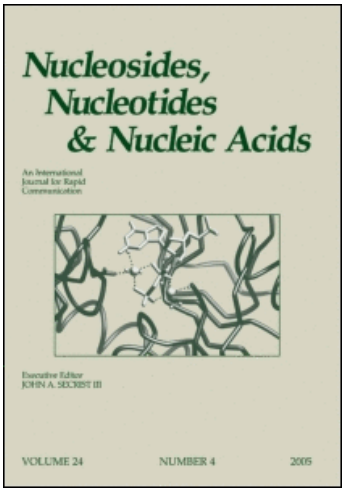

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Preparation of 8-Hydroxy-dGTP and 2-Hydroxy-dATP by a Phosphate Transfer Reaction by Nucleoside-Diphosphate Kinase

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PREPARATION OF 8-HYDROXY-dGTP AND 2-HYDROXY-dATP BY A PHOSPHATE TRANSFER REACTION BY NUCLEOSIDE-DIPHOSPHATE KINASE

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ABSTRACT: Here we show a simple method for the preparation of modified nucleoside triphosphates using nucleoside-diphosphate kinase. 8-Hydroxy-2'-dGDP and 2-hydroxy-2'-dADP were incubated with ATP and nucleoside-diphosphate kinase. By the transfer of the γ-phosphate of ATP, the cognate triphosphates were formed. This method is applicable to the preparation of modified nucleoside triphosphates that are labeled at the γ-phosphate and/or are difficult to purify.

Modifications of deoxyribonucleoside triphosphates by endogenous and exogenous agents, in addition to those within DNA, are believed to be involved in mutagenesis and other processes. For example, 8-hydroxy-2'-deoxyguanosine 5'-triphosphate (8-OH-dGTP) and 2-hydroxy-2'-deoxyadenosine 5'-triphosphate (2-OH-dATP), which are produced by reactive oxygen species, induce mutations *in vivo*[1]. Thus, the preparation of modified nucleoside triphosphates is required to elucidate their biological roles in cells. Particularly, radioactive nucleoside triphosphates will be useful to study the metabolism of modified nucleotides.

Nucleoside-diphosphate kinase (NDPK, EC 2.7.4.6), which is present ubiquitously and is considered a housekeeping enzyme, catalyzes the transfer of the γ-phosphate from a nucleoside triphosphate to a nucleoside diphosphate[2,3]. Since this enzyme appears to display little specificity for different bases[4,5], we think that it may be utilized for the preparation of modified deoxyribonucleoside triphosphates. We report here the preparation of 8-OH-dGTP and 2-OH-dATP from the cognate diphosphates by this enzyme.

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MATERIALS AND METHODS

Nucleotides.

2-Hydroxy-2'-deoxyadenosine 5'-diphosphate (2-OH-dADP), 8-hydroxy-2'-deoxyguanosine 5'-diphosphate (8-OH-dGDP) and authentic 8-OH-dGTP were synthesized from dADP, dGDP and dGTP, respectively, by the Fe (II)-EDTA treatment as described[6].

NDPK reactions.

The mixture of 2000 pmol of 8-OH-dGDP or 2-OH-dADP and 200 pmol of ATP was incubated with one unit of yeast NDPK (Sigma) in a buffer solution of 83 mM triethanolamine, 17 mM $MgCl_2$, and 67 mM KCl, pH 7.6 in a total volume of 20 μl at 37 °C for one hr (10:1 reactions). For 30:1 reactions, the mixture of 6000 pmol of 8-OH-dGDP and 200 pmol of ATP was incubated with two units of NDPK in a total volume of 40 μl. The reaction mixture was injected onto a TSK-gel DEAE-2SW column (ϕ4.6 X 250 mm, Tosoh). The elution was carried out with 20% acetonitrile, 75 mM sodium phosphate buffer (pH 7.0) at a flow rate of 1 ml/min.

RESULTS AND DISCUSSION

Ten- or 30-fold excess amount of 8-OH-dGDP was incubated with 200 pmol of ATP and yeast NDPK at 37 °C and the reaction mixture was analyzed by anion-exchange HPLC using TSK-gel DEAE-2SW. As shown in FIG. 1E-H, the treatment of 8-OH-dGDP and ATP with NDPK yielded a compound that eluted after ATP and showed a spectrum similar to 8-OH-dGTP (data not shown). We confirmed the formation of 8-OH-dGTP by the NDPK reaction by coinjection of the reaction mixture with authentic 8-OH-dGTP (FIG. 1I). Moreover, the yields of 8-OH-dGTP were 9 and 3% of the starting 8-OH-dGDP when ten- and 30-fold, respectively, excess amounts of 8-OH-dGDP were used (data not shown). These values are coincident with the ratio of ATP to 8-OH-dGDP added to the reaction vessels, indicating that almost all of the ATP molecules were utilized as phosphate donors. Thus, the γ-phosphate of ATP appears to be transferred by NDPK very efficiently. Similarly, 2-OH-dADP was converted to 2-OH-dATP by this enzyme (FIG. 1J).

This new method will be applied to the preparation of these and other base-modified nucleoside triphosphates that are labeled with ^{32}P or ^{33}P at the γ-phosphate. To date, modified nucleotides labeled with ^{32}P or ^{33}P at the γ-position must be prepared from unmodified γ-radiolabeled nucleotides, and the low yields of the desired compounds may make it difficult to carry out experiments in which large amounts of tracers are necessary. In our new method, only the mixing of "cold" modified nucleoside diphosphates with [γ-^{32}P] or [γ-^{33}P]ATP and NDPK is required to obtain the γ-labeled

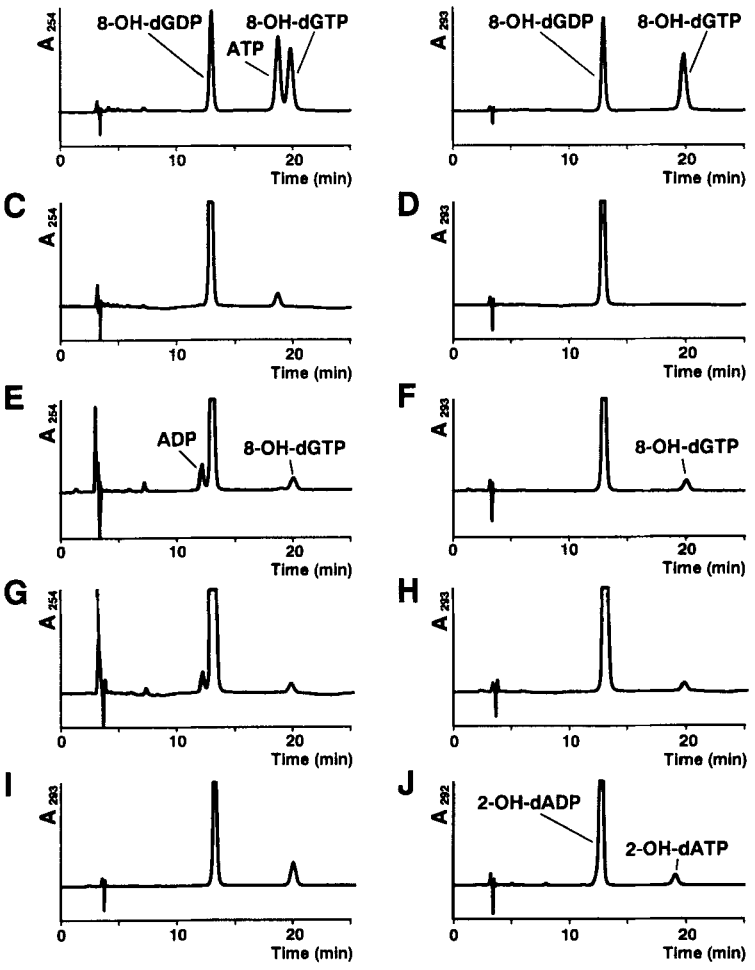

FIG. 1. Anion-exchange HPLC analysis of the phosphorylation reactions catalyzed by NDPK. (A)(B) Authentic nucleotides, 8-OH-dGDP, 8-OH-dGTP, and ATP. (C)(D) The mixture of 2000 pmol of 8-OH-dGDP and 200 pmol of ATP (10:1) was incubated without NDPK. (E)(F) The same mixture was incubated with NDPK (one unit) at 37°C for one hr. (G)(H) The mixture of 6000 pmol of 8-OH-dGDP and 200 pmol of ATP (30:1) was incubated with NDPK (two units). (I) The 10:1 mixture was treated with NDPK and was injected onto an HPLC column with authentic 8-OH-dGTP. (J) The mixture of 2000 pmol of 2-OH-dADP and 200 pmol of ATP (10:1) was incubated with NDPK (one unit) at 37°C for one hr. (A)(C)(E)(G) Monitored at 254 nm. (B)(D)(F)(H)(I) Monitored at 293 nm. (J) Monitored at 292 nm.

cognate nucleoside triphosphates. Indeed, we could prepare [γ-^{33}P] 8-OH-dGTP and [γ-^{33}P] 2-OH-dATP (data not shown). This simple method will be useful for the preparation of short-lived radioactive compounds. Also, sugar-modified nucleotide analogues may be labeled by this method because NDPK catalyzes the kination reactions

of both deoxyribo- and ribonucleoside diphosphates. These base- or sugar-modified nucleotides can be used as substrates in binding and hydrolysis experiments of nucleotide binding proteins as G-proteins and ATPases which are involved in many important biological processes, in order to study the substrate specificities. This simple method may be useful in preparation of base-modified nucleotides for studies of MutT-like proteins, which remove damaged nucleoside triphosphates. Moreover, this method can be an alternative preparation protocol of modified nucleoside triphosphates that are difficult to purify after direct reaction of a nucleoside triphosphate precursor.

In conclusion, this one-step preparation method for modified nucleoside triphosphates will be very useful in investigations of the biological roles of the nucleotides.

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