

**Artigo Original**

recebido em 13/11/2002 e aceito em 08/04/2003

**Radioiodination of vasoactive intestinal peptide (VIP) using different oxidative electrophilic substitution agents**

*Marcação do peptídeo intestinal vasoativo (VIP) com radioiodo utilizando diferentes agentes oxidantes em substituição eletrofílica*

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**Abstract**

Vasoactive Intestinal Peptide (VIP) is a linear 28 residue polypeptide with a wide range of biological activities. Various tumor cells express significant amounts of VIP receptors and this became the basis for use of radiolabelled VIP for the in vivo localization of adenocarcinomas. The VIP molecule contains two tyrosine residues, in positions 10 and 22, susceptible to iodination. This work describes the influence of the oxidation agent in the radiochemical purity and in the production of different labelled forms of VIP, analysed by high performance liquid chromatography (HPLC). Three labelling procedures using oxidant agent were employed. In the first one, Iodogen was prepared in the form of pre-coated reaction vial. In the second procedure, Iodogen was introduced as a suspension and the iodinations were allowed to proceed for 30 minutes at room temperature with gentle stirring. The third labelling procedure used Chloramine T as oxidant agent after a few minutes of reaction (less than 3 minutes) at room temperature and with gentle stirring, the reaction was terminated by the addition of sodium methabisulfite solution. The radiochemical purity was determined by HPLC (RP C18, 5 µm, 4.6 x 50 mm) eluted isocratically with 73:27 % TFA (trifluoroacetic acid 0.1% aqueous solution: acetonitrile) with a flow rate of 0.5 mL/minute. Free radioiodine was also determined by horizontal zone electrophoresis. Radioiodinated VIP was obtained with high labelling yield and radiochemical purity, in a short reaction time, when using Chloramine T as oxidant agent. The proposed isocratic HPLC system allows the separation of labelled VIP of high specific activity, necessary for receptor-mediated diagnostic procedures with radiopharmaceuticals.

**Keywords:** Protein radioiodination, Radiopharmaceuticals, VIP

**Resumo**

O Peptídeo Intestinal Vasoativo (VIP) é um peptídeo linear composto de 28 aminoácidos com um amplo espectro de atividades biológicas. Várias células tumorais expressam quantidades significativas de receptores para VIP, provendo a base para o uso do peptídeo na localização de adenocarcinomas. A molécula de VIP contém dois resíduos de tirosina, nas posições 10 e 22, susceptíveis à iodação. Este trabalho descreve a influência do agente oxidante na pureza radioquímica e nas diferentes formas de VIP obtidas, analisadas por cromatografia líquida de alta eficiência (CLAE). Três procedimentos de marcação foram estudados. Nos dois primeiros foi utilizado como agente oxidante Iodogen, na forma de um fino filme, aderido ao tubo de reação bem como na forma de suspensão. As reações de iodação foram conduzidas por

30 minutos, à temperatura ambiente e com agitação suave. No terceiro procedimento de marcação empregou-se Cloramina T como agente oxidante e após um curto período de incubação à temperatura ambiente e agitação suave, a reação foi interrompida pela adição de metabissulfito de sódio. A pureza radioquímica das marcações foi determinada por cromatografia líquida de alta eficiência (coluna de fase reversa C18, 5 µm, 4,6 x 50 mm) com mistura de solvente composta de TFA (solução aquosa de ácido trifluoroacético 0,1%) : acetonitrila 73:27 %, com fluxo de 0,5 mL/minuto. A porcentagem de radioiodo nas marcações também foi determinada por eletroforese. O peptídeo radioiodado foi produzido com alto rendimento e pureza radioquímica, em um curto período de tempo, particularmente ao utilizar-se Cloramina-T como agente oxidante. O sistema de cromatografia líquida estudado possibilitou a separação do VIP marcado com alta atividade específica, necessária para procedimentos diagnósticos que envolvem a ligação de radiofármacos a receptores específicos.

**Palavras Chave:** Peptídeo vasointestinal ativo, Radiofármacos, Radioiodação de proteínas.

## Introduction

Vasoactive Intestinal Peptide (VIP) is a linear 28 residue polypeptide with a wide range of biological activities including vasodilation, secretion of various hormones, immunomodulation and promotion of growth and proliferation of normal and malignant cells (Virgolini *et al.*, 1994; Virgolini *et al.*, 1995).

Various tumor cells express significant amounts of VIP receptors providing a basis for use of radiolabeled VIP for the *in vivo* localization of adenocarcinomas, breast cancer, melanomas, neuroblastomas and pancreatic carcinomas (Dagar *et al.*, 2001; Hennesius *et al.*, 2000; Li *et al.*, 2001; Reubi *et al.*, 1999; Virgolini *et al.*, 1994; Virgolini *et al.*, 1995; Virgolini *et al.*, 1997; Zia *et al.*, 2000).

The VIP molecule contains two tyrosine residues, in positions 10 and 22, that are theoretically equally susceptible to iodination. Furthermore, the methionine residue in position 17 is susceptible to oxidation by common agents used in oxidative electrophilic labelling procedures such as Chloramine T and Iodogen. The oxidation of Met<sup>17</sup> to methionine sulfoxide only marginally decreases the biological activity of VIP, as previously determined (Martin *et al.*, 1986).

This work describe the influence of the oxidation agent in the radiochemical purity and in the production of different labelled forms of VIP, analysed by high performance liquid chromatography (HPLC).

## Materials and Methods

Natural human Vasoactive Intestinal Peptide (VIP) and Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenyl glycouril) was purchased from Sigma, Chloramine T

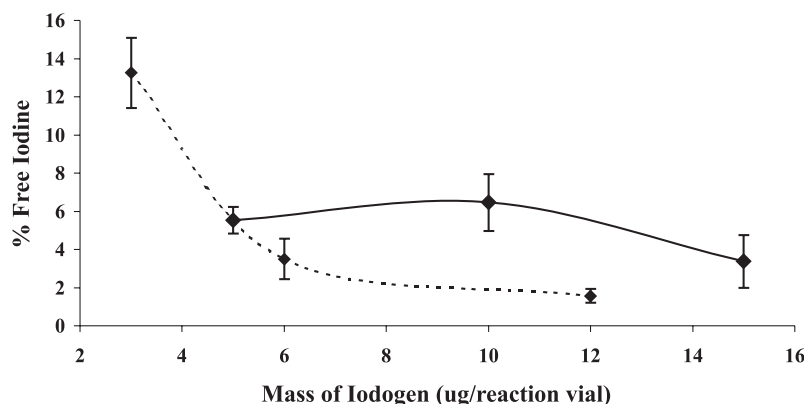
(N-chloro-p-toluene-sulfonamide, sodium salt trihydrate) and sodium metabissulfite was purchased from Merck. [<sup>131</sup>I]NaI were obtained from Nordion (Canada) and processed at IPEN/CNEN (São Paulo, Brazil), in 0.01M NaOH solution.

## Labelling procedures

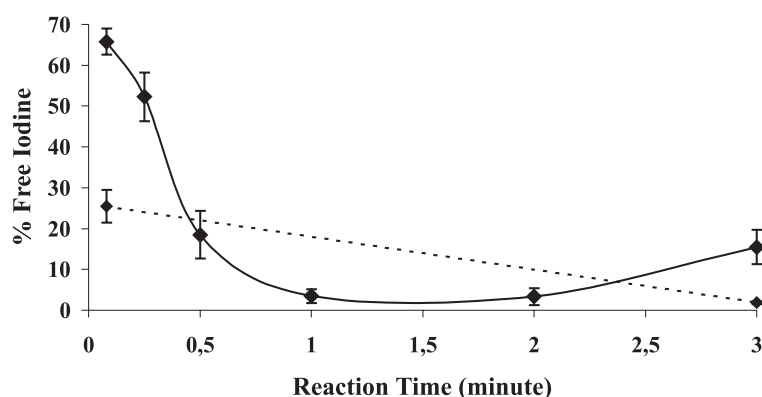
All labelling procedures employed VIP (12.5-25.0 µg) dissolved in 20 µL of 0.2M phosphate buffer pH 7.5 and [<sup>131</sup>I]NaI solution (3.7-7.4 MBq/10 µL). Three labelling procedures, based in the *in situ* production of electrophilic radioiodine species (direct labelling) were studied. In the first one, (Salacinski *et al.*, 1981), Iodogen was dissolved in dichloromethane (1 mg/mL), and 5 to 15 µL of this solution dispensed in the bottom of a polypropylene iodination vial and evaporated to dryness under a nitrogen stream. The vials were stored at -85°C. In the second procedure, (Virgolini *et al.*, 1995), Iodogen was used as a suspension freshly prepared by dissolving 6 mg of Iodogen in 100 µL of acetone and vortex-mixing with ideal volume (4900 µL) of phosphate-buffered saline pH 7.5, 0.2M (PBS). An ideal volume of this suspension containing 3, 6 or 12 µg of Iodogen was used in labelling procedures. The labelling mixtures were composed by the VIP and [<sup>131</sup>I]NaI as described above, Iodogen (in the appropriated form) and also 5 mL of KI (0.10 ng) and 40-50 µL of 0.2 M phosphate buffer pH 7.5. The iodinations were allowed to proceed for 30 minutes at room temperature with gentle stirring and only in the first case, the reaction was terminated by removing the mixture from the reaction Iodogen pre-coated vial. The third procedure, (Marie,1985), was developed using 5 µL of Chloramine T (1.0 mg /mL distilled water) as oxidant agent and after few minutes of reaction (5 seconds to 3 minutes) at room temperature and with gentle stirring, the reaction was terminated by the addition of 5 µL of sodium methabisulfite solution (2.0 mg/mL distilled water).

## Radiochemical purity determination

The reaction mixtures were analyzed by a HPLC system (column RP C18, 5 µm, 4.6 x 50 µm, Waters) eluted isocratically with 73% aqueous TFA (trifluoroacetic acid 0.1% solution) and 27% acetonitrile with a flow rate of 0.5 mL/minute. Free radioiodine was also determined by horizontal zone electrophoresis (Amershan) on Whatman n° 1 paper, 0.05M barbital buffer, pH 8.6, using a field of 300 V for 40 minutes. The labelled peptide remained in the origin and free radioiodine migrates 9 to 11 cm.



**Figure 1.** Influence of the Iodogen mass on labelling of VIP (25 µg) with (—) Iodogen suspension and (¾) Iodogen pre-coated tube



**Figure 2.** Influence of reaction time on the labelling of 12.5 µg VIP (¾) and 25 µg VIP (—) using Chloramine T as oxidant agent

## Results

The influence of Iodogen mass on labelling yield was evaluated when using pre-coated Iodogen tubes and Iodogen suspension. The percent of free radioiodine was determined by zone electrophoresis and the HPLC profile of all labelling mixtures was also evaluated to verify the radiochemical species that was formed (Figure 1). Radiochemical purity was also related to reaction time and mass of the peptide as evidenced on Figure 2.

Radioactive HPLC profiles of the labelling conditions employing Iodogen as oxidant agent are quite similar when using Iodogen in the form of pre-coated tube or as suspension (Figure 3), with four peaks with retention time (Rt) 6.8, 7.8-7.9, 8.4-8.6 and 11.3 minutes.

When using excess of Iodogen as suspension, another peak with Rt 16.8 minutes can be eventually observed and can be attributed to the iodination of the Iodogen. This hypothesis was confirmed by the HPLC profile obtained with a labelling procedure performed including all reagents, except the peptide (Figure 4).

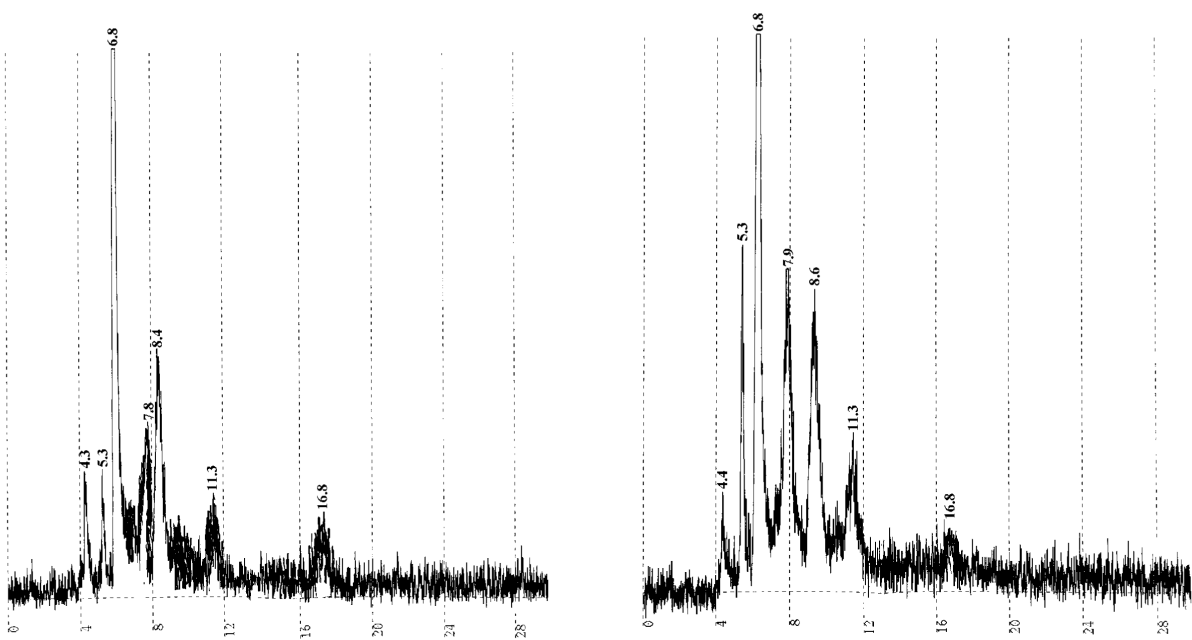
Radioactive HPLC profile employing Chloramine T as oxidant agent presents only two peaks, with Rt 6.8 and 8.4 minutes (Figure 5).

In the isocratic HPLC system studied, the retention time of unreacted VIP is 5.9 minutes (Figure 6) and of the free iodine is 5.3 minutes (Figure 7).

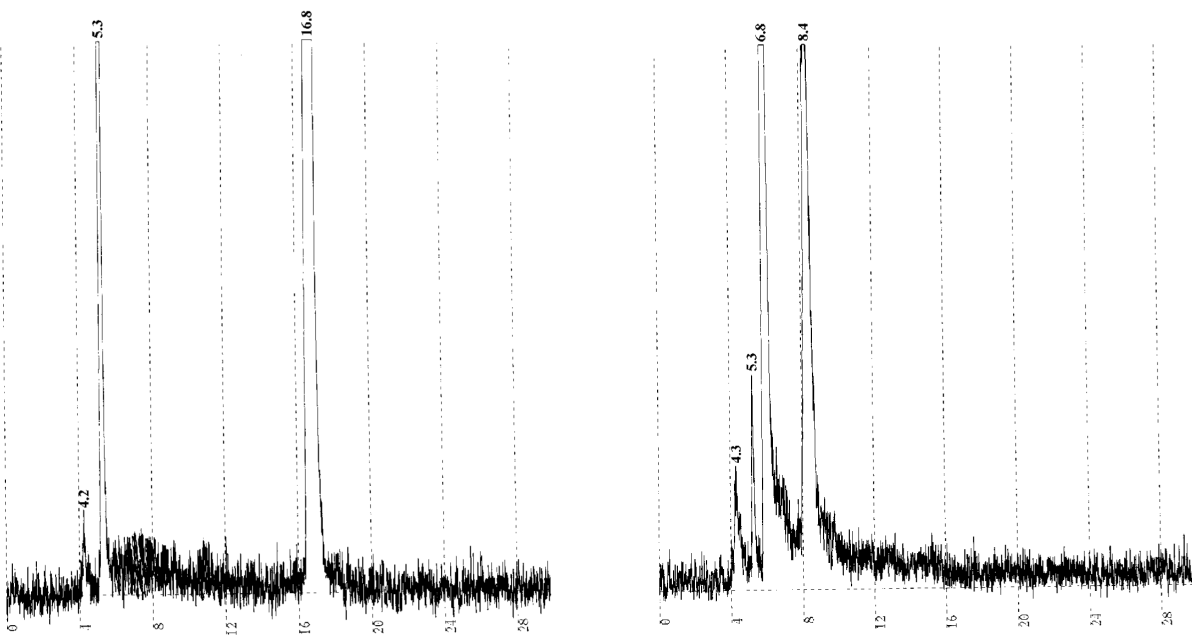
## Discussion

In this work, the mass of Iodogen was studied to obtain labelled VIP with high radiochemical yield. Using Iodogen as suspension, a smaller mass was necessary when compared to Iodogen used as a pre-coated tube. Only 6 mg of Iodogen suspension results in high radiochemical yield (about 97%), equivalent to 15 mg of Iodogen in a pre-coated tube form.

When considering time reaction using Chloramine T as oxidant agent, only 2 minutes for labelling conditions employing 12.5 µg of the peptide and 3 minutes when using 25 µg of peptide was necessary to obtain radiochemical purity superior to 98%.

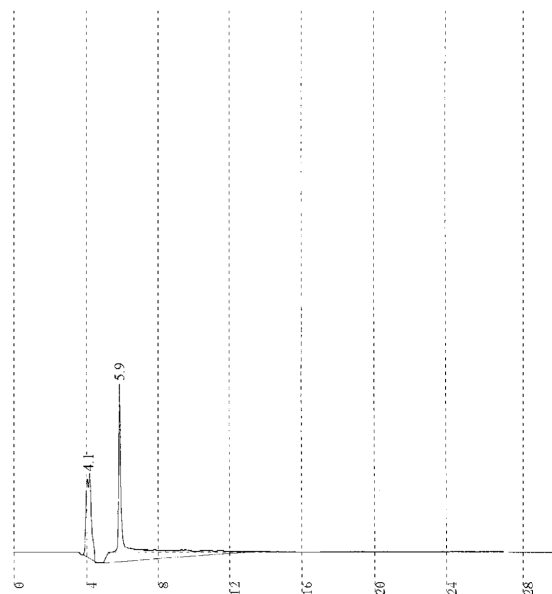


**Figure 3.** Radioactive HPLC profile of radioiodinated VIP using Iodogen pre-coated vial (25 µg VIP, 6 µg Iodogen, 30 minutes reaction time upper panel) and Iodogen suspension (25 µg VIP, 15 µg Iodogen, 30 minutes reaction time lower panel).



**Figure 4.** Radioactive HPLC profile of reaction products with Iodogen suspension and without VIP (6 µg Iodogen, 30 minutes reaction time).

**Figure 5.** Radioactive HPLC profile of radioiodinated VIP using Chloramine T as oxidant agent (25 µg VIP, reaction time of 3 minutes).



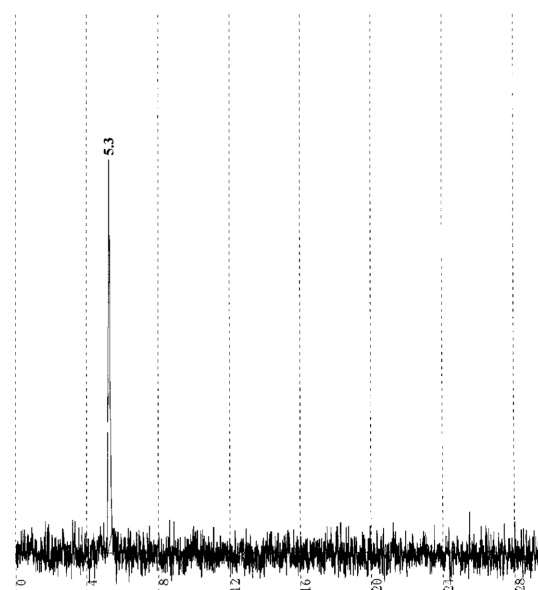
**Figure 6.** UV HPLC profile of VIP (Absorbance at 230 nm).

Good radiochemical purity were obtained using Chloramine T as oxidant agent with low reaction time (1 minute). Chloramine T is a very strong oxidant agent and the time reaction has to be controlled to prevent damage to the peptide. Labelling procedures employing less than 3 minutes presented no alteration in the HPLC profiles.

In the eletrophilic oxidative radioiodination of VIP, as previously described by Virgolini *et al.* (1994, 1995), two forms of radioiodinated VIP can be observed in the HPLC profile, that correspond to VIP-Tyr<sup>10</sup> and VIP-Tyr<sup>22</sup>, respectively. In our studies, when using Chloramine T as oxidant agent, two radiochemical forms were observed and the retention time of both radioactivity species were greather than the cold VIP and free radioiodine, making possible the separation of radiolabelled VIP with high radiochemical yield and high specific activity.

However, when using Iodogen as oxidant agent, two more radioactivity peaks could be observed in the HPLC profile. Considering the possibility of the oxidation of the methionine residue (Met<sup>17</sup>), as previously described by Martin *et al.* (1986), in both radioiodinated forms (VIP-Tyr<sup>10</sup> and VIP-Tyr<sup>22</sup>), to produce the VIPox-Tyr<sup>10</sup> and VIPox-Tyr<sup>22</sup>, four radioactivity peaks in theory could be observed in the HPLC profile. In fact, in this work, four peaks were observed when using Iodogen as oxidant agent.

The differences observed in the radioactivity HPLC profile, when comparing Chloramine T and Iodogen



**Figure 7.** Radioactive HPLC profile of [<sup>131</sup>I]NaI

methods, can be related to the oxidative potential of both oxidants agents. Probably, when using Chloramine T as oxidant agent, due to its high oxidative potential, only the VIPox species could be observed.

Martin *et al.* (1986) showed two major peaks in the HPLC profile when labelling VIP employing Chloramine T as oxidant agent and a third peak, with intermediary retention time, that was converted in the first peak with time. The authors also determined that the oxidation of the Met<sup>17</sup> in VIP reduced only minimal de affinity of the peptide to the cell.

This study showed that labelled VIP can be obtained by direct oxidative radioiodination using Iodogen or Chloramine T as oxidant agent. When using Chloramine T, the labelled peptide is obtained in a short reaction time and high radiochemical yield. In this case, only two radiochemical species are formed and this fact facilitates the HPLC purification process.

Using Iodogen as oxidant agent, four radiochemical species were obtained. The influence of these radiochemical forms in the interaction of the radiopharmaceutical with tumor cell receptors will be investigated in further studies.

The proposed isocratic HPLC system permitted the separation of labelled VIP of high specific activity such as is necessary for receptor mediated diagnostic procedures.

### Acknowledgment

This research was supported by the International Atomic Energy Agency (IAEA) and IPEN-CNEN.

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