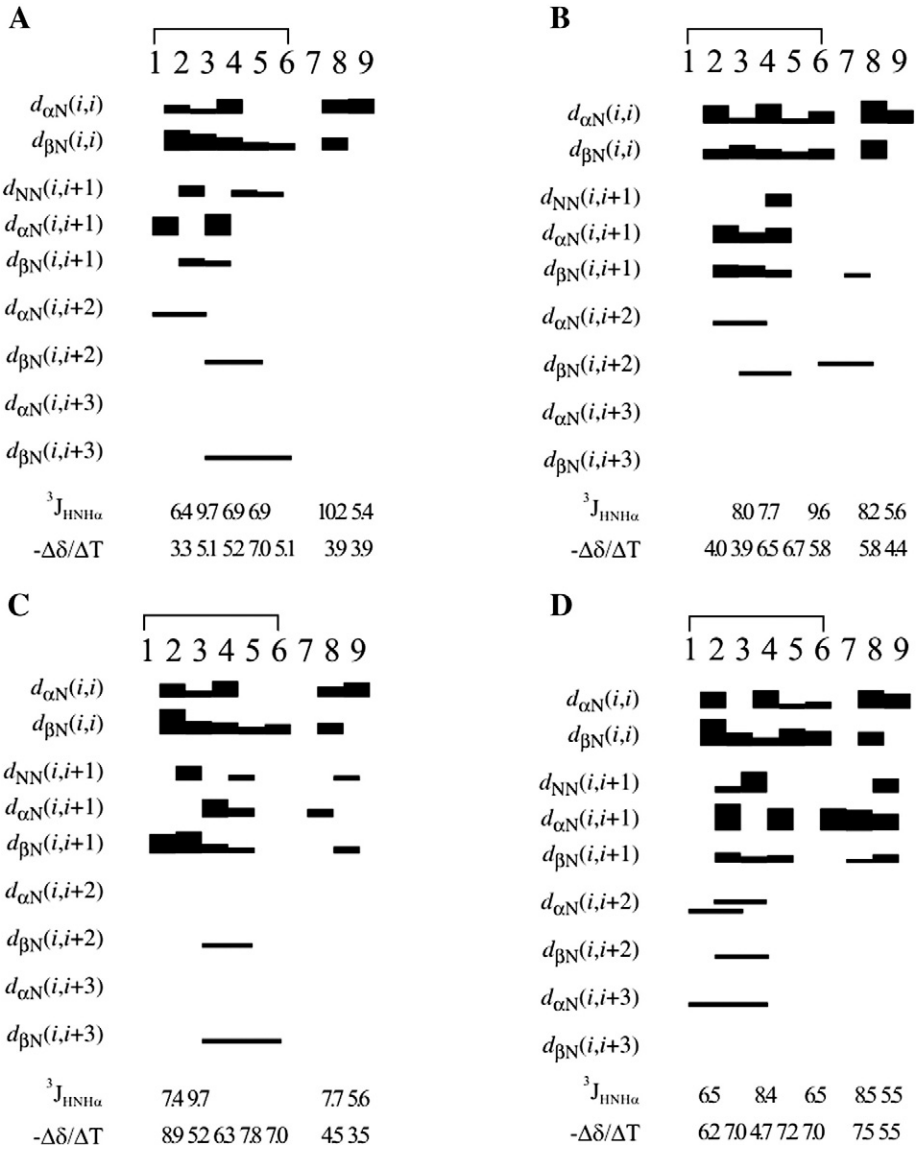

Fig. 2. The NOE effects corresponding to the interproton distances, $^3J_{HNH\alpha}$ coupling constants and the temperature coefficients of the backbone amide atoms of (A) [L-Igl^2]AVP (**I**), (B) [D-Igl^2]AVP (**II**), (C) [Mpa^1,L-Igl^2]AVP (**III**) and (D) [Mpa^1,D-Igl^2]AVP (**IV**).