

Preparation and Radiolabeling of Vasopressin 1b Ligands for Receptor Studies

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Background

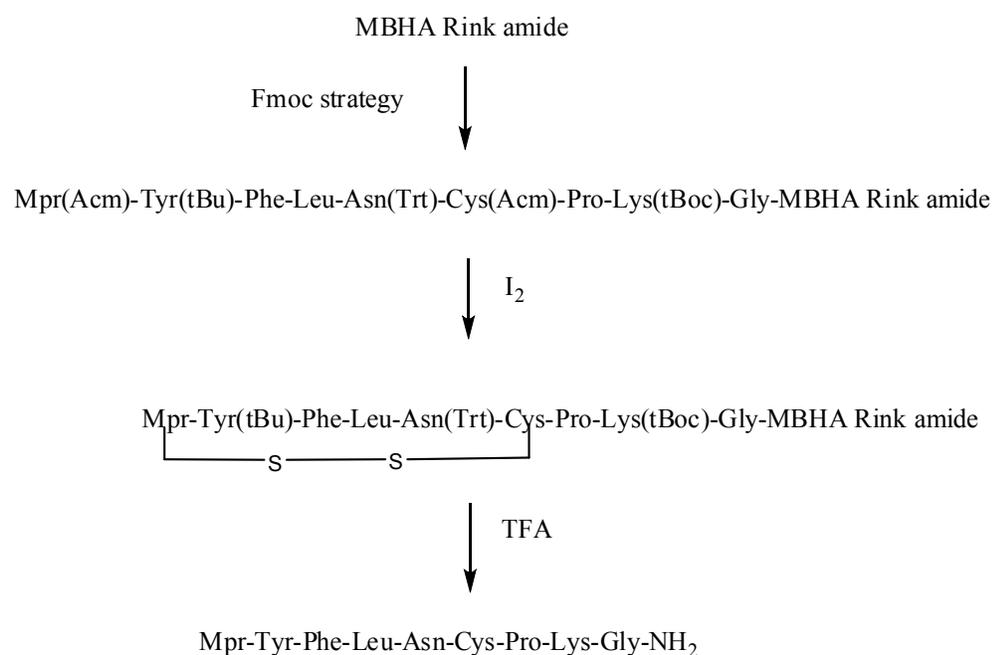
Oxytocin (Oxt) and vasopressin (Avp) are thought to exert their behavioral effects on parturition/lactation and plasma osmolarity regulation, respectively, through Oxtr, vasopressin 1a (Avpr1a) and vasopressin 1b (Avpr1b) receptors in the central nervous system (CNS).¹ The Avpr1b receptor was discovered in the anterior pituitary² and is believed to have important roles in social behavior patterns including reduction of aggression in male mice, as shown by studies in knockout mice.³ Peptide agonist and antagonist analogs have been used to study rodent receptor binding and one non-peptide antagonist underwent development for stress-related disorders,⁴ although its lipophilicity was found to hinder its use in Avpr1b receptor studies.⁵ Recent research has identified several Avp peptide analogs that may overcome this problem,⁶ and radiolabeled versions of these peptide analogs are required for studies of their tissue receptor interactions. Several potential peptide candidates exist, perhaps the best being d[Leu⁴]AVP and d[Leu⁴, Lys⁸]VP.⁶ The latter shows the best overall specificity when compared with the Oxtr, Avpr1a and vasopressin 2 receptors (Avpr2), although as there is little or no Avpr2 in the mouse CNS, the d[Leu⁴]AVP may prove a superior ligand as it has a higher specificity compared to Oxtr.

Chemistry

d[Leu⁴]AVP may be radiolabeled with high specific radioactivity as an iodinated (I-125) version by labeling the tyrosine residue in position 2, although radioiodine incorporation is likely to increase the peptide's lipophilicity by around one log unit. A tritiated version may also be considered for autoradiography studies without concern for an increase in lipophilicity leading to higher non-specific binding. The d[Leu⁴]AVP vasopressin analog [Mpr-Tyr-Phe-Leu-Asn-Cys-

Pro-Lys-Gly-NH₂] (Scheme 1) is a nonapeptide containing one cyclic disulfide ring and a C-terminal amide. The presence of a tyrosinyl- residue in the 2-position suggests that an iodine-125 radiolabeled version could be readily made by electrophilic substitution while preparation of a cold (non-radioactive) 2-iodotyrosyl-d[Leu⁴]AVP would serve as a standard and also as a precursor to the tritiated peptide by a standard dehydrohalogenation reaction.

Scheme 1. Synthesis of d[Leu⁴]AVP peptide.



Experimental

General. Protected amino acids and O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were purchased from CEM (Matthews, NC) and 4-methylbenzhydrylamine (MBHA) Rink amide resin from EMD Biosciences (San Diego, CA). The synthesis of the peptide sequence was carried out on a CEM Liberty automated peptide synthesizer at a 0.1 mmol scale using standard Fmoc-based solid-phase chemistry. Iodine-125 as Na¹²⁵I in 10⁻⁵ M NaOH solution (pH 8-11) was purchased from Perkin-Elmer (Waltham, MA).

Synthesis of d[Leu⁴]AVP: Fmoc-protected amino acids (0.5 mmol) were sequentially coupled to the 4-methylbenzhydrylamine (MBHA) Rink Amide resin (167 mg) at a 0.6 mmol/g

substitution level using HBTU as a peptide coupling reagent. After the peptide was assembled on the machine, the resin was transferred to a manual peptide synthesis vessel for the cyclization reaction and washed with dimethylformamide (DMF, 3x10mL), dichloromethane (DCM, 3x10 mL) and methanol (MeOH, 3x10 mL). Iodine (254 mg, 1 mmol) in MeOH (10 mL) was added and the reaction was shaken for 2 hours, then washed extensively with MeOH (3 x 10 mL), DCM (3 x 10 mL), DMF (3 x 10 mL) and DCM (3 x 10 mL) and dried. For cleavage of the peptide from the resin, a cleavage mixture (9.5 mL trifluoroacetic acid, 0.25 mL water, 0.25 mL anisole) was added, and the reaction mixture was stirred at room temperature for 1 hour and filtered. The filtrate was taken to dryness *in vacuo*. Cold diethyl ether was added to the residue and the peptide was precipitated and collected by filtration. The crude peptide was purified by mass spectral-directed HPLC purification and the final deprotected peptide was obtained as a white solid (8 mg, 7.6%).

Synthesis of d[Leu⁴]AVP[2-iodo-tyrosine]: d[Leu⁴]AVP (1.3 mg, 1.23 μmol) was dissolved in a mixture of methanol and water (200 μL, 1:1, v/v), and an iodine-methanol solution was added (50 μL, 3.13 mg/mL). The vial was shaken for 4 hours and the crude product was purified using a Beckman System Gold HPLC equipped with a Vydac protein & peptide C18 column (4.6×250 mm; Cat. No.218Tp54). The solvent system consisted of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) with a linear gradient of solvent B from 5-60% over 25 minutes at a 1 mL/minute flow rate. d[Leu⁴]AVP[2-iodo-tyrosine] eluted at 17.2 minutes and fractions collected containing d[Leu⁴]AVP[2-iodo-tyrosine] were combined and lyophilized. The target peptide was obtained as a white solid (0.4mg, 31%) at a purity over 98%. MALDI-TOF-MS:[M+H]⁺ 1181.2, calculated: 1180.142.

Radiosynthesis of d[Leu⁴]AVP[2-¹²⁵I-tyrosine]: The radioiodination of d[Leu⁴]AVP was performed in an iodogen-coated tube (Thermo Scientific, Waltham, MA). The peptide (2 mg/mL in water; 20 μL) was transferred into the iodogen vial, followed by addition of 0.1 M phosphate buffer (70 μL, pH 7.0) and sodium [¹²⁵I]iodide solution (10 μL, 37 MBq, 3.7 GBq/mL). The reaction was allowed to continue for 15 minutes with vortex mixing every 2 minutes. Then, to quench the reaction, a tyrosine solution (50 μL, 10 mg/mL) was added and the mixture was allowed to stand for at least 2 minutes. The I-125-radiolabeled peptide was purified using the same method as used for the non-radioactive analog above. Quality control of d[Leu⁴]AVP[2-¹²⁵I-tyrosine] was also performed using the same HPLC conditions as above. d[Leu⁴]AVP[2-¹²⁵I-

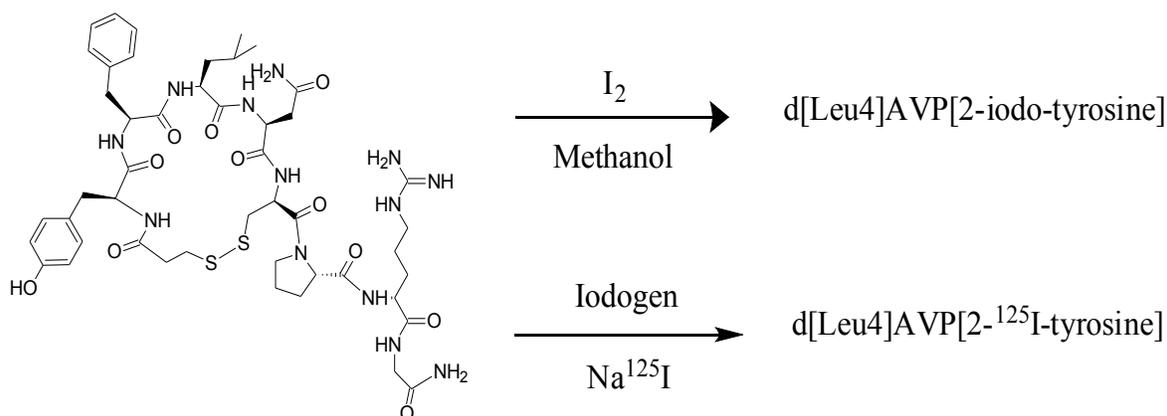
tyrosine] eluted at 16.9 minutes with a radiochemical purity over 96%. The radiochemical yield was 68%, the specific activity was 1.843 Ci/mg, and a 0.328 μM concentration was obtained.

Radiolytic Stability: During storage of d[Leu⁴]AVP[2-¹²⁵I-tyrosine], accumulation of free iodine over time was observed. To prevent radiolysis, potassium iodide (KI), ascorbic acid and BSA were tested as radiolysis inhibitors. Their effect on stabilization of d[Leu⁴]AVP[2-¹²⁵I-tyrosine] is shown in Table 1. Based on the results, 1% BSA was chosen as the preferred anti-radiolytic protector.

Table 1. Stability of d[Leu⁴]AVP[2-¹²⁵I-tyrosine] when stored alone or with the addition of the radioprotectants.

Additives	Time (days)			
	0	1	2	7
None Added	96	92%	80%	81%
KI	96	68%	83%	78%
Ascorbic acid	96	91%	88%	88%
BSA 1mg/mL	96	96%	96%	90%

Scheme 2. Iodination and radioiodination of d[Leu⁴]AVP.



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