SYNTHESIS OF A ^{125}I LABELLED AZIDO-SUBSTITUTED GLIBENCLAMIDE ANALOGUE FOR PHOTOAFFINITY LABELLING OF THE SULFONYLUREA RECEPTOR

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SUMMARY

N-[4-(2-{4-Azido-2-hydroxy-5-[^{125}I]iodobenzamido}ethyl)benzenesulfonyl]-N'-cyclohexylurea has been prepared as a novel photoaffinity ligand for the sulfonylurea receptor. This involved synthesis of a 4-azidosalicyloyl analogue of glibenclamide and treatment of the analogue with Na^{125}I and chloramine-T. Purification by HPLC gave the radiolabelled sulfonylurea in 56% radiochemical yield and high specific activity (2130 Ci/mmol).

Keywords: Glibenclamide analogue, radioiodination, photoaffinity, sulfonylurea receptor

INTRODUCTION

Insulin-releasing sulfonylureas, e.g. tolbutamide and glibenclamide (**6**, Figure 1), are applied in the treatment of non-insulin-dependent diabetics (1-3). This effect of sulfonylureas is due to their binding to a high affinity receptor site in the plasma membrane of the pancreatic β-cell. Up to now there are no sequence data for the sulfonylurea receptor. A putative sulfonylurea receptor of M_r 150000 was purified 2500-fold from brain (4). Using [^{3}H]glibenclamide or an ^{125}I-labelled glibenclamide analogue to photolabel binding proteins in microsomal membranes from insulin-secreting cells, Kramer et al. (5), Aguilar-Bryan et al. (6), and Nelson et al. (7) presented evidence that a polypeptide of M_r 140000 represents the high affinity sulfonylurea receptor.

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However, when intact insulin-secreting cells were photolabelled by an iodinated glibenclamide derivative, examination of the labelled proteins was in favour of a M_r 65000 sulfonylurea receptor (8). One possible explanation for the discrepancies could be different conditions of photolabelling. It has been suggested that glibenclamide and its ^{125}I-labelled analogue are photoreactive because they contain benzene rings and an aromatic O-methyl ether (5,6). However, the nature of the photo-reaction is unknown, and even under optimized conditions of UV irradiation less than 1% of the occupied receptors were photocoupled to the radioligand (7).

Phenylazide groups of several ^{125}I-labelled drugs have been shown to mediate photolabelling of receptor sites with high efficiency (9-12). We therefore prepared a ^{125}I-labelled azido-substituted glibenclamide analogue and found that this novel probe showed 10-fold higher efficiency of photoincorporation into the sulfonylurea receptor than previously used probes (13). Here we provide the details of the synthesis of the new tool for investigation of the sulfonylurea receptor.

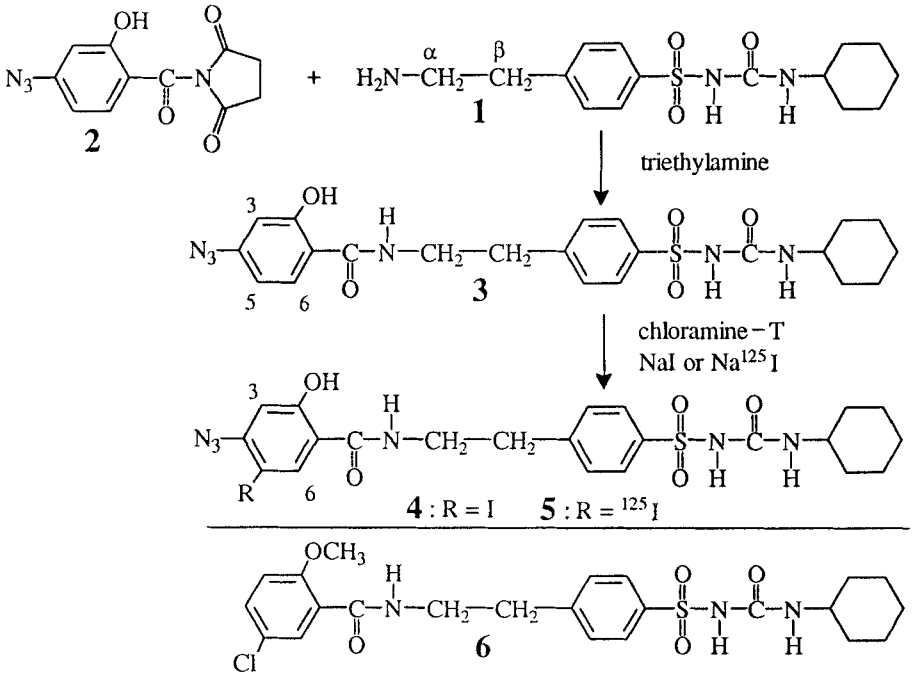

Figure 1. Structure of glibenclamide (6) and synthesis of the glibenclamide analogues (4) and (5).

RESULTS AND DISCUSSION

The synthetic route used to prepare the desired ^{125}I-labelled azido-substituted glibenclamide analogue (5) is shown in Figure 1 and involved reactions similar to those reported previously for

the preparation of iodoazidogalactosyl digitoxigenin (14). The amino-substituted sulfonylurea (**1**) was synthesized using the method reported by Vicentini and Guarneri (15) with minor modifications. Compound **1** was reacted with commercially available 4-azidosalicylic acid N-hydroxysuccinimide ester (**2**) to produce the azidosalicyloyl-substituted sulfonylurea (**3**). Compound **3** was then iodinated using non-radioactive NaI and chloramine-T to examine the reaction conditions and the purification and to characterize the purified final products by spectroscopy. In addition to compound **4**, a 3,5-diiodinated derivative of **3** was formed. When using a fourfold excess of **3** over NaI, the yield (based on **3**) amounted to 14% and less than 1% for **4** and the diiodinated product, respectively. Therefore, we also used a fourfold excess of **3** over $Na^{125}I$ when performing radioiodination. Purification by reversed-phase HPLC gave the desired ^{125}I labelled photoaffinity ligand (**5**) in 56% radiochemical yield.

EXPERIMENTAL

Unless stated otherwise, chemicals (pure grade) for syntheses were purchased from Aldrich Chemical Company (Milwaukee, WI) and were used as received. Solvents were dried and distilled before use. All melting or decomposition points were determined on a Tottoli melting point apparatus (Büchi, Switzerland) and are uncorrected. Reversed-phase HPLC was performed on a Merck-Hitachi system (type 655A-12, Darmstadt, Germany) equipped with a UV detector and an injection valve (type 7125, Rheodyne Inc., Cotati, CA) with a 1.4 mL (for preparative HPLC) or 50 µL sample loop. A Nucleosil® 300-C_{18} 7 µm column (250 x 10 mm, Macherey-Nagel, Düren, Germany) was used for preparative HPLC and an ODS-Hypersil 5 µm column (250 x 4 mm, Bischoff, Leonberg, Germany) for all other HPLC separations. IR spectra were obtained as KBr pellets on a model 297 spectrometer (Perkin-Elmer Corp., Norwalk, CT). The ^1H NMR spectra were recorded at 200 MHz on a model VXR-200 spectrometer (Varian, Palo Alto, CA). Chemical shifts are reported in part per million (δ) relative to tetramethylsilane. Positive-ion fast atom bombardment (FAB) mass spectra were measured on a Finnigan MAT 95 spectrometer with *m*-nitrobenzyl alcohol as a matrix. Elemental analyses were performed by the analytical laboratory of the University of Göttingen.

N-[4-(2-{4-Azido-2-hydroxybenzamido}ethyl)benzenesulfonyl]-N'-cyclohexylurea (3). With the method of Francia et al. (16), 4-(2-acetamidoethyl)benzenesulfonamide was prepared from 2-phenylethylamine, with an overall yield of 52%. N-[4-(2-Aminoethyl)benzenesulfonyl]-N'-cyclohexylurea (**1**) was prepared from 4-(2-acetamidoethyl)benzenesulfonamide using a minor modification of the method of Vicentini and Guarneri (15), with an overall yield of 43%.

To 6 mL dimethylformamide containing **1** (32.5 mg, 100 μmol) and triethylamine (28 μL, 200 μmol) was added 4-azidosalicylic acid N-hydroxysuccinimide ester (**2**, 27.6 mg, 100 μmol, Sigma, St. Louis, MO) at 0 °C. After stirring in the dark for 2 h at 0 °C and then overnight at room temperature, the reaction mixture was poured into icewater, followed by acidification with 2 M HCl. The resulting precipitate was collected by filtration and washed with cold water. A white solid (39.0 mg) was obtained with a yield of 80%, mp 165-170 °C (dec); IR(KBr): ν 2120 (N_3) cm^{-1}; FAB MS: m/z 487[M+1]$^+$; ^{1}H NMR((CD_3)$_2$SO): δ 1.00-1.68 (m, 11H, cyclohexyl-*H*), 2.96 (t, 2H, *J* = 7 Hz, β-C*H*$_2$), 3.57 (dt, 2H, *J* = 7,7 Hz, α-C*H*$_2$), 6.30 (d, 1H, *J* = 8 Hz, exchangeable N*H*-cyclohexyl), 6.58 (d, 1H, *J* = 1 Hz, aromatic C_3-*H*), 6.65 (dd, 1H, *J* = 8, 1 Hz, aromatic C_5-*H*), 7.40-7.90 (4H, AA'BB' type, SO_2-aromatic *H*), 8.80 (t, 1H, *J* = 7 Hz, exchangeable N*H*-CH_2), 8.83 (d, 1H, *J* = 8 Hz, aromatic C_6-*H*). Anal. calcd. for $C_{22}H_{26}N_6O_5S$: C, 54.31; H, 5.39; N, 17.27. Anal. found: C, 54.14; H, 5.39; N, 17.32.

N-[4-(2-{4-Azido-2-hydroxy-5-iodobenzamido}ethyl)benzenesulfonyl]-N'-cyclohexylurea (4). To 1 mL dimethylformamide containing **3** (73.0 mg, 150 μmol) and NaI (22.5 mg, 150 μmol) was added chloramine-T (34.1 mg, 150 μmol) at room temperature. After stirring in the dark for 1 h at room temperature, the reaction mixture was dissolved in 11 mL of the mobile phase (60% acetonitrile, 40% water, 0.07 trifluoroacetic acid) used for preparative HPLC, and 1 mL samples were separated isocratically at a flow rate of 5 mL/min. Monitoring of the eluate at 254 nm showed a retention time of 6.8 min for **3** and product peaks at 10.0 and 17.3 min. The fractions containing the peak at 10.0 min were combined and evaporated to dryness. A pale yellow solid (38 mg) was obtained with a yield of 62%; IR(KBr): ν 2120 (N_3) cm^{-1}; FAB MS: m/z 613[M+1]$^+$; ^{1}H NMR((CD_3)$_2$SO): δ 1.00-1.68 (m; 11H, cyclohexyl-*H*), 2.95 (t, 2H, *J* = 8 Hz, β-C*H*$_2$), 3.56 (dt, 2H, J = 8,8 Hz α-C*H*$_2$), 6.30 (d, 1H, *J* = 8 Hz, exchangeable N*H*-cyclohexyl), 6.82 (s, 1H, aromatic C_3-*H*), 7.40-7.95 (4H, AA'BB' type, SO_2-aromatic *H*), 8.24 (s, 1H, aromatic C_6-*H*), 8.86 (t, 1H, *J* = 8 Hz, exchangeable N*H*-CH_2), 10.22 (s, 1H, exchangeable SO_2N*H*), 12.79 (s, 1H, exchangeable O*H*).

N-[4-(2-{4-Azido-2-hydroxy-3,5-diiodobenzamido}ethyl)benzenesulfonyl]-N'-cyclohexylurea. Using the separation of the reaction mixture described above, the fractions containing the peak at 17.3 min were combined and evaporated to dryness. A pale yellow solid (8 mg) was obtained with a yield of 11%; IR (KBr): ν 2120 (N_3) cm^{-1}; FAB MS:m/z 739[M+1]$^+$; ^{1}H NMR((CD_3)$_2$SO): δ 1.00-1.68 (m; 11H, cyclohexyl-*H*), 2.95 (t, 2H, *J* = 8 Hz, β-C*H*$_2$), 3.56 (dt, 2H, J = 8,8 Hz, α-C*H*$_2$), 6.30 (d, 1H, *J* = 8 Hz, exchangeable N*H*-cyclohexyl),

7.40-7.95 (4H, AA'BB' type, SO_2-aromatic *H*), 8.36 (s, 1H, aromatic C_6-*H*), 8.86 (t, 1H, *J* = 8 Hz, exchangeable N*H*-CH_2), 10.22 (s, 1H, exchangeable SO_2N*H*), 12.79 (s, 1H, exchangeable O*H*).

N-[4-(2-{4-Azido-2-hydroxy-5-[^{125}I]iodobenzamido}ethyl)benzenesulfonyl]-N'-cyclohexylurea (**5**). To a NENSURE®-vial containing 2 mCi of Na^{125}I (2130 Ci/mmol, 0.94 nmol) in 20 µL of 10 µM NaOH (NEZ-033A, NEN, Dreieich, Germany) was added **3** (3.75 nmol, 15 µl of a 0.25 mM solution). The latter solution was prepared by dissolving 1.22 mg **3** in 8 mL of 10 mM NaOH, adding 1.95 mL of 0.5 M sodium phosphate buffer (pH 7.4) and adjusting the pH to 7.4 with 2 M HCl. Iodination was started by addition of 15 µL of 0.25 mM chloramine-T dissolved in 0.1 M sodium phosphate buffer (pH 7.4). After 15 min at room temperature in the dark, the reaction was quenched by addition of 1 µL of 0.14 M ß-mercaptoethanol and mixing. Separation of the total reaction mixture was carried out immediately by HPLC (ODS-Hypersil 5 µm column). The mobile phase (60% acetonitrile, 40% water, 0.07% trifluoroacetic acid) was used isocratically with a flow rate of 2 mL/min. The retention times of the radioactive peaks representing **5** and the 3,5-diiodinated derivative of **3** were 4.8 and 9.8 min, respectively, and were identical with the retention times of the corresponding authentic samples. The radioactivity of the diiodinated derivative was 4% of the radioactivity of **5**. A minor radioactive peak was eluted just ahead of **5**. This peak amounted to about 5% of the radioactivity of **5** and was excluded from the fractions containing **5**. The retention time of **3** was 3.0 min, and non-radioactive UV peaks were not detected between 3.0 and 15.0 min. The fractions (333 µl) containing **5** were collected, counted (1.12 mCi) and stored at 4° C (used for up to 4 weeks). As monitoring of the eluate did not reveal chemical or radiochemical impurities in the fractions containing **5**, a specific activity of 2130 Ci/mmol can be assumed. The radiochemical yield based on ^{125}I was 56%.

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