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Exchange Labelling of ATP with Tritium Gas**

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Abstract

Adenosine 5' triphosphate (ATP) in aqueous solution has been labelled by exchange with tritium gas in the presence of palladium oxide catalyst. Comparison with our experiments using Pd/BaSO₄ as the catalyst shows that we have obtained product with higher specific activity and improved chemical purity. ³H NMR spectroscopy of the tritiated ATP shows labelling in both the C-8 and C-2 positions, and the integral ratio of these positions was found to vary from 3:1 to 1:1 under different reaction conditions.

1. INTRODUCTION

We required high specific activity ATP for biological studies, and the preferred site of labelling was the 2 position on the Adenine ring. Heterogeneous metal-catalyzed exchange in solution with tritium gas provides a convenient method for labelling nucleosides and nucleoside mono-, di- and triphosphates, [1] and seemed the obvious first approach. In this technique, the compound is stirred in solution in the presence of tritium gas and a metal catalyst, and palladium oxide supported on barium sulphate has often been the catalyst of choice. Even though exchange is fast in some cases, most adenosine derivatives require a long reaction time (16-21 h) during which nucleoside phosphates tend to hydrolyze, resulting in poor chemical yields and the necessity for extensive purification. The use of a catalyst promoting faster exchange was sought to overcome this disadvantage, with the additional aim of tritium incorporation into a stable position. We have used unsupported palladium oxide as the catalyst for exchange labelling of adenosine 5'-triphosphate (ATP) with tritium gas.

2. RESULTS AND DISCUSSION

The exchange reactions were carried out by exposing a solution of ATP in phosphate buffer (pH 8.6) to a catalyst and an atmosphere of tritium gas. After reaction the catalyst was filtered off and the reaction mixture analyzed by HPLC. The products were eluted with diammonium phosphate, under conditions (Experimental) which resolved ATP from the major hydrolysis products, adenosine 5'-diphosphate (ADP) and adenosine 5'-monophosphate (AMP).

Our initial studies employing 5% palladium oxide supported on barium sulphate were conducted for comparison with the results obtained using unsupported palladium oxide, and the data are summarized in Table 1.

Table 1
Metal-Catalyzed Exchange of ATP^a with 20% T/H

R _x No.	Catalyst ^b (mg)	R _x Time (h)	Product Distribution (%) ^c				Yield mCi	S.A. Ci/mM	Dist. of ³ H ^d	
			ATP	ADP	AMP	Others			C-8	C-2
1	A (200)	1.75	95.7	1.9	-	2.3	11.7	0.75	88	12
2	A (200)	22	66.0	25.0	0.6	8.9	39	4.0	^e	
3	B (40)	3	92.0	8.0	-	-	117	3.5	74	26
4	B (40)	6	92.5	7.5	-	-	125	7.4	67	33
5	B (80)	3	91.1	8.9	-	-	135	8.4	70	30
6 ^f	B (80)	3	-	-	-	-	188	10.7	53	47

- a). ATP (25 mg) in phosphate buffer (pH = 8.6, 1 mL).
 b). Catalyst system A = 5% palladium oxide on barium sulphate and B = unsupported palladium oxide.
 c). Based on HPLC and UV analysis, similar values were obtained from radioactivity measurements.
 d). Based on measurement of integrals in the ³H NMR spectra of the labelled ATP.
 e). The resonances were broadened, hence accurate measurement of integrals was not feasible.
 f). 100% T₂ gas.

Table 2
Literature Results for Metal-Catalyzed Exchange of ATP^a with Tritium Gas

R _x No.	Catalyst (mg)	R _x Time (h)	Product Distribution (%)				Yield mCi	S.A. Ci/mM	Dist. of ³ H	
			ATP	ADP	AMP	Others			C-8	C-2
1 ^b	50	72	90.0	-	-	10.0	76	-	-	-
2	50	16	90.0	-	-	10.0	84	13.0	-	-
3 ^c	50	16	70.0	-	-	30.0	120	-	-	-
4 ^d	125	16	80.0	-	-	20.0	290	-	-	-
5 ^e	100	1	-	-	-	-	349	20.0	-	-
6 ^f	50	4	-	-	-	-	675	12.0	78	22
7 ^g	50	2	-	-	-	-	97	5.6	52	48

- a). PdO on BaSO₄ catalyst. ATP (10 mg) in phosphate buffer (0.1M, pH = 8.0, 2.5mL). Chemical yields were estimated at 50-60%, with losses thought to be due to adsorption on the catalyst (Reference 1).
 b). ATP (10 mg) in water (pH = 8.0, 2.5mL).
 c). ATP (10 mg) in phosphate buffer (0.1M, pH = 10.0, 2.5mL).
 d). ATP (25 mg) in phosphate buffer (0.1M, pH = 10.0, 2.5mL).
 e). Data from Reference 2, Table 3: ATP (10 mg) in phosphate buffer (0.1M, pH = 10.0, 2.5mL).
 f). Exchange results from Reference 2, Table 3: Adenosine (30mg) in phosphate buffer (0.1M, pH = 8.2, 1.5mL). NMR data are from Reference 3.
 g). As in f), with Adenine-b-D-araboside (10mg) in phosphate buffer (0.1M, pH = 8.2, 2.0mL).

The 1.75 hour exchange reaction using Pd/BaSO₄ resulted in ATP of low specific activity (S.A. = 0.75 Ci/mmmole), with the majority of the tritium in the C-8 position. A 22 h reaction time resulted in significant decomposition to ADP and other products (Table 1, reaction 2), and therefore to a lower recovery of ATP. These results are similar to those obtained by others using carrier free tritium gas and Pd/BaSO₄ and compiled in Table 2 [1-3].

In contrast, exchange reactions using unsupported palladium oxide resulted in largely unhydrolyzed ATP (Table 1, #3). The specific activity (3.5 Ci/mmmole) was comparable to the 22 h Pd/BaSO₄ reaction, and 26% of the tritium was in the stable C-2 position. The ³H NMR spectrum of this tritium labelled ATP shows resonances at δ 8.54 (s) and 8.24 (s) arising from labelling of C-8 and C-2 positions respectively (Fig 1A). This represents an increase of a factor of ten for the C-2 position, when compared with reaction 1 in Table 1 (e.g. 0.88 vs 0.08 Ci/mmmole). Longer reaction times or an increase in the amount of catalyst did not appreciably affect the tritium distribution between the C-2 and C-8 positions, but increased the total specific activity (Table 1, #3, #4). By a combination of these approaches it was possible to increase the specific activity at the C-2 position by a factor of 25-30 (cf. #1 and #5, Table 1), while still observing little hydrolysis of the ATP.

A PdO catalyzed exchange reaction using 100% tritium gas resulted in tritium labelled ATP with a specific activity of 10.7 Ci/mmmole (Table 1, #6). There are two issues of interest in this result: by extrapolation of the reaction conditions from reaction 5 we may have expected a five-fold increase in specific activity when using 100% T₂ in place of the 20% T/H mixture, and comparison of the tritium NMR spectrum (Figure 1B) with products from a 20% T/H reaction shows a markedly different distribution. The reasons for these differences are unknown, but one contributing factor could be back-exchange of the C-8 position during methanolic workup. Good kinetic data exist for the base-catalyzed exchange of the C-8 and C-2 positions of a number of adenine derivatives, and the C-8 is known to be rapidly exchanged [4,5]. Such issues were not further investigated in this work, but one approach may be to study the exchange reactions with deuterium, with and without methanolic workup of products.

For our studies, a concurrent approach was to synthesize high specific activity ATP tritium labelled specifically at the C-2 position by tritidehalogenation of 2-bromo-adenosine 5'-triphosphate. While our desired, highly tritiated, C-2 labelled ATP was produced by this dehalogenation approach, it is interesting to note that the same inconsistency in exchange levels at the C-8 position was observed between 10% T/H and carrier free tritium experiments as noted for the PdO exchanges. A comparison of the tritium NMR spectra for a 10% experiment and a 100% experiment is shown in Figures 1C and 1D, and the C-8 contribution is markedly reduced in the spectrum of the high level tritiation.

3. EXPERIMENTAL

ATP was obtained from Boehringer Mannheim GmbH. HPLC was performed using a Waters Model 510 pump with automated gradient controller, a 1/4" x 25 cm Supelco LC-18 column, a Hewlett Packard 1040A diode array spectrometer for UV analyses, and a Raytest Ramona-5-LS radiometric detector for radioactivity measurements. The HPLC conditions were established using reference samples. The chemical yield and specific activity of the product were determined by UV analysis and radioactivity measurements. NMR spectra were recorded on IBM Instruments Inc. AF-300 spectrometer (¹H at 300 MHz, ³H at 320 MHz).

Tritium Labelling of ATP: ATP (25 mg, 0.041 mmole) was dissolved in phosphate buffer (pH 8.6, 1 mL), catalyst (PdO or 5%Pd/BaSO₄) was added, and the substrate was hydrogenolyzed under 1 atm of tritium gas. The catalyst was filtered off, washed with water (2 x 2 mL) and the filtrate was lyophilized. The residue was analyzed by HPLC with a mobile phase of 0.1M diammonium phosphate adjusted to pH 6.0 with phosphoric acid, flow rate 2 mL/min. The chemical yield, specific activity and ³H NMR integral data obtained from different reactions are given in Table 1.

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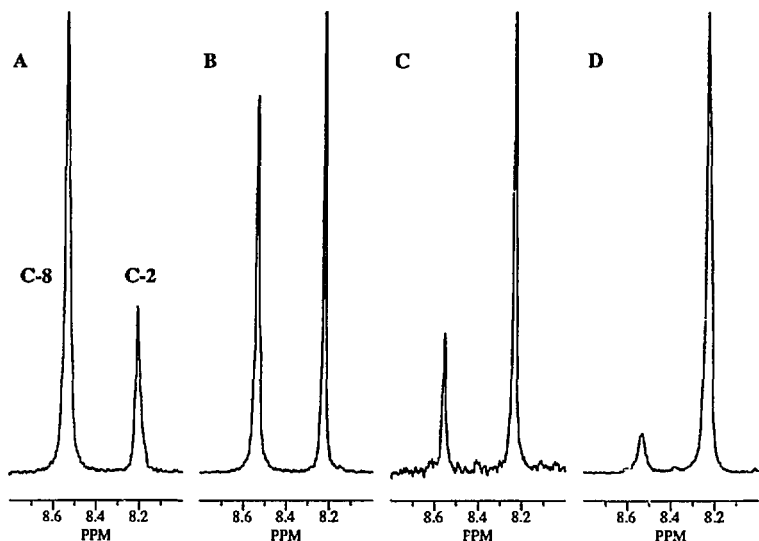


Figure 1. 320 MHz tritium NMR spectra of labelled ATP in D_2O . Products A&B were obtained by PdO-catalyzed exchange with tritium gas for 3 hours. A). 20% T/H, B). 100% T_2 . Products in C&D were obtained by PdO-catalyzed dehalogenation of 2-Br-ATP for 3 hours C). 10% T/H, and D). 100% T_2 .