137. Synthesis of [Sar[1], Val[5], (4'-Azido-3',5'-ditritio)Phe[8]] Angiotensin II, a Photoaffinity Label for the Isolation of Angiotensin II Receptors

Communication 1[1])

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Summary

The synthesis of [Sar[1], Val[5], (4'-azido-3',5'-ditritio)Phe[8]] angiotensin II from a iodinated precursor peptide is described. The principal problems of this synthesis and their resolution are discussed: i) The β-induced autophotolysis of the highly tritiated (73 Ci/mmol) and photosensitive label, and ii) the absorption problems encountered during the purification of microgramm quantities. Such photoaffinity labels are being used for specific labeling and isolation of peptide hormone receptors.

Within a few years the photoaffinity labeling technique has gained an ever increasing importance for studying peptide-hormone receptors and other ligand-receptor interactions [1–3]. The unequivocal results obtained recently with [Sar[1], Val[5], (4'-azido)Phe[8]] angiotensin II (**5**) on the angiotensin II (AT)-receptor in rabbit aorta strips [4] and other tissues [5] have opened the way to attempts to isolate this receptor. Introduction of radioactivity in the label in form of ^{125}I was ruled out by the important negative influence on biological activity of AT analogues by iodination [6]. Due to the low number of specific receptors on a single target cell (1000–10,000) it is necessary to use labels with the highest possible specific radioactivity. We therefore prepared [7] an iodinated precursor analogue [Sar[1], Val[5], (4'-amino-3',5'-diiodo)Phe[8]]AT (**1**) which has been tritiated catalytically with pure $^3H_2$ to 73 Ci/mmol. This [Sar[1], Val[5], (4'-amino-3',5'-ditritio)Phe[8]]AT (**2**) was stable in liquid nitrogen for more than two years. The conversion of non-radioactive [Sar[1], Val[5], (4'-amino)Phe[8]]AT (**4**) into **5** has already been reported [6]. We preferred

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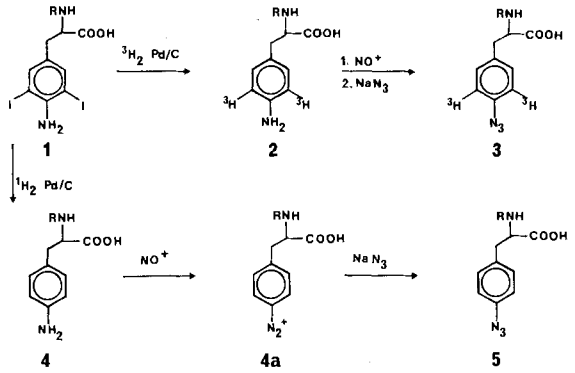
Scheme



R = Sar - Arg - Val - Tyr - Val - His - Pro -

this approach (see *Scheme*) to the synthetic incorporation of preformed (4′-azido-3′,5′-ditritio)Phe, which had been done earlier but which used lower specific activities [8].

Modifications of small quantities of non-radioactive **4** into **5** showed that gel-filtration alone did not remove all salts and that an additional purification, a partition chromatography, was necessary to obtain pure **5**. The conversion of several μg of **2** into **3** with the same purification system gave an yield of only 4% **3**. It was suspected that a major part was absorbed to the glass columns and the synthesis was repeated with plastic columns for the purification (plexiglass for gel-filtration and teflon for partition chromatography). With this modification the final yield was improved to 43%. Determination of specific activity with the *Lowry*-test gave the same specific activity in **3** as in **2**.

Upon lyophilisation and storage in the dark at −200° for one day the product **3** was completely decomposed as shown by TLC. and biological activity. On TLC. the product showed almost the same pattern as photolyzed **5** [5] and it was suspected that the product had suffered autophotolysis due to ^{3}H-β-induced chemiluminescence. Another research group had observed similar problems with an azido- and tritium-containing analogue of naphthalene [9]. When **3** was stored in aqueous solution and in the presence of the scavenger (4′-amino)Phe [3], no deterioration of the product was observed after one year if kept in the dark and at −200°. The biological activity of **3** and the irreversible inhibition of the AT-response upon irradiation of treated tissues was the same as described with **5** [4].

The isolation of the AT-receptor from arterial smooth muscle and adrenal cells is under investigation at present and will be published elsewhere.

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Experimental Part

General. Inorganic chemicals were purchased from *Alfa-Ventron,* solvents were distilled before use. TLC. were performed on *Merck* precoated silicagel plates (type G60-F254) in the solvent systems BAW = butanol/acetic acid/water 10:2:3; BAWP = butanol/acetic acid/water/pyridine

15:3:12:10; BIWCL = butanol/2-propanol/water/monochloroacetic acid 65:15:20:3. The spots were visualized with UV. fluorescence, *Pauly* reagent and for radioactive substances with autoradiography on *Ilford* HP4 film. All works were carried out exclusively under incandescent illumination and, if possible in the dark. Scintillation counting was performed with *Aquasol* and in a *Intertechnique* counter. Specific activities were determined with the *Lowry*-test, using an internal standard obtained with the corresponding non-radioactive analogue.

Peptide purification. Peptide quantities below 1 mg were initially purified with 0.8×60 cm glass columns containing *Sephadex* G15 or *Sephadex* G25 fine. For improved yields gel-filtration on G15 was carried out in acrylglass tubings 0.3×60 cm, and eluted by gravity with 0.2N degassed acetic acid. Partition chromatography on G25 fine was carried out in a 0.2×50 cm teflon tube, equilibrated with the lower phase of butanol/acetic acid/water 4:1:5 and eluted by gravity with the upper phase. Fraction of 0.5 ml were collected on drop-mode with a fraction collector *Redi-Rack* from *LKB*, Bromma, Sweden. Peptide fractions were determined with TLC. and/or scintillation counting, peptide purity with TLC. and autoradiography.

Sar-Arg-Val-Tyr-Val-His-Pro-(4'-amino-3',5'-ditritio)Phe (**2**). The stirred mixture of 5 mg of [Sar^1,Val^5,(4'-amino-3',5'-diiodo)Phe^8]AT (**1**, MW 1237.97, 4 μmol), 0.5 ml of dimethylacetamide and 2.0 mg of 10% Pd/C was tritiated for 30 min at 600 torr with carrier-free tritium gas. After removal of the volatile tritium and the catalyst the product was resuspended in water and evaporated several times in order to remove any exchangeable radioactivity. The crude product was filtered through *Sephadex* G15 with 0.1N formic acid and 1.2 mg of product was collected. TLC. with autoradiography was homogenous and identical with **4**; Rf BAW, 0.19; BAWP, 0.41; BIWCL, 0.08. Yield of **2**: 1.2 μmol or 30%, 1.93×10^{11} dpm or 87.6 mCi. This product was dissolved in 15 ml water and divided into 30 equal portions, stored in liquid nitrogen. After two years of storage only minor impurities were detectable.

Sar-Arg-Val-Tyr-Val-His-Pro-(4'-azido-3',5'-ditritio)Phe (**3**). An example of a synthesis with improved yield is described as follows: 40 μg of **2** (40 nmol, 2.92 mCi or 6.5×10^9 dpm) were filtered over G15 and the central peak fractions were united and lyophilized. The resulting 21 nmol of pure **2** were dissolved in 150 μl water at 0°, 150 μl of 1N HCl was added, followed by 50 μl of a 0.1M solution of $NaNO_2$. After stirring for 10 min the iodine-starch test was positive and 300 μl of a 0.1M solution of sulfamic acid was added in order to destroy the excess of nitrite. Compound **3** was obtained by addition of 50 μl of a 0.1M solution of NaN_3 and the reaction mixture was brought to pH 3.5 with 1M sodium acetate. The product was lyophylized, immediately dissolved in 100 μl 0.2M acetic acid, and filtered over G15. The radioactive peptide fractions were pooled, partially liophylized and subjected to partition chromatography. The peptide fractions were again pooled and the purity was assessed by TLC. and autoradiography. Rf BAW 0.43; BAWP, 0.51; and BIWCL, 0.19, identical with **5**. The product was partially evaporated i.V. and diluted to 1 ml with 1 mM (4'-amino)Phe-solution. Yield of **3** was 1.6×10^9 dpm, peptide quantity 9.0 μg or 43% of the pre-purified **2**. At 0.2 μM this peptide gave maximal contraction of rabbit aorta strips [4] and 65% irreversible specific inactivation of the AT-response upon irradiation [7]. Purity control after 1 year by TLC. and autoradiography showed that only slight decomposition had occurred. An identical synthesis carried out with 24.0 mg **4** and 1.2×10^6 dpm **2** (specific activity 23.3 μCi/mmol) gave 9.1 mg **5** with $(4.55 \pm 0.12) \times 10^5$ dpm **3** (specific activity 23.5 ± 0.6 μCi/mmol) indicating that no tritium exchange took place.

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