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SYNTHESIS OF A NEW ^{125}I-LABELED TYROSINE METHYL ESTER CONJUGATE OF ADENYLYL-(2'-5')-ADENYLYL-(2'-5')-[2',3'-DI-O-(2-CARBOXYETHYL)ETHYLIDENE]ADENOSINE

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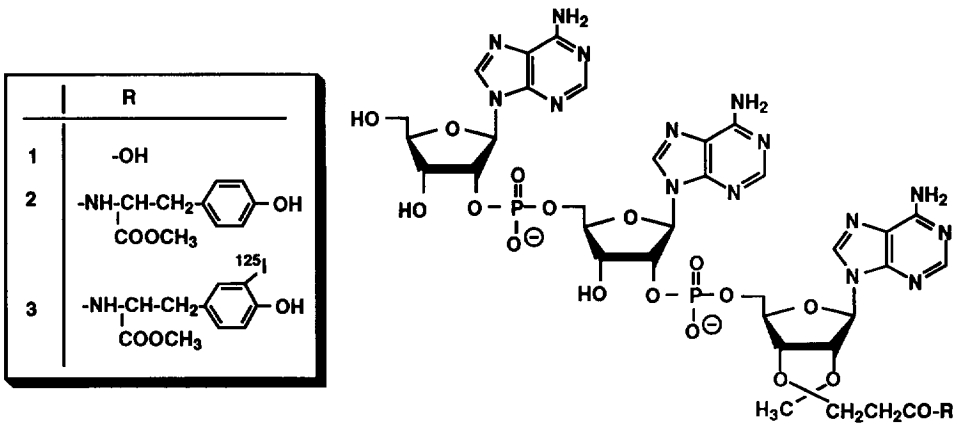
Abstract. A novel ^{125}I-labeled conjugate of 2',5'-oligoadenylate trimer for radioimmuno assay of 2',5'-oligoadenylates have been obtained by the reaction of adenylyl-(2'-5')-adenylyl-(2'-5')-[2',3'-di-O-(2-carboxyethyl)-ethylidene]adenosine (**1**) with tyrosine methyl ester followed by iodination of intermediate **2** with ^{125}I in the presence of chloramine-T. ^{125}I-labeled conjugate **3** was isolated by column chromatography on a TSK-gel HW-40 in 73% yield. Specific activity for the synthesized conjugate was approximately 1760 Ci/mmol.

Introduction.

It is known that components of the 2-5A system are widely distributed in tissues and cells, and their activity varies with physiological conditions[1]. The functions of the 2-5A system outside the interferon treatment suggesting that it may be involved in the normal regulation of cellular RNA levels. However, the real importance and exact role of the 2-5A system is still unresolved. To examine its possible roles it is essential to have direct, sensitive assays for 2',5'-oligoadenylates, both 5'-phosphorylated and nonphosphorylated. The assays based on the inhibition of cell-protein synthesis through the activation of the 2-5A dependent RNase L[2], or on the cytotoxic activity of nonphosphorylated derivatives[3], are relatively insensitive and not suitable for the routine analysis of large numbers of samples[4]. The radioimmuno assay, using antibodies against 2',5'-oligoadenylates, is the most convenient method for this purpose. The major difficulty in the development of this assay lies in the synthesis of radioactive 2',5'-oligoadenylates of sufficiently high specific activity to give the required sensitivity. Some radioimmuno assays for 2',5'-oligoadenylates (5'-phosphorylated and nonphosphorylated) have been developed using [8-^{14}C]-A2'p5'A2'p5'A[5], $(A2'p)_2A$-[^{32}P]pCp, and $ppp(A2'p)_2A$-[^{32}P]pCp[4,6,7], ^{125}I-labeled conjugates of alanyltyrosine methyl ester with periodate-oxidized 5'-triphosphates trimer and tetramer A2'p5'$(A2'p)_n$5'A[8,9], and ^{125}I-labeled A2'p5'A succinyl tyrosine methyl ester[10,11], as radiolabeled probes. Each of these compounds has a serious drawback which consists in low specific activity of [8-^{14}C]-A2'p5'A2'p5'A, high difference in the structure of periodate-oxidized and [^{32}P]pCp containing derivatives with determined 2',5'-oligoadenylates, and insufficient stability of succinyl group of ^{125}I-labeled A2'p5'A succinyl tyrosine methyl ester. To overcome these disadvantages we developed a new sensitive radioimmuno assay for 2',5'-oligoadenylates, using stable immuno-

genic and ^{125}I-labeled conjugates attached to the adenylyl-(2'-5')-adenylyl-(2'-5')-[2',3'-di-O-(2-carboxyethyl)-ethylidene]adenosine (**1**) at the 2'-terminal carboxyl function. This type of conjugate allows to detect phosphorylated and nonphosphorylated 2',5'-oligoadenylates[12,13] at physiological concentrations (i.e. nanomolar range). Here we describe the synthesis of a new ^{125}I-labeled tyrosine methyl ester derivative of 2',5'-A_3 trimer.



Chemical Syntheses

Adenylyl-(2'-5'-)adenylyl-(2'-5')-[2',3'-di-O-(2-carboxyethyl)ethylidene]adenosine tyrosine methyl ester conjugate (2)[18]. To a solution of bis-triethylammonium salt of trimer **1**[14] (6.3 mg, 4.7 μmole) in a mixture of DMF (300 μl) and NEt_3 (8.3 μl, 6.03 mg, 60 μmole), butyl chloroformate (7.8 μl, 8.21 mg, 60 μmole) was added at 0°C, and the reaction mixture was stirred at room temperature for 40 min. Then a suspension of tyrosine methyl ester hydrochloride (6.96 mg, 30 μmole) and NEt_3 (4.17 μl, 3.03 mg, 30 μmole) in DMF (300 μl) was added, and the reaction mixture incubated at room temperature for 15 h. To the resulting homogeneous mixture, a saturated solution of ammonia in methanol (100 μl) was added and the mixture evaporated. The residue was dissolved in water (2 ml) and applied onto a DEAE-cellulose SS-23 column (2x16 cm; HCO_3^--form). The product was eluted with a linear gradient of aqueous (0.01/0.3 M) TEAB buffer solution. The product containing fractions were collected and lyophilized to give a residue (2.6 mg; 36%) of conjugate **2**. UV[17] (H_2O): λ_{max} 260 nm, ε = 36900. ^{1}H-NMR[17] (D_2O): 8.17 (s, 1H), 8.11 (s, 1H), 7.91 (s, 2H), 7.83 (s, 1H) and 7.75 (s, 1H) (2-H, 8-H); 7.01 (d, 2H, o to PhOH, J=9.6 Hz); 6.68 (d, 2H, m to PhOH, J=9.6 Hz); 6.10 (d, 1H, 1'-H, $J_{1',2'}$=4.8 Hz); 5.94 (d, 1H, 1'-H, $J_{1',2'}$=3.0 Hz); 5.84 (d, 1H, 1'-H, $J_{1',2'}$=1.2 Hz); 5.05 (m, 2H, 2'-H); 3.83 (s, 3H, OCH_3); 2.46 (m, 2H, CH_2CH_2CONH); 2.13 (m, 2H, CH_2CH_2CONH); 1.38 (s, 3H, CCH_3). Anal. Calcd for $C_{57}H_{84}N_{18}O_2P_2$: C, 48.78; H, 6.03; N, 17.96. Found: C, 48.59; H, 6.01; N, 17.88.

^{125}I-labeled adenylyl-(2'-5')-adenylyl-(2'-5')-[2',3'-di-O-(2-carboxyethyl)ethylidene]-adenosine tyrosine methyl ester conjugate (3). To a solution of bis-triethylammonium salt of conjugate **2** (1.4 mg, 1.0 mmol) in 15 ml of 0.1 M sodium phosphate buffer (pH 7.5), a solution of Na^{125}I (100 MBq) in 5 ml of 0.1 M sodium hydroxide, and chloramine-T (20 mg, 71 mmol) in 10 ml of sodium phosphate buffer were added. The reaction mixture was stirred at room temperature for 15 min, diluted with a solution of $Na_2S_2O_5$ (20 mg, 120 mmol) in 10 ml of sodium phosphate buffer. The resulting solution was applied onto a TSK-gel HW-40 column (1x25 cm), and the product eluted with sodium phosphate buffer, containing 0.1% of bovine serum albumin. The product containing fractions were collected and lyophilized to give 73 MBq (73%) of ^{125}I-labeled conjugate **3** with 98% of radiochemical purity and a specific radioactivity of 1760 Ci/mmol.

Results and Discussion

For the synthesis of radioactive labeled probe **3** trimeric adenylyl-(2'-5')-adenylyl-(2'-5')-[2',3'-di-O-(2-carboxyethyl)ethylidene]adenosine (**1**) was used as a starting compound[14] which implicates some structural advantages in this approach. Firstly, the synthesis of 2',3'-di-O-(2-ethoxycarbonylethyl)ethylidene-N^6-benzoyladenosine - a key intermediate for the preparation of trimer **1** - resulted in formation of only one diastereomer with the unequivocally defined structure of the *endo* conformation of the methyl group, and the *exo* conformation of the carboxyethyl side chain[15]. Secondly, this modification keeps the ribofuranose ring at the 2'-terminal nucleoside intact and does not influence the spatial arrangement of the oligomer **1** vs. trimer 2'-5'A_3. This conclusion follows from the identity of the ORD-spectra of both oligomers (data not shown). Thirdly, we have found that compound **1** does not undergo remarkable degradation upon incubation with rabbit serum at 37°C for 24 h.

The carboxyl function of trimer **1** was activated by treatment with butyl chloroformate to the intermediary mixed anhydride which on treatment with L-tyrosine methyl ester gave the desired conjugate **2** in 36% isolated yield after anion-exchange column chromatography. The structure of this compound was confirmed by UV and ^{1}H-NMR spectroscopy as well as elemental analysis.

Trimer **2** was iodinated with ^{125}I by conventional chloramine-T method[16] to give ^{125}I-tyrosine methyl ester conjugate of adenylyl-(2'-5')-adenylyl-(2'-5')-[2',3'-di-O-(2-carboxyethyl)ethylidene]adenosine (**3**), isolated in 73% yield by column chromatography on TSK-gel HW-40. The specific radioactivity of the ^{125}I-labeled compound was 1760 Ci/mmol which suggests that nearly all the molecules of trimer **3** were iodinated with ^{125}I. This novel conjugate was stable upon storage in a freezer for 2 months. Conjugate **3** was used as a labeled probe of which 90% was bound to an excess of antibodies and fully displaceable by trimer **2**.

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17. ^{1}H-NMR spectrum was obtained on *Bruker WM-360* spectrometer and is reported relative to tetramethylsilane (δ-scale in ppm). Ultra-violet spectrum was recorded with *Specord UV-VIS* spectrophotometer. Elemental analysis was performed by the Microanalytical Laboratory at the Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences in Minsk.
18. Butyl chloroformate was purchased from *Fluka* (Switzerland), tyrosine methyl ester hydrochloride from *Reanal* (Hungary), bovine serum albumin, DEAE-cellulose SS-23 from *Serva*, and TSK-gel HW-40 from *Toyo Soda MFG, Co., Ltd.* (Japan).

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