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CATALYTIC TRITIATION OF SPLENOPENTINE AND DIACETYLSPLENOPENTINE VIA THE DI-IODO-TYROSINE DERIVATIVES

J. Oehlke[+], E. Mittag*, H.-J. Klebsch[+] and H. Niedrich[+]

+ Institute of Drug Research, Academy of Sciences of GDR, Alfred-Kowalke-Str. 4, Berlin, DDR-1136 and

* Central Institute of Nuclear Research, Rossendorf, GDR

Summary

Tritium labelled splenopentine (Arg-Lys-Glu-Val-Tyr-OH) and diacetylsplenopentine (N_{α}-ac-Arg-N_{ε}-ac-Lys-Glu-Val-Tyr-OH) were prepared by the catalytic dehalotritiation of the corresponding diiodopeptides obtained by ICl-iodination. Under conditious which proved to be optimal in foregoing model deuterations, a specific radioactivity of about 90% of the theoretical one was achieved. The labelling result was influenced noticeably by a transfer of solvent hydrogen directly to the substrate analogously as found in the dehalodeuterations of simple amino acid derivatives.

Key words: ^{3}H-Tyrosine peptides, catalytic tritiation, solvent hydrogen transfer

Introduction

The immunomodulating peptides splenopentine and its diacetyl derivative labelled with tritium were required for investigations of receptor-ligand interactions.

Tyrosine containing peptides can be tritiated easily by ICl-iodination and subsequent catalytic dehalotritiation /1/, but the resulting labelling

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may differ noticeably, caused by unwanted hydrogen incorporation into the reaction product. Analogous hydrogen incorporations during dehalodeuterations of halogenated derivatives of phenylalanine and tyrosine could be minimized simply by decreasing the catalyst-to-substrate ratio, avoiding solvents with exchangeable hydrogens and by evaluation of the catalyst not only by its dehalogenation activity but also by its ability to incorporate solvent hydrogen into the substrate /2,3/.

In the present work we have tried to optimize the peptide tritiation on the basis of these model results. Additional tritiation experiments were performed to obtain information about the influence of a direct transfer of solvent hydrogen to the substrate which was shown in the dehalodeuterations /2,3/ to be as important for the labelling result as a dilution of the reacting gas by solvent hydrogen.

Results and discussion

The reaction of the peptides with equimolar amounts of ICl at a pH of about 2 led to a nearly quantitative yield of dihalogen products without noticeable oxidative side reactions. The iodinated splenopentine could be purified easily by means of reversed phase silicagel as described for the purification of iodinated insulin octapeptide-B_{23-30} /4/. For the diacetyl derivative no purification was required after the separation from the iodination mixture.

The results of the catalytic tritations accomplished with the iodinated peptides show differences which depend on the catalyst-to-substrate ratio and on the solvents used as has been previously found in deuterations of halogenated derivatives of phenylalanine and tyrosine /2,3/ (table). The decrease of 30% or more in the observed specific radioactivity when using a 5-fold catalyst-to-substrate ratio or using H_2O or D_2O instead of dimethyl-acetamide (DMA) illustrate the practical importance of minimizing these influences also for tritiations.

In the present case the reaction conditions, being optimized according

to the model investigations, led to a specific radioactivity of about 90 % of the theoretical one (table), taking into account a tritium content in the gas of about 90% and an isotopic effect of about 7, as derived for the dehalodeuteration of di-iodo-tyrosine /3/.

Table: Specific radioactivity of the labelled peptides and amount of tritium activity in the solvent after catalytic tritiation of iodinated splenopentine and diacetylsplenopentine in the presence of 10% Pd/Al_2O_3 using about 90% $^3H_2$-gas.

Peptide	solvent	mg/peptide mg catalyst	spec. activity of the ^{3}H-peptide (GBq/mmol)	^{3}H-activity in the solvent (GBq)
Di-iodo-splenopentine	H_2O/Et_3N 0.01 N	0.2/2.7	560	16
Di-iodo-splenopentine	D_2O/Et_3N 0.01 N	1.6/2.9	920	14
Di-iodo-diacetylsplenopentine	DMA/Et_3N 0.01 N	2.7/20	1175	43
Di-iodo-diacetylsplenopentine	DMA/Et_3N 0.01 N	0.5/20	855	83

A comparison of the specific radioactivies of the labelled peptides with the corresponding tritium activity incorporated into the solvent during the tritiation (table) implies that as found in the model deuterations /2,3/ a direct transfer of hydrogen from the solvent must be a major reason for the different degrees of labelling. To explain the found differences of the specific activities only by a dilution of the reacting gas with solvent hydrogen, a solvent radioactivity corresponding to a drop of the ^{3}H-content in the gas of about 90 % to at least 85 % would be needed, taking into account an isotopic effect of about 7 as derived for the dehalodeuteration of di-iodo-tyrosine /3/. Under the conditions used here (about 300 µmol ^{3}H-gas, see Experimental) such a drop in the tritium content of the reacting gas would correspond to a solvent radioactivity of about 30 GBq, which is

more than twice the total solvent radioactivity found after reaction in water (table).

This discrepancy is further increased by additional observations implying that the greatest part of the determined solvent radioactivity should not be attributed to a gas-solvent exchange, but is caused by a reduction of oxygen absorbed or bound on the catalyst similarly as found by Cerny et al. /5/, which ist not connected with a dilution of the reacting gas. Thus the solvent radioactivity seems to correlate with the catalyst amount rather than with the exchangeability of solvent hydrogen (table). Furthermore during the tritations in DMF a drop in the gas pressure corresponding to a consumption of about 30 and 40 µmols respectively of $^3H_2$ was observed, which is in accordance with the found solvent radioactivity and with the finding that more than 90 % of this radioactivity could be attributed to tritiated water by treatment with the molecular sieve 3 Å. Based on such an assumption the difference in the solvent radioactivities after reaction in DMA could be interpreted by an inhibition of the reduction of oxygen in the presence of higher amounts of substrate or halogenide formed during the reaction.

Experimental

Materials and methods

TLC was performed on silicagel 60 plates (Merck, FRG) preferably with pyridine/CH_3COOH/H_2O/CH_3OH/ethylacetate 2.7/0.8/1.5/1/1, n-butanol/pyridine/CH_3COOH/H_2O 10.5/6/1/7.5 and n-butanol/CH_3COOH/H_2O/ethylacetate 1/1/1/2.

Electrophoresis was carried out on paper type FN 7 (VEB Papierfabrik Niederschlag, GDR) at 25 V/cm in 7 % acetic acid.

Fluorescence measurements were done using a specol spectrometer equipped with a fluorescence additive (VEB Carl Zeiss, Jena, GDR). Splenopentine and diacetylsplenopentine were gifts form Dr. Klaus Forner (Inst. Drug res., Berlin, GDR).

Dimethylacetamide (Merck-Schuchard, FRG) was distilled and stored over molecular sieves 10X and 3A before use. Tritium gas (tritium content

80-90 %) was purchased from Techsnab-export (USSR) and stored in the form of uranium tritide.

Palladium on alumina (10 %) catalyst (Engelhard, Hannover, FRG) was selected after a deuteration of N-acetyl-di-iodo-tyrosineamide according to /3/.

Arg-Lys-Glu-Val-I_2-Tyr-OH

To 33 mg splenopentin (47 µmols), dissolved in 9 ml H_2O were added 0.47 ml of a 0.2 M solution of iodine monochloride in 1 M hydrochloric acid (containing 12 % NaCl). After 2 h the yellow hue of the solution was removed by addition of about 0.06 ml of 0.1 M $Na_2S_2O_3$ solution. The reaction mixture was then passed through a short column (1 x 5 cm) containing 3 g of reversed phase silicagel Li-chroprep RP 18 (25-40 µm; Merck, FRG) by means of low pressure. The silicagel was eluted successively with 40 ml H_2O, 30 ml CH_3OH/H_2O, 3/2, 10 ml CH_3OH, 20 ml CH_3OH/CH_3COOH 7/3 and 10 ml CH_3OH/CH_3COOH 2/3. The CH_3OH/H_2O 3/2 and CH_3OH/CH_3COOH 7/3 -fractions were combined and evaporated to dryness. The residue was dissolved in CH_3OH, reprecipitated by addition of ether and was then filtered off and washed with ether. Yield 30 mg (66 %). $\varepsilon_{310\ nm}$ = 4900 (0,1 m borate buffer, pH 8,0). The substance was shown to be chromatographically pure by tlc in three solvent systems.

N_ε-ac-Arg-N_ε-ac-Lys-Glu-Val-I_2Tyr-OH

To 78 mg diacetylsplenopentine (100 µmols), dissolved in 30 ml H_2O, was added 1 ml of a 0.2 M solution of ICl in 1 M hydrochloric acid (containing 12 % NaCl). After stirring for 1 h the precipitated iodination product was filtered off, washed with water and dried in vacuo over P_2O_5.
Yield 57 mg (55 %). $\varepsilon_{310\ nm}$ = 4200 (0.1 M borate buffer, pH 8.0). The product contained three minor impurities (5-10 %) by tlc but was used without furter purification for the tritiation.

Tritiation procedure

In all cases the same batch of tritium gas and catalyst was used. To

obtain information about the minimal amount of catalyst meeded for quantitative dehalogenation, the iodinated peptides were dissolved in water or DMA respectively and were treated with deuterium (produced by electrolyzing D_2O, containing 5 % H_2SO_4) at normal pressure in the presence of different catalyst amounts. A twofold of the catalyst-to-substrate ration, which led to a quantitative deahlogenation after one hour of deuteration was used as the minimal catalyst-to-substrate ratio for the tritiations (table).

For the tritiation, the di-iodinated splenopentine was dissolved in 0.5 ml of H_2O or D_2O (see table) and then 5 µl of a 10 percent solution of triethylamine in H_2O or D_2O were added. To this solution, 10 % palladium on alumina (Engelhard, Hannover, FRG) was added and the reaction vessel was connected to the tritiation manifold and was then cooled by liquid nitrogen and evacuated (p = 0.1 Pa). The same procedure was adopted in the case of di-iodinated diacetylsplenopentine which was dissolved in 0.5 ml of DMA/Et_3N 0.01 M. After the introduction of tritium gas (0.3 mmol), the reaction mixture was agitated by means of a magnetic stirrer for 60 minutes at ambient temperature and a tritium pressure of about 60 kPa.

After stopping the reaction, 1.5 ml H_2O was added, a sample of the solution was taken for counting the solvent radioactivity, and then the catalyst was centrifuged off and washed with 10 ml water. The combined filtrate were freeze-dried 4 times using water to remove labile tritium. The remaining solid was dissolved in water and then purified by paper electrophoresis.

The tritiated peptides were eluted from the paper using 1 % acetic acid and stored at -20 °C in 1% acetic acid/Ethanol 1/1 at a radioactive concentration of about 150 MBq/ml. According to UV-measurements, yields of about 70 % were obtained in all cases (relating to the amount of iodinated peptides used).

The products have been proved to be radiochemically pure to more than 90 % and identical with splenopentine and diacetylsplenopentine, respectively, by tlc using the three solvents given under Materials and methods and following radioscanning.

The specific radioactivity of the labelled peptides was determined by

measuring the extinction in 1 % acetic acid at 275 nm (ε_{275} = 1300) and liquid scintillation counting.

For splenopentine this estimation was confirmed by fluorescence measurements after reaction with fluram. For this purpose, an amount of about 5 - 20 MBq of the labelled peptide was dissolved in 2.0 ml of 0.05 M borate buffer of pH 8. To this solution 0.5 ml of a solution of fluram (Hoffmann-La Roche, Basel, Switzerland) in dioxane (2 mg/10 ml) was added under shaking, and the fluorescence intensity at 365 nm was measured within 30 minutes. The reference was treated in the same manner.

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