Re 20 - *Radioiodination of protein using prosthetic group: a convenient way to produce labelled proteins with in vivo stability*

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Radiolabeled peptides can provide new approaches for radiopharmaceutical development. Several prosthetic groups have been developed for radioiodination of proteins in order to minimize *in vivo* dehalogenation. In this work, the prosthetic group N-succinimidyl 4-[^{131}I]iodobenzoate was obtained by an alternative procedure that employs Cu(I) assisted radioiododebromination to produce p-[^{131}I]iodobenzoic acid. The p-bromobenzoic acid (50 mL of a 0.2 M solution in DMSO) was placed in a tightly-sealed 2 mL glass vessel followed by addition of CuCl (100 mL of 0.01M solution in DMSO) and 5mL of [^{131}I]NaI solution (3.7 – 7.4 MBq). The reaction was conducted at 165°C, for 60 minutes. Radiochemical purity of the labelling mixtures was determined by chromatographic method using Whatmann 3MM paper and chloroform:acetic acid (9:1) as solvente. The p-[^{131}I]iodobenzoic acid was produced with a radiochemical purity of 92.73 ± 1.51 (N=6). The p-radioiodobenzoic acid was purified by a Sep-Pack C-18 cartridge (Waters) to remove free iodine. To the reaction vial containing purified p-radioiodobenzoic acid 10 mL of 2N NaOH was added and the solvent was dried in a nitrogen stream, followed by the addition of 100 mL of TSTU 0.2M in acetonitrile. The reaction was conducted at 60°C for 15 minutes. The HPLC profile of the final product, using silica column (10 mm; 250 x 4.6 mm, Waters) eluted with hexane:ethyl acetate:acetic acid (70:30:0.2) at a flow rate of 1 mL/ min revealed that the purified N-succinimidil-4[^{131}I]iodobenzoate was obtained with a radiochemical purity of 98.19 ± 1.14 (N=6). The N-succinimidyl-4-[^{131}I]iodobenzoate was placed in a reaction vial and the solvent was evaporated to dryness under nitrogen stream. Human IgG (200 mg/50mL of borate buffer pH 8.5) was added to this reaction vial and the reaction proceeded for 30 minutes at room temperature and gentle stirring. The reaction was terminated by the addition of 300 mL of 0.2M glicine in borate buffer pH 8.5. Human IgG was also labelled by direct method using Iodogen: 10mg of Iodogen (previously prepared as a pre-coated reaction vial), 100 mg of IgG/0.5M phosphate buffer pH 7.5 and 30-50 mL of [^{131}I]NaI (3.7-7.4 MBq) reacted at room temperature for 30 minutes with gentle stirring. Radiochemical purity of labelled proteins was evaluated by ITLC-SG using methanol 85% as solvent. The proteins labelled by both prosthetic group and Iodogen methods were purified by Sephadex G25 gel column (5 x 1 cm) eluted with PBS. Swiss mices (normal group and animals with inflammation focus developed on the right thigh by tupertine injection) were injected with IgG radioiodinated with [^{131}I]SIB and by direct method. The comparison of results showed a fast blood clearance, better target organ/background relation and low uptake in thyroid and stomach ($P < 0.01$) for the protein labelled with SIB, what suggests a greater *in vivo* stability. The potential of this thechnique may be further investigated for labelling proteins, specially peptides, to produce radiopharmaceuticals of clinical interest with radioiodine. Topic of interest: New radiopharmaceuticals